ISSN(Print):2328-3491, ISSN(Online): 2328-3580, ISSN(CD-ROM): 2328-3629



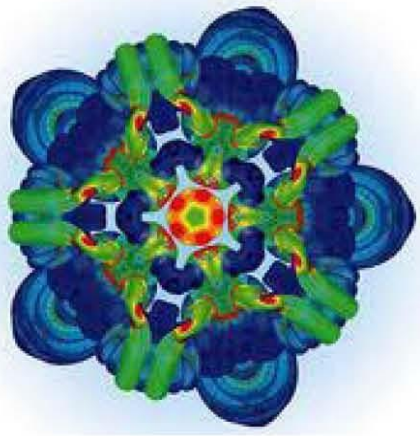
National Conference on- Innovations in Bio, Chemical and Food Technology, February 02, 2019

Organised by:
Laboratory for Bioremediation Research and Unit Operations Laboratory, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

A I J R S T E M

American International Journal of Research in Science, Technology, Engineering & Mathematics





International Association of Scientific Innovation and Research (IASIR)
(An Association Unifying the Sciences, Engineering, and Applied Research)
STEM International Scientific Online Media and Publishing House
Head Office: 148, Summit Drive, Byron, Georgia-31008, United States.
Offices Overseas: Germany, Australia, India, Netherlands, Canada.
Website: www.iasir.net, E-mail (s): iasir.journals@iasir.net, iasir.journals@gmail.com, aijrstem@gmail.com

ISSN (PRINT): 2328-3491
ISSN (ONLINE): 2328-3580
ISSN (CD-ROM): 2328-3629

AIJRSTEM

Special Issue:
National Conference on Innovations in Bio, Chemical and Food Technology (IBCFT -2019), February 02, 2019

Organised by:
Laboratory for Bioremediation Research and Unit Operations Laboratory, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore 641049, Tamil Nadu, INDIA

.

American International Journal of Research in Science, Technology, Engineering & Mathematics

International Association of Scientific Innovation and Research (IASIR)
(An Association Unifying the Sciences, Engineering, and Applied Research)
STEM International Scientific Online Media and Publishing House
Head Office: 148, Summit Drive, Byron, Georgia-31008, United States.
Offices Overseas: Germany, Australia, India, Netherlands, Canada.
Website: www.iasir.net, E-mail (s): iasir.journals@iasir.net, iasir.journals@gmail.com, aijrstem@gmail.com

Editorial Board Members of the National Conference- on Innovations in Bio, Chemical and Food Technology (IBCFT 2019)

CHIEF EDITOR

Dr. N. Sivarajasekar, Organizing Secretary,
Laboratory for Bioremediation Research and Unit Operations Laboratory,
Department of Biotechnology, Kumaraguru College of Technology,
Coimbatore – 641049, Tamilnadu, India.

EDITORS

Dr. N. Selvaraju,
Department of Bioscience and Bioengineering,
IIT Guwahati, Guwahati

Dr. K. Ravi Shankar,
Department of Biotechnology,
Anna University Trichy, Tiruchirappalli

Dr. M. Matheswaran,
Department of Chemical Engineering,
National Institute of Technology Trichy, Tiruchirappalli

Dr. M. Thirumarimurugan,
Department of Chemical Engineering,
Coimbatore Institute of Technology

Dr. Swapnil A Dharaskar,
Department of Chemical Engineering,
Pandit Deendayal Petroleum University, Gandhinagar, Gujarat

Dr. M. Rajasimman,
Department of Chemical Engineering,
Annamalai University, Annamalai Nagar, Chidambaram

Dr. K. Saravanan,
Department of Chemical Engineering,
Kongu Engineering College, Erode

Dr. R. Baskar,
Department of Food Technology,
Kongu Engineering College, Erode

Dr. J. Prakashmaran,
Department of Food Science and Nutrition,
Periyar University, Salem

Dr. Gopikrishnan,
Department of Food Process Engineering,
Karunya University, Coimbatore

Organizing Committee Members of the National Conference- on Innovations in Bio, Chemical and Food Technology (IBCFT 2019).

Dr. P.Ramalingam, Professor, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. R.Baskar, Associate Professor, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. Vinohar Stephen Rapheal, Associate Professor, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. K.Kumaresan, Associate Professor, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. T. Sathishkumar, Assistant Professor – III, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. S. Nithyapriya, Assistant Professor (Sr.G), Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Mr. D.R. Manimaran, Assistant Professor (Sr.G), Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Mr. P. Muthukumaran, Assistant Professor (Sr.G), Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. A. Thirumurugan, Assistant Professor - II, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. K. Kumaravel, Assistant Professor - II, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

Dr. K. Ram, Assistant Professor - II, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore

TABLE OF CONTENTS

Conference Proceedings of the "National Conference- on Innovations in Bio, Chemical and Food Technology 2019 (IBCFT 2019)", Kumaraguru College of Technology, Coimbatore 641049, Tamil Nadu, INDIA

Special Issue of IBCFT 2019

S. No.	Paper Code	Title	Page No.
		Chemical Science and Technology	
1	IBCFT-001	Removal of Cr(VI) from Waste Water Using Cocos Nucifera –Batch Studies **G.Surendran, S. Kavitha, K.Balasubramani, J. Praveen Kumar, S.S.Baral**	1-8
2	IBCFT-014	Environmentally Benign Encapsulated Functional Ionic Liquids As Catalysts For Synthesis Of Phenolic Compound Based Antioxidant **Pandian Elavarasan, Sreedevi Upadhyayula**	9-12
3	IBCFT-015	Techno-Economic Analysis for the Production of Ethanol from Ipomoea batatas (sweet potato) **Deenadayalan Karaiyagowder Govindarajan, Yogesan Meganathan, Gowthama Prabu Udayakumar and Ram Kothandan**	13-24
4	IBCFT-018	Hydrogen Production from Distillery Wastewater Using Anaerobic Sequencing Batch Reactor (AnSBR) **S.Sharmiladevi, Paviyadharshini.S.A, Rasha Ali**	25-30
5	IBCFT-031	Crystal Violet Dye Removal Using Activated Carbon Leucaene Leucocephala By Adsorption Method **A. Nagaveni, M. Anusuya, E. Jayanthi, N. Renugadevi**	31-36
6	IBCFT-033	Ag/TiO_2 Nanocomposite: Synthesis, Characterization, Photocatalytic And Antimicrobial Activities **E. Jayanthi, M. Anusuya and A. Nagaveni**	37-43
7	IBCFT-035	Optimizing the activation process of red mud for cadmium (II) adsorption - Comparison of the results from response surface methodology and artificial neural network **C. Maheswari, Anjali Dasari**	44-52
8	IBCFT-038	Experimental Analysis On Pulp And Paper Industry Wastewater By Ozonation **Dynisha.S , J.Thirumal, Ashik vivek.A**	53-56
9	IBCFT-040	Experimental Investigation of Solar Bio-mass Integrated Dryer System **Ravinth Kumar A, Sam Solomon D P, Senthil Kumar K, Rashik M**	57-61
10	IBCFT-058	Bacterial Leaching of Heavy Metals from Sewage Sludge: Studies on pH Optimization **Venkatesa Prabhu. S, Gizachew Assefa. K, Abel W. Ourgessa, Ababo Geleta. G, Assefa Alene**	62-67
11	IBCFT-059	Eco-friendly mosquito repellent fabric using dye derived from Curcuma aromatica Salisb **A.M. Ananda kumar , K. Anvar, R. Rakkimuthu, P. Sathishkumar, D. Sowmiya, J. Alahupreeti**	68-72

12	IBCFT-061	Ruthenium(II) complexes of benzothiazolamine Schiff bases: Synthesis, characterization and in vitro cytotoxicity **R. Valarmathi, K. Sampath, C. Jayabalakrishnan**	73-77
13	IBCFT-062	Mathematical Modeling of Commensal Relation between Streptococcus thermophilus and Lactobacillus bulgaricus **Sivaprakash. B**	78-81
14	IBCFT-069	Heat Transfer Studies and Development of Nusselt Number Correlation Using Al_2O_3–CMC and SA-Based Nanofluid on Plate Type Heat Exchanger **A. Raj Kumar, M. Thirumarimurugan, E. Sudalai Manikandan**	82-90
15	IBCFT-070	Simulation Studies in an Anaerobic Sequential Batch Reactor for the Treatment of Textile Dyeing Industry Effluent **T.Senthilkumar, S.Rajamohan, B.Gopalakrishnan, M.Rajasimman**	91-96
16	IBCFT-079	Preliminary analysis of Toxic Metal Contaminants in Common Food Products By Laboratory Chemical Reagents **S.Dinesh kumar, K.Manimaran, A.Swedha, V.Kavishree, K.Krishnaveni**	97-101
17	IBCFT-083	EVALUATION OF POLLUTION PARAMETERS IN POULTRY WASTEWATER FROM LOCAL CHICKEN SHOPS **M.Udhaya Ganga, Dr.Bakyaraj, Dr.G.Nagamaniammai, J.Suresh Kumar**	102-105
18	IBCFT-084	Anti Bacterial, Sem, Damage Probability and Beam Displacement Of Glycinium Maleate Nano Particles **S JEYAKUMAR, K SENTHILKANNAN, PRASANTH K, S JAIPRIYA**	106-109
19	IBCFT-108	Membrane Bioreactors For Treatment Of Textile Effluent **Abarna Varshini. A, Madhumitha. M, Oviya Soundari. N, Sudhamathivadhani. E, Sagarika, Sivarajasekar. N**	110-114
20	IBCFT-109	Industrial Wastewater Treatment Using Membrane Bioreactors **Mithra, Deepthi Suresh, Uthish, Keerthana, Poornima, Bharati Priyamvatha, Sivarajasekar. N**	115-118
21	IBCFT-110	Quantum Dots- Applications and Recent Advancements **Vasudha Priyadharshini, N. Sivarajasekar**	119-122
22	IBCFT-118	Concise review on removal of commercial textile dyes from water **Dhiranesh. N**	123-125
23	IBCFT-119	The b – chromatic coloring for Silicate and Benzenoid Networks **K. P. Thilagavathy, K.R.Aranganayagam and A. Santha**	126-129
Bio-Science and Technology			
24	IBCFT-002	Optimization of Endoglucanase production using mixed substrate by Trichoderma viride **S.Kavitha, Janani.B, J.Praveen Kumar, K.Balasubramani, G.Surendran**	130-135
25	IBCFT-004	Fluorescent Biomarkers in Environmental Monitoring **Lekha K, Kerensa Miriam Sheen, and Kumaravel K**	136-139

26	IBCFT-005	Outcome Ambulatory and Pre and Post Walk Test Values of Task-Oriented Training on Physical Appropriateness Using Locomotive Vector, Time Factor of Stroke Survivors **R RANADEVAN, S JEYAKUMAR, SHARMILA R, SANTHI K, SUGANYA K, T RAJESHWARI, K SENTHILKANNAN**	140-146
27	IBCFT-007	Enhanced Production of Pectinase from Aspergillus awamori by Solid State Fermentation Using Rice Bran **S.B.RISWANALI**	147-151
28	IBCFT-008	Biosynthesis of Cholesterol Lowering Drug from Aspergillus terreus by Solid State Fermentation **Subhagar Seraman, Aravindan Rajendran**	152-156
29	IBCFT-009	Packed Entrapped Microbial cells for Treatment of Simulated Textile Wastewater **S.Rengadurai, B.Preetha**	157-159
30	IBCFT-010	Microbial Production of Xylitol in optimized conditions from Rice straw **S.Ramesh, S.Subhagar, R.Muthuvelayudham**	160-164
31	IBCFT-011	CHEMICAL COMPOSITION of ETHANOL EXTRACT of DIOSYPROS MONTANA ROXBERG LEAVES AND ITS BIOLOGICAL ACTIVITES **Saranya.K.S., Karthigai Priya. M, Poonkodi. K**	165-169
32	IBCFT-012	Evaluation of Phytoconstituents of Feronia limonia and Its in Vitro Cytotoxicity **Karthigai Priya M, Saranya. K .S, Poonkodi. K**	170-174
33	IBCFT-013	Statistical screenings of medium components for the production of Chitinase by Trichoderma viride **SUDHAKAR.P, NAGARAJAN.P**	175-178
34	IBCFT-016	Evaluation of phytoconstituents of Andrographis paniculata and its IN VITRO antioxidant activity against DPPH and ABTS assay **Jeevitha N, Nandha kumar K, Vasantha Mohan S, Pradeep K, Velliangiri Prabhu**	179-183
35	IBCFT-022	PHYTOCHEMICAL ANALYSIS AND INVITRO ANTICANCER ACTIVITY OF ETHANOL EXTRACT OF WRIGHTIA TINCTORIA **Mini.R, Vimaladevi.K**	184-187
36	IBCFT-025	Recent Trends and Prediction of Long Intergenic Non-coding RNA – a Review **Cashlin Anna Suveetha G, Abirami SK, Jeyapriya Rachana S, Ram Kothandan**	188-192
37	IBCFT-030	Studies on the antioxidant activity of the fern Hemionitis arifolia (Burm.) Moore **Dr. R. Rakkimuthu, P. Naveenraj, M. Susila, A. Sindhusha, R. Indu**	193-198
38	IBCFT-037	GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM DIFFERENT PLANTS AND IDENTIFYING ITS ANTI-BACTERIAL ACTIVITY **S.Kiruthika, Dr. Lali Growther**	199-203

39	IBCFT-054	Effect of silver nanoparticles of Ulva fasciata in germination of selected pulses **D. Sowmiya, R. Rakkimuthu, P. Sathishkumar, A. M. Anandakumar, S. Sinju, B. Vanisree**	204-207
40	IBCFT-055	Quantitative phytochemical screening of Filicium decipiens Wight & Arn. Leaves **P. Sathishkumar, D. Kaviya, R. Rakkimuthu, A.M. Anandakumar, D. Sowmiya, S. Sabirafathima**	208-212
41	IBCFT-060	Scanning, Imaging and High Frequency Phononic Analysis for Healing Effect of Controlling and Minimising the Impact on Brain of The Stroke Patients - an Ethicality View **R RANADEVAN, A.UMA, S JEYAKUMAR**	213-216
42	IBCFT-067	Production of Biodiesel from Anacardium occidentale Shell Coat Oil **Nivetha. K, Prethika Murugesan, Suhanika.P, Dr. N. Sivarajasekar**	217-221
43	IBCFT-068	STUDIES ON BIOGAS PRODUCTION FROM DAIRY WASTE BY COGENERATION **Deepamalini. P, Dr. N. Sivarajasekar**	222-226
44	IBCFT-080	Thermal death kinetics analysis for effective sterilization of microorganisms and shelf life improvement of milk **Elakya .M.V, Suvetha. R, G. Bharath**	227-231
45	IBCFT-087	Extraction of Phytoconstituents from Rocella montagnei and Evaluation of Its Anti Apoptotic Potential **R.Niroshini, V.Evangelin Shaloom, B.Lakshmi Priya, R.Shyam Kumar**	232-240
46	IBCFT-094	Molecular Docking Study of Apigenin-7-O-β-D-Glucuronide with Viral Oncoproteins **C.Nirmala, Sambhasan Banerjee, M.Sridevi, R.Rajeshwari**	241-249
47	IBCFT-101	Advanced Controllers in Bioprocess **Dhivya Priya.K.S, Kabila.N, Hemalatha.G, Vyshali.P, Geeta.A.M, Livya.M, Sivarajasekar. N**	250-252
48	IBCFT-102	Nanobiotechnology and clinical diagnosis of Diabetes mellitus **Swathi. G, Kanish Siddarth. R, Manopriya. M, Sivarajasekar. N**	253-256
49	IBCFT-103	Nanotechnology in Cancer Diagnostics and Treatment **Senthilrani.N, Shruthi.A,Chrislyne Antonette Fernandez.J, Srividya Priyadarshini, Niveda, Aishwarya, Sanjana, Sivarajasekar. N**	257-261
50	IBCFT-104	Thermal Biosensors and Their Applications **S.Vasuki, V.Varsha, Mithra, R.Arul Dharshni, S.Abinaya, R.Deva Dharshini, N. Sivarajasekar**	262-264
51	IBCFT-105	Antimicrobial peptides from plants and their mode of action **Yamuna S, Nithesh Kumar V, Bala Subramaniam D and Kumaravel K**	265-269
52	IBCFT-106	Effect of biofertiizer, farmyard manure and vermicompost on seed germination, plant growth and vigor of Solanum lycopersicum L. (Tomato) **Kousalya Mangai. S, A. Jenifer, A.S.Agalya, R. Akshara. B, Boobalakannan, K.V.J. Gowsika, S. Abarna and P. Muthukumaran**	270-273

Food Science and Technology			
53	IBCFT-019	Transglutaminase Cross-Linked With Casein and Gelatin Coating for Fruits and Vegetables **Sowndharya G, S Daniel Robert, Lakshmi Anand**	274-277
54	IBCFT-021	Effect of drying methods and infusion of functional ingredients on the quality characteristics of coconut based snack **Dr.M. Sivasakthi, Dr.Narayanasamy Sangeetha**	278-284
55	IBCFT-023	Optimization of Process Parameters for The Osmotic Dehydration of Banana in Sugar Solution **P.Manivannan, G.Kalaiarasi, M.Rajasimman**	285-296
56	IBCFT-027	Quality Assessment and Shelf Life Evaluation of Momordica charantia Based Candies **P. Divya, M. Priyadharshini, S. Tamilarasu, S. Poornima, B. Mythili Gnanamangai, K.S. Ghanapathy Ram, M. Sanjeev Kumar, R.C. Gowtham**	297-301
57	IBCFT-032	Production and Quality Evaluation of Herbal Wine **Dr.M. Sivasakthi, M.Rajeswari**	302-306
58	IBCFT-046	Review on Non-Dairy Based Ice Cream with Fortification **S. Mythra, A. Sangeetha, C. Raagul, T. Surya**	307-310
59	IBCFT-047	Influence of wall material on microencapsulation of Nigella sativa Seed Oil **Gothainayagi, Josephine Nirmala Many**	311-315
60	IBCFT-049	Formulation of Ice Cream from Peanut (Arachis hypogaea) Milk **Sharmila R, KalaiRajan P, Nandhini A, Shenbagam T**	316-319
61	IBCFT-051	Development of Value Added Health Mix using Pumpkin Seeds and Almonds **Dr. B. Premagowri**	320-324
62	IBCFT-064	Effect of Hydrocooling and application of Anti-Microbial Extract on Quality attributes of Selected Tropical Fruits **R.Renu, Dr Kavita Waghray, Dr P.Dinesh Sankar Reddy**	325-331
63	IBCFT-065	Study on Drying Characteristics of Guava Leaves **K Shravya , V Mounika , R Renu**	332-336
64	IBCFT-066	QUALITY EVALUATION OF COATING ON SAPOTA **Sruthi Josyula, Chalamalasetti Hema Sai Samhitha**	337-342
65	IBCFT-071	Physico-chemical composition, Sensory and Shelf-life quality of value added pizza base **Dr.M. Sivasakthi, Najma.P.V**	343-352
66	IBCFT-075	Design and Fabrication of Three-Dimensional Printer for Food Printing **DINESH.E, Revathi.R**	353-356
67	IBCFT-076	Production of extruded products using millets and Amarphophallus paeoniifolius (elephant foot yam) flour **Anita S, Vigneshwaran S, Srinivasan R, Karthikeyan K, Nanda kishor MV**	357-360

68	IBCFT-078	Identification of Food Adulterants in Some Selected Food Items using Chemical Qualitative Analysis **R. Ashokmithran, G. Saravanakumar, S. Lavanya, S. Nithya, K. Krishnaveni**	361-365
69	IBCFT-081	Sensory and Textural Properties of Gluten Based Chewing Gum **Suresh kumar.J, G.Nagamaniammai, Shwetha.H.S**	366-369
70	IBCFT-086	Effect of Honey on Functional Compounds and Sensory Attributes of Natural Amla(Emblica officinalis Gaertn) Milk shake **Hisham Mammu, G. Nagamaniammai**	370-373
71	IBCFT-107	Biosensors for Food Allergens Detection **Senthilrani.N, Jenifer.A, Kousalya Mangai.S, Subhangini Thithima, Vivega, Seema Afra, Mega varshini, Sivarajasekar. N**	374-377
72	IBCFT-111	Development of Therapeutic Rts **Nithya Priya S, Anushya Menon K C, Kousalya P, Ruthi Muhinda K**	378-382
73	IBCFT-112	Development Of Omega-3 Enriched Traditional Indian Sweet **Nithya Priya S, Geetha P, Priyanka S, Ramakrishnan V, Veeragayathri P V, Sanjana R, Subha Priya C, Sakthi Priyadharsini A, Joel John Varghese**	383-387
74	IBCFT-113	Imaging Techniques in Food Processing **Pradeepika.T , Sivapriya.S , Gayathri.V, Keerthana.K.J, Sivarajasekar. N**	388-394
75	IBCFT-116	Microcontrollers in Food Process Engineering **Surendhiran P, Bala subramaniam D, Nithesh kumar V, Somasundaram K, Karthick balaji K.R, Sivarajasekar. N**	395-403
76	IBCFT-117	Conversion of Alkaline Batteries into Fertilizers **Hari Chandra Mouli.R, Kanimozhi.Ra, S.Sharmiladevi O.Prasanth kuttan**	404-407
77	IBCFT-118	Efficiency Rate Analysis of Tubers Using Zero Wastage-Review **Caroline Arunagiri, Aishwarya Jayakumar, Fahima firdouse Thajudeen, Pooja Kanakarajan, Preethaa Arokiyaaswamy, Rajshri Naveen**	408-412

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Removal of Cr(VI) from Waste Water Using *Cocos Nucifera* –Batch Studies

G.Surendran [1*], S. Kavitha [1], K.Balasubramani [1], J. Praveen Kumar[1], S.S.Baral [2]
[1]Department of Petrochemical, JCT College Engineering and Technology, Coimbatore, India.
[2]Department of Chemical Engineering, BITS,Pilani-Goa Campus, India.
*Corresponding Author -E-mail: surendrang@yahoo.com

Abstract: The presence of heavy metals in the environment is of major concern because of their toxicity, bio-accumulating tendency, threat to human life and the environment. Although all the heavy metal waste water treatment techniques can be employed to remove heavy metals, they have their inherent advantages and limitations. Among all these methods, adsorption process is considered better than other methods because of convenience, easy operation and simplicity of design. In the present paper, a new adsorbent i.e. Cocos Nucifera (coconut leave) is used for the removal of Cr(VI) from waste water. In order to study the effect of different parameters on adsorption process, batch adsorption experiments were carried out by varying different adsorption parameters. The influence of pH, contact time, initial chromium concentration and adsorbent dosage on the removal of Cr(VI) from the solutions and also the uptake capacity has been investigated. Experimental results were fitted to different adsorption kinetic model such as Pseudo first-order/Lagergren, First-order reversible, Ritchie's second-order and Pseudo second-order kinetic model to find out the best fit. FTIR analysis and SEM image before and after adsorption were analysed. The adsorption process was found to follow pseudo second-order kinetics. The best adsorption isotherm and rate controlling mechanism is also established.

Keywords : *Cr(VI), Coconut leaves, uptake, kinetics, isotherm, FTIR, SEM*

I. Introduction

Cr(VI) is highly toxic material which comes out in many of the chemical industrial effluents. Many researchers have done their work in removing this toxic material by various processes. The aim of this study was to explore a new adsorbent which will have high adsorption capacity at higher pH. The present study explored the use of natural, environmental friendly and easily available coconut tree (**Cocos Nucifera**) leaves as an adsorbent to remove the Cr(VI) from wastewater. Batch adsorption experiments were carried out in-order to study the effect of different parameters on adsorption process. The influence of pH, contact time, initial chromium concentration and adsorbent dosage on the removal of Cr(VI) from the solutions has been investigated. The removal efficiency and uptake capacity have been correlated with the initial Cr(VI) concentration, adsorbent dosage as well as the contact time.

II. Materials and Methods

The adsorbent used in this study is a natural coconut leave known as *Cocos Nucifera* which grows profusely in coastal region of west India. The waste coconut leave (after removal centre stick) was collected and cleaned with distilled water thrice and dried for 48 hours. In order to remove the remaining moisture after drying, the coconut leave was kept in an oven at 80^0C for 24 hours. The dried coconut leave was then ground into powder form and sieved (British Standard Screens) to different size fractions. The sieved powdered coconut leave samples were then stored in an airtight container for further use. A stock solution was then prepared by dissolving known quantity of potassium dichromate in double distilled water. In order to meet the prescribed concentration for experiments, the solution was further diluted as required. The initial pH of the solution was maintained by adding the dilute weak Acetic acid or NH_4OH (concentration of 0.1 N) made as appropriate before adsorption. The complex agent, Di-Phenyl Carbazide (DPC) solution was ready by dissolving requisite amount of Di-phenyl Carbazide in acetone and kept in an air tight volumetric flask in a dark place until further use. 6N H_2SO_4 solution was prepared from the concentrated solution by using the appropriate amount of distilled water and kept in a standard flask for use in the spectro-photometric analysis of Cr(VI) concentration. All the chemicals used for experiments were obtained from Merck of standard analytical grade.

Methods

Adsorption experiments were conducted by using 500 ml prepared chromium known concentration solution in a beaker at constant temperature. The mixtures were well mixed using a magnetic stirrer (REMI maker) with speed controller. 5 ml of sample was collected at regular time intervals and filtered through a Whatman42 filter paper. In order to maintain the solid to liquid ratio, the samples were collected under dynamic conditions. The residual Cr(VI) concentration in the filtrate was analysed using an UV spectrometer (Hach DR5000, model 1201) at a wavelength of 540 nm after complexing with metal ion by 1,5 Di-Phenyl Carbazide under acidic conditions.

Percentage adsorption of Cr(VI) was calculated as follows

$$E\% = \frac{(C_o - C_t)}{C_o} x\ 100 \qquad q_e = \frac{V(C_o - C_e)}{W} \qquad (1)$$

where E, C_o and C_t denote percentage adsorption and Cr(VI) concentration (mg L^{-1}) at the beginning and after time t, V is the volume (L) of the solution and C_e is equilibrium chromium concentration, q_e is uptake capacity (mg/g) of chromium at equilibrium was calculated from the difference of metal concentration in the solution before and after adsorption is respectively.

Characterization

In order to find out the morphological changes in the adsorbent, the powdered adsorbent both before and after adsorption was investigated by the Scanning electron microprobe images are (SEM JXO-8100) at 1000X magnification. These results were presented in Fig. 1 and 2. From the micrograph image, it is clearly observed that there is a change in surface morphology before and after adsorption.

FTIR Spectroscopy was conducted to identify existing functional groups in the adsorbent before and after adsorption. The samples were finely powdered with 200 mg of moisture less KBr in a mortar and placed in10 mm diameter disks, under 10 tons of pressure for FTIR analysis. The adsorbent spectra with the array of 400- 4000 cm^{-1} wave number were measured. The FTIR spectra of the adsorbents display a number of absorption peaks, signifyingthe complex nature of the adsorbent. The percentage transmittance and important peaks of the adsorbents before and after adsorption is shown in Table 1. As can be observed in Fig.3 the spectrum of coconut leaves before and after the adsorption process displays substantial drop in the intensity at an absorption bands of 3132 cm^{-1} which relates the hydroxyl stretching vibrations and hydrogen bonding of celloligo-saccharides and their degradation products [1]. The adsorption bands observed at 1269.16 and 1261.45 cm^{-1}corresponds to CN stretching and CN vibration. The band at 1045 $cm^{-1}$was found to be OH group bending, whereas the band at 898 cm^{-1} indicates C-O-C stretching and CH deformation [2]. From Table 1, it can be observed from the adsorption peaks that, either a reduction or shift in adsorption band after adsorption signifying that all the functional groups have played major role. These band shifts specify that there is OH bending, CN stretching/bending and NH stretching particularly play an important role in chromium bio-sorption on the adsorbent. Analogous results have been observed by various researchers.

III. Results and Discussion

Effect of Contact time

Adsorption experiments were carried out over 600 min to find the equilibrium contact time. During the experiment, the other adsorption parameters such as stirring speed, adsorbate concentration, dose, particle size and temperature of the solution were kept constant 100 ppm, 20g/L, 52 BSS and 30^0C, respectively. The results are shown in Fig 4. It can be seen from the figure that the kinetics was very fast during the first 30 min followed by a slower kinetics. During the faster phase, 80% of the total adsorption was reached. The adsorption process attained equilibrium within 6 h and beyond that there was hardly any change in concentration. The initial faster rate may be due to the higher free surface available initially [3]. Once the available free surface is clogged, then the adsorbate molecules penetrate through the pores and get adsorbed inside the pore, which is known as intra-particle diffusion. The intra-particle diffusion accounts for the slower kinetics at the later stage

Effect of pH

pH is an important parameter affecting the adsorption process. Experiments were carried out by varying pH between 1.0 and 3 to find out its effect on the percentage of adsorption and Cr(VI) uptake capacity of the adsorbent [4],[5]. The results are shown in Fig.5 below. It was observed that the maximum adsorption takes place at pH of 1.0. Sorption capacity also increases with decreasing the pH. pH plays a vital role in the adsorption process [6]. As the current studies were in the pH range 1.8–3.0, we can assume all the Chromium in the form of $Cr_2O_7^{2-}$.sequentially to observe the consequences of pH on the removal of Cr(VI) and uptake capacity, the experiments were considered by

varying pH between 1.0 and 3. The results were revealed in Fig.5.It is clear, that the maximum adsorption takes place at pH of 1.0 and the sorption capacity increases with decrease in the pH. The percentage of adsorption increases from 4 to 95.5 with decreasing pH of solution from 3 to 1 respectively. Further the final pH of the Cr(VI) solution increases after the adsorption. It is widely claimed in literature that the removal of Chromium is through a mechanism, where then negatively charged Cr(VI) ions were combine to the cationic groups of bio-adsorbent [7].

Effect of dose

The trend of adsorption at varying doses of adsorbent is shown in Fig.6. The percentage of Cr(VI) removal increased from 45 to 100 with increase in adsorbent dose from 1.0 to 10 g/L. The trend was as per expectations since more active sites of adsorbent were exposed when the adsorbent dose increased [8], [9]. However, the Cr(VI) uptake capacity of the adsorbent decreased from 4.4 mg/g to 1 mg/g when the adsorbent increases from 1 g/L to 10 g/L. This is mainly because of the fact that with increase in adsorbent dose the number of active site available for adsorption increases whereas limited number of adsorbate molecule present for the adsorption [10].

Effect of Particle size

The effect of adsorbent particle size was studied under constant initial Cr(VI) concentration, pH, speed, dosage and temperature. Initially the crushed coconut leaves have been screened in BSS (British Standard Screen) sieves with different mesh screen. Experiments were carried out by varying the particle size between 52 BSS to 150 BSS. It was found that initial the percentage adsorption as well as uptake increases with decrease in particle size whereas afterword there is no change in percentage adsorption. This is may be due to the fact that limited number of adsorbate particle present for the large adsorbent surface [11]. Particles size 100– 150 mesh shows in Fig 7, the maximum adsorption capacity. The percentage adsorption is increases from 91.9% to 100% with increase in mesh size from 52 BSS to 150 BSS and the Cr(VI) uptake increases from 1.8 mg/g to 2 mg/g.

Effect of Initial Cr(VI) concentration

Assessment of the effect of initial concentration on the adsorption of Cr(VI) on coconut leaves was carried out by varying the initial Cr(VI) concentration from 10 mg/L to 100 mg/L and the results is shown in Fig 8. It was observed that the percentage adsorption decreased with the increase of adsorbate concentration whereas uptake capacity increases with increase initial Cr(VI) concentration [12]. The percentage adsorption decreases from 91 % to 40 % with increase in adsorbate concentration from 10 mg/L to 100 mg/L. The uptake of the adsorbent increases from 1.82 mg/g to 8 mg/g when adsorbate concentration from 10 mg/L to 100 mg/L. This is fact that at fixed adsorbent dose, the number of active sites to accommodate the adsorbate ions increases with higher initial concentration. So the loading is faster with higher concentration of adsorbate.

Kinetic Model

It is very important to find the rate at which adsorption takes place for a given system in order to design the continuous column. In order to investigate the mechanism of adsorption, various kinetic models have been suggested. Numerous kinetic models have described the reaction order of adsorption systems based on solution concentration [13].

Lagergren's pseudo first-ordermodel

The Lagergren equation is possibly the earliest known rate law describing the rate of adsorption in the liquid-phase systems. This model describes liquid-solid phase adsorption systems and is derived based on the adsorption capacity of the adsorbent [14]. So, the kinetic equation expressed as follows:

$$\frac{dq}{dt} = k\,(q_e - q) \qquad (3)$$

Where q_e is the uptake at equilibrium, q is uptake at any time t, k is the rate constant. Equation (3) integrated with boundary condition resulted in the following equation:

$$\log(q_e - q) = \log q_e - \frac{k}{2.303}t \qquad (4)$$

The values of '*k*' were interpreted from the slope of the graph between $\log(q_e - q)$ and '*t*' for different adsorption parameters under the experiments has been studied. The correlation coefficients and rate constants are given in Table 2. The correlation coefficients were in acquiescence with the pseudo first-order kinetics.

Ritchie's second order model

Ritchie reported model for the adsorption of gases and liquid systems. Considering that the rate of adsorption depends exclusively on the fraction of sites which are empty at time t, then

$$\frac{q_e}{q_e - q_t} = 1 + kt \qquad (6)$$

Where q_e is the uptake capacity at equilibrium, q_t is the uptake capacity at any time t. The above equation was fitted to the investigational dataand model parameters were calculated along with the R^2 values. The evaluated value of the kinetic model parameters has been presented with correlation coefficient R^2 in Table 2.

First Order Reversible model

The following bio-sorption reaction mechanism assumed that, Chromium adsorption follows the first order reversible reaction, which gives as

A ↔B

$$\ln(1 - U_t) = -(k_1 + k_2)t \quad (8)$$

Where $U_t = \frac{(C_{A0} - C_A)}{(C_{A0} - C_{Ae})}$

and C_A is concentration of the adsorbate in solution (mg/L), C_{AO} is initial concentration of the adsorbate, and C_{Ae} is concentration of the adsorbate in the solution at equilibrium condition. Model parameters and R^2 values are calculated from the graph between $\ln(1-U_t)$ vs t and shown in Table 2.

Pseudo second order model

The pseudo second order kinetics is usually associated with the situation where the rate of uptake capacity depends on the square of the driving force, that's nothing but the accessible fraction of the active sites. The rate of reaction depends on the quantity of metal ions on the surface of the adsorbent and the quantity of metal ions adsorbed at equilibrium.

$$\frac{t}{q} = \frac{1}{k_2 {q_e}^2} - \frac{t}{q_e} \quad (10)$$

The values of model parameters along with R^2 are calculated from the plot between t/q vs t/qe and is reported in Table 2.

The rate constant along with the R^2 values are evaluated and reported in Table 2. From the table it was observed that Cr(VI) adsorption by coconut leaves follows the pseudo second order kinetic model.

Equilibrium Adsorption Isotherm

The equilibrium separation between the two phases is an important criterion to be considered for the successful representation of the dynamic adsorptive separation of the solute from solution into an adsorbent [15]. An adsorption isotherm plays a major role, which relates the surface properties and affinity of the adsorbent to compare the various pollutants uptake capacity. In order to find out the mechanism of Cr(VI) adsorption by the coconut leave and determine the maximum uptake capacity of the adsorption three popular adsorption isotherms were used to fit the experimental data.

Freundlich isotherm model

Freundlich isotherm which relates the uptake capacity with equilibrium concentration widely used isotherms for including bio-sorbents. This equation is outlined below

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (11)$$

where, K_F and 1/n are the Freundlich constants. The q_e (mg/g) is the uptake capacity and C_e(mg/L) is the adsorbate concentration in solution at equilibrium correspondingly. The values of Freundlich constants are calculated from the graph between log q_e vs. log C_e and are shown in Table 3. From the table values it clear that it is not following the Freundlich Isotherm model.

Langmuir isotherm model

The equilibrium relationship between a bulk liquid phase and coverage of molecules on a solid phase at a fixed temperature was given by Langmuir isotherm [16].

Based on the above assumptions, the Langmuir equation is given by:

$$\frac{C_e}{q_e} = \frac{1}{Q_0 K} + \frac{C_e}{Q_0} \quad (12)$$

Where, C_e and q_e are the concentration (mg/L), uptake capacity (mg/g) at equilibrium. Q_0 and K are related to uptake capacity and energy of adsorption and are the Langmuir constants. The model parameters along with the regression coefficients are calculated from the plot between C_e/q_e vs C_e and are shown in Table 3. From the table it is observed that experimental data follows the Langmuir isotherm model, as the R^2 values are close to1. 0.

Temkin Isotherm

Temkin Isotherm is derived based on the assumption that the heat of adsorption decreases linearly. The equation for Temkin isotherm model is given by:

$$q_e = B \ln A + B \ln C_e \quad (13)$$

Where, q_e is the uptake capacity in mg/g, Ce is the equilibrium adsorbate concentration in mg/L and B= RT/b. in the model equation T(K) is the absolute temperature and R is the universal gas constant. The constant b is related to the heat of Cr(VI) adsorption [17]. From the Table 3, it is evident that our experiment does not fall on the Temkin isotherm because of low values of regression coefficients.

The values of isotherm parameters along with the R^2 values are reported in Table 3. From the Table 3, it is clear that adsorption follows the Langmuir adsorption isotherm.

Rate controlling mechanism

On porous adsorbents, the adsorption of Cr(VI) us governed by four steps in series. The four steps are bulk diffusion in solution followed by film diffusion, surface diffusion and intra-particle diffusion. Either of the above individual steps or combinations of the steps may control the rate. In case of low stirring speed, the mixing is poor and hence external transport, i.e., bulk diffusion and film diffusion can be the rate limiting steps. In the current adsorption process, the adsorbent used is porous in nature and therefore pore diffusion is also anticipated additionally to surface diffusion. The time variation experimental data can be used to find out the rate limiting step in the sorption procedure [18]. In order to minimise the resistance to external transport, batch adsorption is carried out at higher agitation speeds. At higher stirring speeds, the resistance to the external transport reduces significantly due to reduction in thickness of the film adjoining the adsorbent. Thus, at this stirring speed, it can be safely assumed that external transport isn't the rate limiting step. Therefore, either intra-particle or surface diffusion is the rate-determining step. As they happen simultaneously, among the two transport processes, the slower one will be the rate limiting step. The intra-particle diffusion model can be interpreted as follows:

$$q_t = k_{id} t^{1/2} \quad (15)$$

where q_t= mg of adsorbate adsorbed per gram of adsorbent (mg/g), t = time of contact (min) and k_{id} = coefficient for intra-particle diffusion (mg/g $min^{1/2}$). Linear plots are drawn between q_t versus $t^{1/2}$ and the k_{id} values are determined from the slope. Outcome of various experiments are given in Table 4. The R^2 values in the table specify that, intra-particle diffusion is the rate determining steps for the adsorption process. Further analysis of data shows non-linear behaviour of time versus percentage adsorption plot for different sorption parameters. Therefore, it is assumed that numerous steps may well influence the Cr(VI) sorption process. So, the entire sorption process is occurring in two different steps: the early curved portion is the surface diffusion and the latter linear portion is the intra-particle diffusion. With the assumption that adsorbent particles are spherical in geometry the following equations can be used for surface and intra-particle diffusion:

$$\frac{q_t}{q_e} = 6\left(\frac{D_1}{\pi a^2}\right)^{1/2} t^{1/2} \quad (16)$$

$$ln\left(1 - \frac{q_t}{q_e}\right) = ln\left(\frac{6}{\pi^2}\right) - \left(\frac{D_2 \pi^2 t}{a^2}\right) \quad (17)$$

For equation 16, a plot between q_t/q_e and $t^{1/2}$ is drawn for the initial curved portion. The values of diffusivity for surface diffusion 'D_1' and regression coefficientR^2 at different sorption parameter are calculated from the slope of the curve and are shown in Table 4. In order to calculate, 'D_2' values, plots were drawn between $\ln(1-q_t/q_e)$ and 't' under diverse adsorption parameters. The values of D_2 were calculated from the slope of the curve and are given in Table 4, which showed D_2 is always greater than that of the D_1. Therefore, surface diffusion is the slowest steps and hence is the rate limiting steps.

I. Conclusion

A new, readily available and environmental friendly adsorbent is used for the treatment of Cr(VI) contaminated wastewater. Experimental results show the ability to remove the Cr(VI) by using locally available coconut leaves. It has been observed that under the optimum condition (pH: 3.0, Chromium concentration 10 mg/L, adsorbent doze 5 g/L, temperature 30ºC and particle size of 52 BSS), the sorption efficiency 91% and this has been increased to 100% at adsorbent dose of 10 g/L. Kinetic analysis revealed that pseudo second order kinetic model is better as compared to all other kinetic models. Experimental results show that it follows the Langmuir isotherm and surface diffusion is the rate limiting step.But the adsorption takes place at low pH of 1.5 and treated effluent water has got pH of 2.2.

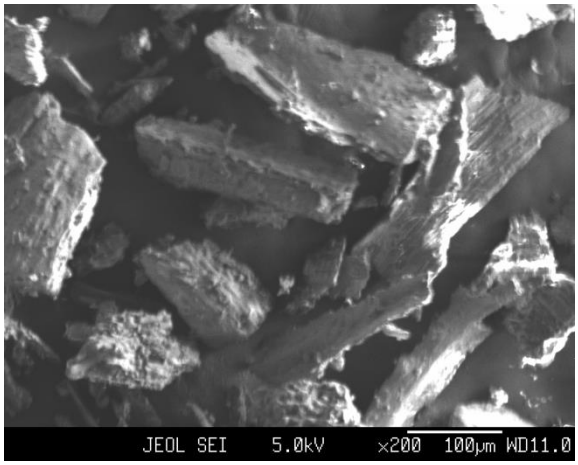


Fig. 1

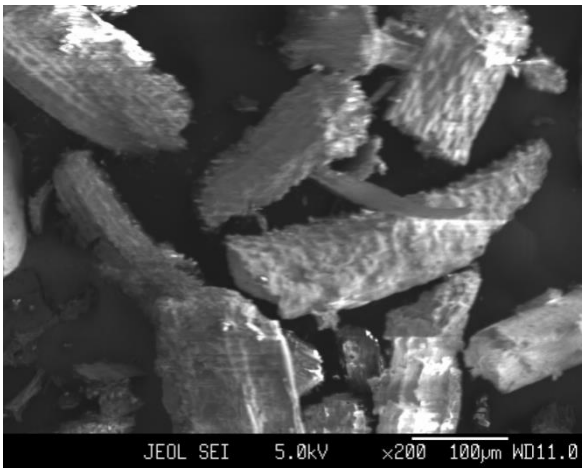


Fig. 2

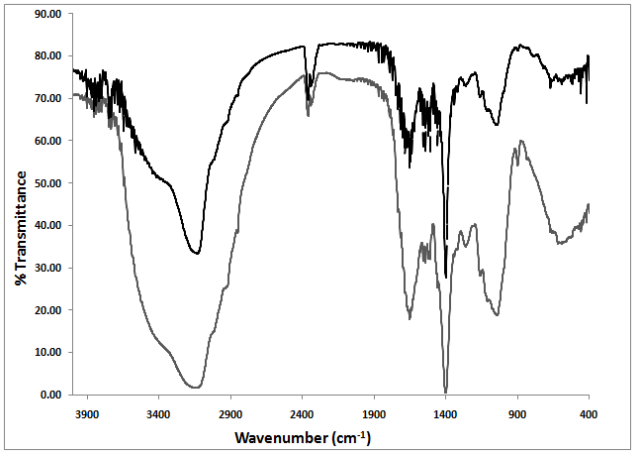


Fig 3 FTIR Analysis

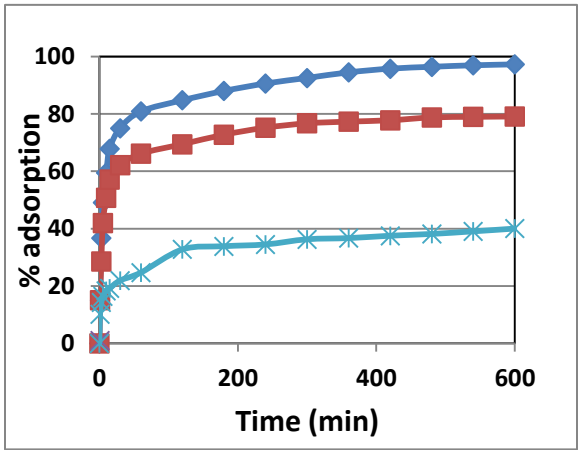


Fig. 4 Effect of contact time

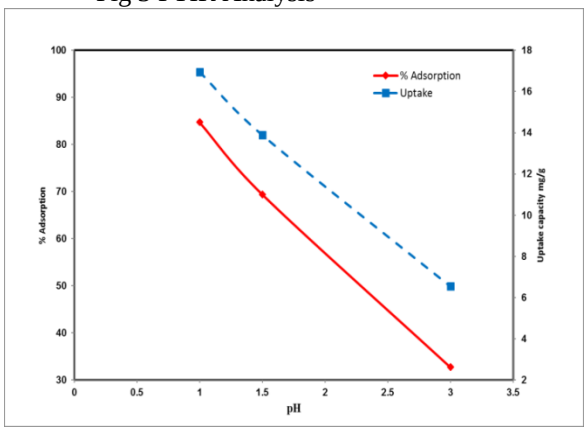


Fig. 5 Effect of pH

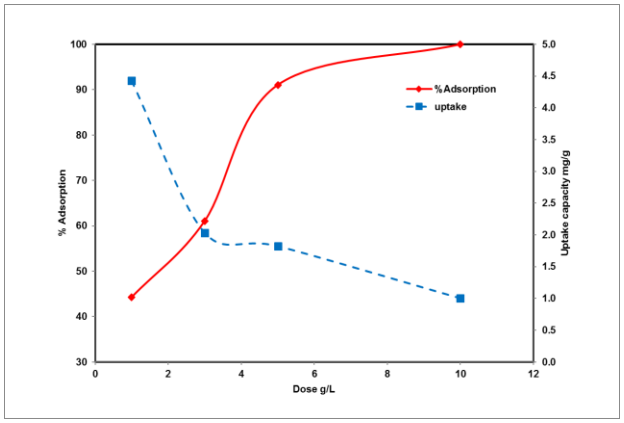


Fig. 6 Effect of Adsorbent Dose

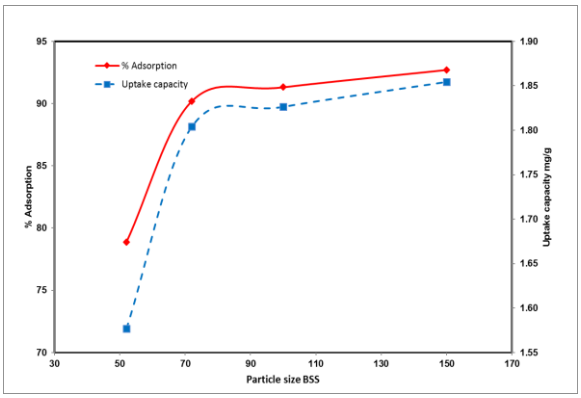


Fig. 7 Effect of Particle Size

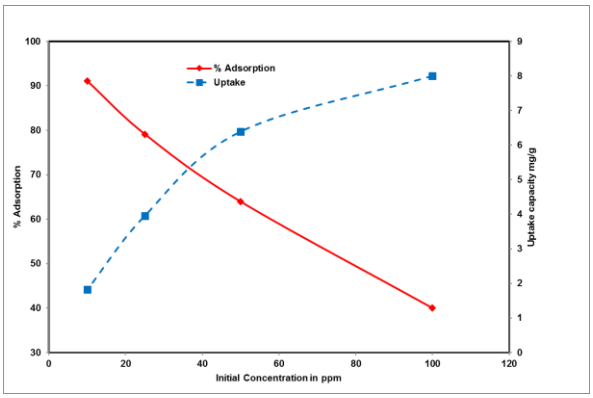


Fig. 8 Effect of Initial Cr(VI) Concentration

Table 1: FTIR Spectrum Analysis of the adsorbent before and after adsorption

IR peak number	Adsorption band			% Transmittance			Group Assigned
	Before	After	Diff.	After	Before	Diff.	
1653				53.67	17.80	35.87	C=N Vibration mode
1400	1400.32	1398.39	1.93	27.91	0.58	27.33	NH Stretching and bending vibration of N=O group
1269	1261.45	1269.16	7.71	73.05	35.07	37.98	CN stretching and CN vibration
1111	1107.14	1111	3.86	67.01	22.17	44.84	CN Stretching
1045	1041.56	1045.42	3.86	63.67	18.91	44.76	OH bending
898	896.83	898.83	2.0	81.24	54.26	26.98	C-O-C stretching and CH deformation

Table 2 : Adsorption Kinetic models parameters (sample)

		Pseudo 1st order		**Richie's 2nd order**		**Pseudo 2nd order**		**First order reversible**		
Parameter	**Values**	**k**	**R^2**	**k**	**R^2**	**k**	**R^2**	**k_1**	**k_2**	**R^2**
pH	1	0.34	0.96	0.312	0.564	0.005	0.999	32.0	0.010	0.837
	1.5	0.4	0.94	0.59	0.517	0.007	0.999	34.5	0.011	0.867
	3	0.24	0.95	0.05	0.8	0.019	0.997	22.0	0.007	0.870

Table 3: Adsorption isotherm models parameters (sample)

		Freundlich Isotherm			**Langmuir Isotherm**		**Temkin Isotherm**	
Parameter	**Values**	**Slope 1/n**	**log K_f**	**R^2**	**$1/q_{max}$**	**R^2**	**RT/b**	**R^2**
pH	1	0.22	1.35	0.51	0.13	1.0	2.50	0.68
	1.5	0.18	1.20	0.50	0.06	1.0	1.74	0.65
	3	0.35	1.02	0.79	0.05	1.0	1.73	0.88

Table 4: Rate controlling mechanism (sample)

		q_t vs $t^{1/2}$		**q_t/q_e vs $t^{1/2}$**			**$\ln(1-(q_t/q_a))$ vs t**		
Parameter	**Values**	**k_{id} mg/g $min^{(1/2)}$**	**R^2**	**$6(D_1/\Pi\ a^2)^{1/2}$**	**D_1 mm^2/time 10^{-6}**	**R^2**	**$D_2\ \Pi/a^2$**	**D_2 mm^2/time 10^{-3}**	**R^2**
pH	1	0.93	0.81	0.09	3.72	0.93	0.45	0.79	0.98
	1.5	0.74	0.80	0.11	5.41	0.95	0.50	0.87	0.94
Size(BSS)	52	0.09	0.90	0.07	2.50	0.95	0.37	0.65	0.84
	72	0.09	0.85	0.08	3.31	0.94	0.45	0.79	0.93
	100	0.09	0.84	0.08	3.17	0.93	0.38	0.66	0.96
	150	0.08	0.82	0.09	3.47	0.96	0.38	0.66	0.99

References

1. G.Surendran and Saroj S. Baral, "Biosorption of Cr(VI) from wastewater using Sorghastrum Nutans L. Nash". Chemistry and Ecology, ISSN: 0275-7540 , 1029-0370 (Online), Taylor and Francis, https://doi.org/10.1080/02757540.2018.1487407
2. D.Ciolacu, F.Ciolacu and V.Popa, "Amorphous cellulose – structure and characterization". Cellulose chemistry and Tech.,vol. 45 (1-2), 2011, pp.13-21 , http://www.cellulosechemtechnol.ro/pdf/CCT1-2(2011)/p.13-21.pdf
3. A. L. Prasad and S.Thirumalisamy, "Evaluation of the use of Acacia nilotica leaf as an ecofriendly adsorbent for Cr (VI) and its suitability in real waste water: study of residual errors", Journal of Chemistry, vol. 7, 2013, http://dx.doi.org/10.1155/2013/354328.
4. M. Aliabadi., K. Morshedzadeh and H.Soheyli, "Removal of hexavalent chromium from aqueous solution by lignocellulosic solid wastes", Int. J. Environ. Sci. Technol, vol. 3, , 2006, pp.321–325 https://link.springer.com/article/10.1007/BF03325940.
5. D.Park, Y.S.Yun , J.M.Park , "Reduction of hexavalent chromium with the brown seaweed Eckloniabiomass", Environ. Sci. Technol., vol.38, 2004, pp. 4860–4864, https://pubs.acs.org/doi/abs/10.1021/es035329
6. S.S. Baral, G.Surendran, N. Das and P.V Rao, "Statistical design of experiments for the Cr(VI) adsorption on weed *salvinia cucullata", Environ. Eng. Manage. J.* vol.12: 2013, pp.465-474. http://www.eemj.icpm.tuiasi.ro/pdfs/vol12/no3/7215_Baral10.pdf
7. S.Karthikeyen., S.Rajgopal , L.R.Miranda, "Cr(VI) adsorption from aqueous solution by Heveabrasilinesissaw dust activated carbon", J. Hazard. Mater., vol.124 , 2005, pp.192–199, DOI:10.1016/j.jhazmat.2005.05.003
8. K.Mohanty, M. Jha, B.C. Meikap, M.N. Biswas, "Biosorption of Cr(VI) from aqueous solutions by Eichhornia crassipes". Che. Eng. J. vol.117, 2006, pp.71–77.
9. L. Yu, S. Shukla, K. Dorris, A. Shukla, J. Margrave, "Adsorption of Cr from aqueous solution by maple sawdust". J. Hazard. Mater. vol.100, 2003, pp53–63,.
10. M. Mukhopadhyay, S.B. Noronha, G.K. Suraishkumar, "A review on experimental studies of biosorption of heavy metals by Aspergillus niger". The Canadian J. Che. Eng. vol.89, 2011, pp 889-900,.
11. D. Park, S.Y.Yun, J.M Park, "Studies on hexavalent chromium bio-sorption by chemically treated biomass of Eckloniasp", Chemosphere., vol. 60 , 2005, pp.1356–1364, DOI:10.1016/j.chemosphere.2005.02.020
12. G. Surendran, B. V. Sasank, S. S. Baral, "Modeling and simulation for the adsorptive removal of Cr(VI) from aqueous solution", Desalination and Water Treatment, vol. 52, 2013, pp.5652-5662, http://dx.doi.org/10.1080/19443994.2013.814001
13. S.J. Allen, P.A .Brown, "Isotherm analyses for single component and multi-component metal sorption onto lignite", J.Chem.Tech. Biotechnol., vol. 62, 1995,pp.17-24, , https://doi.org/10.1002/jctb.280620103
14. R.A.K. Rao, F. Rehman, "Adsorption Studies on Fruits of Gular (Ficus glomerata): Removal of Cr(VI) from Synthetic Wastewater". J. Hazard. Mat. vol. 181(1-3), 2010, pp.405-412,.
15. S. Liang, X. Guo, S. Lautner, B.Saake , "Removal of hexavalent chromium by different modified spruce bark adsorbents", J. Wood Che. Technol., vol.34, 2014, pp.273–290, , doi: 10.1080/02773813.2013.869606
16. T.A.Davis, B.Volesky, A.Mucci, "A review of the bio-chemistry of heavy metal bio-sorption by brown algae", Water Res. vol. 37, 2003, pp.4321-4330, https://doi.org/10.1016/S0043-1354(03)00293-8
17. C.I. Pearse, J.R. Lloyd, J.T. Guthrie, "The removal of colour from textile waste water using whole bacterial cells: a review", Dyes Pigments, vol.58, 2003, pp.179-196,
18. S.S.Baral, N. Das, R.G. Chaudhary, S.N. Das, "A preliminary study on the adsorptive removal of Cr(VI) using seaweed", Hydrilla Verticillata, J. Hazard. Mater, vol.171, 2009, pp. 358-369.

Acknowledgements

The authors are thankful to the JCT engineering college, Coimbatore, India and also, Director, Birla Institute of Technology and Science, Pilani- K. K. Birla Goa Campus, India for permitting to publish the work.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

ENVIRONMENTALLY BENIGN ENCAPSULATED FUNCTIONAL IONIC LIQUIDS AS CATALYSTS FOR SYNTHESIS OF PHENOLIC COMPOUND BASED ANTIOXIDANT

Pandian Elavarasan[1*], Sreedevi Upadhyayula[2]
[1]Department of Chemical Engineering, Annamalai University
Annamalai nagar, Tamil Nadu- 608002, INDIA
[2]Department of Chemical Engineering, Indian Institute of Technology Delhi,
HauzKhas, New Delhi -110016, INDIA
arasu2@gmail.com pe08135@annamalaiuniversity.ac.in

Abstract*: Present investigation is production of tert-butyl cresol (TBC) by reaction of tert-butylation with cresol using encapsulated functional ionic liquid. Ionic liquids have recently emerged as promising, benign and recoverable catalysts for many organic reactions. However, there are still a few problems in their use which come in the way of their commercialization. These problems are, escalated costs due to the need for high purification, hygroscopicity, their requirement in large quantities. This work was undertaken to overcome these problems. Highly dispersed ionic liquid catalysts were prepared through physical confinement or encapsulation of ionic liquids in a silica-gel matrix through a sol–gel process. Three ionic liquids, namely, (N-(4-sulfonic acid) butyl triethylammonium hydrogen sulfate), (1-(4-sulfonic acid) butylpyridinium hydrogen sulfate) and (1-(4-sulfonic acid) butyl-3-methylimidazolium hydrogen sulfate) were used to prepare the silica gel encapsulated catalysts. The loading of ionic liquids in silica gel was 5 - 30 % by weight. They were characterized by NMR, XRD, FTIR and SEM. Thermogravimetric analysis was carried out under inert. The analysis showed that they were thermally stable upto 324ºC. The catalysts were tested foractivity in production of antioxidant TBC in a batch reactor. The conversion of p-cresol was 80% using all the three encapsulated ionic liquids at a temperature of 70ºC with a maximum selective of TBC yield of 87% is achieved.*

Keywords*: Antioxidant, TBC, Ionic liquids, encapsulation*

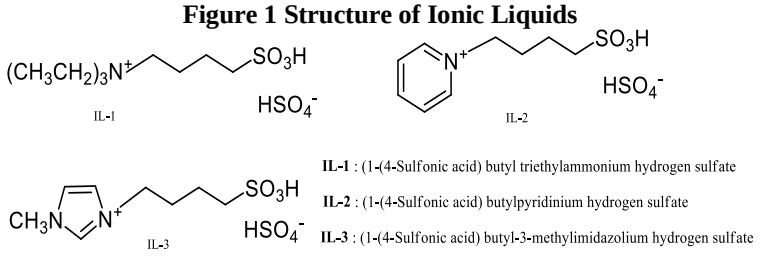
I. Introduction

Alkylation of cresol with *tert*-butanol is most important industrial reaction for antioxidant production [1]. Ionic liquids (ILs) are generally defined as salts melting at or below 100°C. The liquid salts are basically composed of relatively large organic cations and inorganic or organic anions. Any change of the structure or of the length of the carbon side chain of both ions leads to different chemical or physical properties. They have a negligible vapour pressure and are generally assumed to be stable over a wide temperature range. Depending on the application, ionic liquids can be designed to meet the demands. Numerous of these ionic phases have been examined in a variety of different areas reaching from material synthesis to separation science for alternative reaction media. Particularly, ionic liquids are used as catalyst for many of the reaction in homogeneous or heterogeneous reaction at room temperature. These catalysts produce the high selective yield, conversion and recyclable, even though the separation of ionic liquids requires more amount of organic solvent as well as the cost of ILs is high [2-6]. An alternative approach has recently been established which is immobilization of the ionic liquids using solid support [6]. The immobilization ionic liquids show similar or improved chemical behaviors and have simplified separation process being a solid. Sulfonic acid functional ionic liquids have been reported for esterification and alkylation reaction with high conversion with selective product yield. In particular, alkylation of cresol was performed with function ionic liquids. Present study, immobilization of the functional ionic liquids have been sol-gel method have been reported. Highly dispersed ionic liquid catalysts were prepared through physical confinement or encapsulation of ionic liquids in a silica-gel matrix through a sol–gel process. Activity of encapsulated ionic liquids is test with alkylation of *p*-cresol with *tert*-butanol[1-8].

II. Materials and methods

Sulfonic acid functional bronsted acidic ionic liquids were prepared in the laboratory following procedure have been adopted [3-5]. Triethylamine was mixed with 1,4-butane sultone by stirring at 40-80°C for an 12-24 hours. After solidification, the produced zwitterions mass was washed three times using ethyl ether and dried under vacuum (120°C and 0.01 torr). Stoichemetric amount of sulfuric acid is added to the zwitterions and the mixture was stirred at 80°C for 8 hours to obtain ionic liquid. The structures of the ionic liquids are presented in figure 1.

Figure 1 Structure of Ionic Liquids



IL-1 : (1-(4-Sulfonic acid) butyl triethylammonium hydrogen sulfate

IL-2 : (1-(4-Sulfonic acid) butylpyridinium hydrogen sulfate

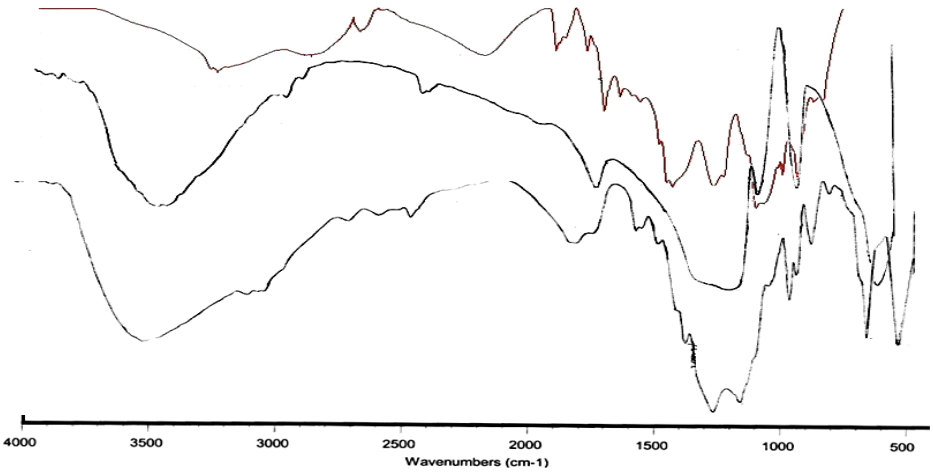
IL-3 : (1-(4-Sulfonic acid) butyl-3-methylimidazolium hydrogen sulfate

A mixture of tetraethoxyorthosilicate (TEOS, 10 ml) and ethanol (7 ml) was heated to 60 0C and the ionic liquids (ILs) (2 to 4 g) were immediately transferred into the TEOS. After the formation of a clear and homogeneous liquid mixture, hydrochloric acid (5 M, 5ml) was added and the mixture gradually coagulated [7]. After aging at 60°C for 12 hours, the resulting solid material was dried in vacuum at 150 °C for 3 h and 3-7 g of solid sample was obtained. A dry solid sample of silica-gel-confined ionic liquid (loading of up to 55% and 40% based on original weight) was obtained, indicating that the ionic liquid added could be completely encapsulated into silica gel. The alkylation experiments were carried out in sealed glass tube with autogeneous pressure at given temperature. A typical batch is containing 10 mmol each of *p*-cresol, *tert*-butyl alcohol and 2 g encapsulated ionic liquids. Reaction temperature was maintained at 70°C and reaction mixture was stirred around 800 rpm for an 8 hours. At the end of reaction, the mixture was cooled and the products were extracted by toluene. A qualitative product analysis was conducted with using a GC-MS and quantitative analyses were conducted by using NUCON GC which is supplied by AIMIL India Ltd using a CHROMSORB-WHP (2 m x 3.175 mm x 2 mm) column and flame ionization detector.

III. Results & Discussion

The decomposition temperature of ionic liquid and encapsulated ionic liquid, the decomposition temperature are same in both cases and only ionic liquid has been decomposed at particular (325 C) temperature was observed thermo gravimetric analysis. FT-IR spectra of IL1 (a) shown in the figure 2. From the IR spectra the silica gel and encapsulated ionic liquid shows the similar peak. XRD of the silica gel and immobilized IL shows amorphous nature. Figure 4 shows the SEM image of silica gel (a) and encapsulated IL (b & c). Average size of silica gel particles is 20 μm and immobilized IL solid are 75-100 μm. The variation in size of the particle shows ILs are entrapped with the silica gel particles.

Figure 2 FT-IR spectra for IL1 (a), pure silica gel (b) and encapsulated ionic liquid (c)



The influence of reaction time on conversion of *p*-cresol and the selectivity towards TBC & DTBC was studied at different temperatures and different reactant mole ratios. Table 1 shows the conversion of *p*-cresol at 70°C, 1:1 molar ratio of p-cresol / TBA and 1:1 catalyst to reactant ratio. The alkylation of *p*-cresol with TBA in the supported IL1 gave a mixture of TBC, 2, 6-*di*-*tert*-butyl-3-methyl phenol (2,6-DTBC) and negligible amount (< 0.2%) of *tert*-butyl-*p*-cresol ether (TBCE). With increase in reaction time, the conversion of *p*-cresol and selectivity to TBC and the yield of TBC increased rapidly and reached maximum after 24 h from figure 4. The conversion of *p*-cresol reached 68.4%, the selectivities to TBC and DTBC reached to 76.2% and 22.4% respectively after 24 h. The increase in the selectivity to DTBC led to decrease in selectivity to TBC with time but the combined selectivities of TBC & DTBC almost remains constant over the time period.

Figure 3 SEM images for pure silica gel (a), encapsulated IL1 (b) and surface of the encapsulated IL1 (c)

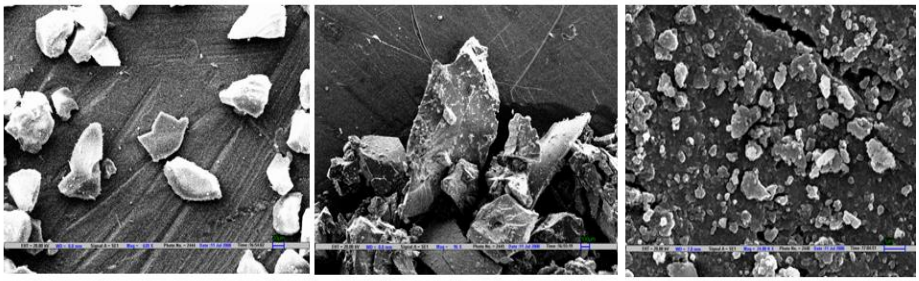


Table 1 Activity of encapsulated IL in alkylation of *p*-cresol with *tert*-butyl alcohol

Catalyst	Conversion of *p*-cresol	Selectivity of 2-TBC	Selectivity of 2, 4-DTBC
IL1	64.5	72.3	25.4
IL2	63.6	76.4	22.5
IL3	63.4	74.8	24.7

Figure 3 Effect of reaction time

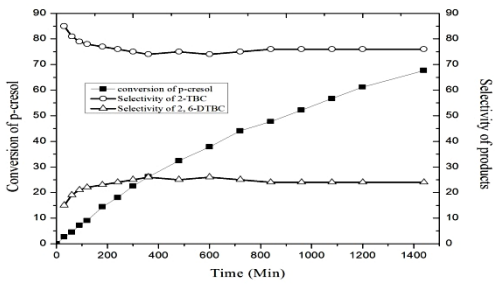


Figure 4 Effect of reaction temperature

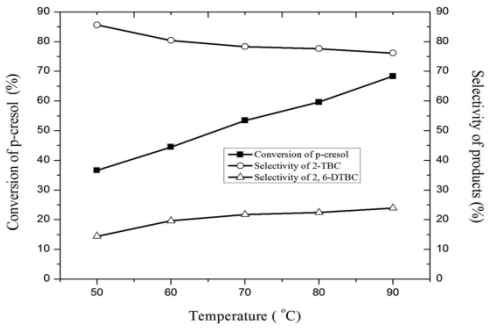
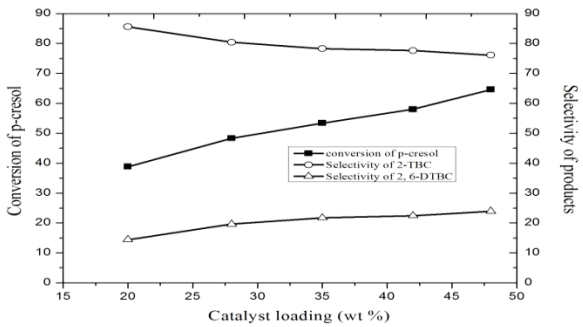


Figure 5 Effect of different IL loading on sol-gel



The reaction was studied at various temperatures from 50°C to 90°C. Figure 5 shows the variation of *p*-cresol conversion and selectivity to different products with temperature. At 50° C the conversion of *p*-cresol is 34.4 % and increased to 69.7 % at 90°Cand 1:1 molar ratio of p-cresol / TBA and 1:1 catalyst to reactant ratio. The selectivity to DTBC is high (14 %) at 50°C and as the temperature is increased, the selectivity to DTBC increased. The highest selectivity to DTBC was found to be 24 % due to higher stability and the availability of solvated *tert*-butyl cation at reaction temperatures. The effect of different loading of IL on supported catalyst on *p*-cresol conversion, TBC and DTBC selectivities were investigated using supported ionic liquid from 2 to 4.8 g at 70°C and TBA / *p* -cresol molar ratio constant at 1:1, which can be shown in Figure 7. As loading IL is increased, the *p*-cresol conversion and DTBC selectivity increased to maximum. Highest conversion of *p*-cresol and highest selectivity of TBC was achieved without any unwanted product, when IL / *p*-cresol molar ratio maintained at 1:1. Using higher amount IL in the reaction led to leaching.

IV. Conclusion

Highly dispersed ionic liquid catalysts were prepared through physical confinement or encapsulation of ionic liquids in a silica-gel matrix through a sol–gel process for production of antioxidant. Three ionic liquids, namely, (N-(4-sulfonic acid) butyl triethylammonium hydrogen sulfate), (1-(4-sulfonic acid) butylpyridinium hydrogen sulfate) and (1-(4-sulfonic acid) butyl-3-methylimidazolium hydrogen sulfate) were used to prepare the silica gel encapsulated catalysts. The loading of ionic liquids in silica gel was 5 - 30 % by weight. They were characterized by NMR, XRD, FTIR and SEM. Thermogravimetric analysis was carried out under inert atmosphere on all the encapsulated ionic liquids in order to determine their exact thermal degradation or decomposition temperature as well as water and other organic impurities. The analysis showed that they were thermally stable upto 324ºC. The catalysts were tested for activity in *tert*- butylation of *p*-cresol with *tert*-butyl alcohol (TBA) in a batch reactor. The maximum conversion of *p*-cresol was 80% at a temperature of 70ºC using all the three encapsulated ionic liquids with a maximum selective yield of 87% monoalkylated product which is TBC.

V. References

[1] P. Elavarasan, K. Kondamudi, S. Upadhyayula, "Synthesis of Antioxidants : Green Chemistry Route," International Journal of Chemical Sciences, Vol S579 (1), Dec 2010, pp. 579-582.

[2] T. Welton, “Room-temperature ionic liquids. Solvents for synthesis and catalysis," Chemical Review, vol. 99(8), July 1999**,** pp 2071–2084, **DOI:** 10.1021/cr980032t.

[3] P. Elavarasan, K. Kondamudi, S. Upadhyayula, "Statistical optimization of process variables in batch alkylation of p-cresol with tert-butyl alcohol using ionic liquid catalyst by response surface methodology.", Chemical Engineering Journal, Vol 155 (1-2), Jan 2009, pp. 355-360. Doi:10.1016/j.cej.2009.07.019.

[4] K. Kondamudi, P. Elavarasan, P. J. Dyson and S. Upadhyayula, "Alkylation of p-cresol with tert-butyl alcohol using benign Bronsted acidic ionic liquid catalyst.", Journal of Molecular Catalysis A: Chemical, Vol 321(1-2), Apr 2010, pp. 34-41. Doi: 10.1016/j.molcata.2010.01.016.

[5] P. Elavarasan, K. Kondamudi and S. Upadhyayula, "Kinetics of phenol alkylation with tert-butyl alcohol using sulfonic acid functional ionic liquid catalysts.", Chemical Engineering Journal, Vol 166 (1), Jan 2011, pp. 340-347. Doi: 10.1016/j.cej.2010.11.001.

[6] P. Elavarasan, K. Kondamudi and S. Upadhyayula, "A Functionalized, Supported Ionic Liquid for Alkylation of p-Cresol with Tert-Butyl Alcohol.", Journal of Chemical Engineering & Process Technology, Vol 7 (1), Jan 2016, pp. 1-7. Doi: 10.4172/2157-7048.1000270..

[7] C. P. Mehnert, “supported ionic liquid catalyst,” Chemistry. Vol 11(1), Dec 2004, pp.50-6. DOI: 10.1002/chem.200400683

[8] C. P. Mehnert, R. A.Cook,N. C. Dispenziere and M. Afeworki, “Supported ionic liquid catalysis--a new concept for homogeneous hydroformylation catalysis,” Journal of American Chemical Society, vol 124(44), Nov 2002, pp. 12932-12933. doi/10.1021/ja0279242

VI. Acknowledgments

This research was supported by QIP-AICTE PhD program at Indian Institute of Delhi, New Delhi, India.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Techno-Economic Analysis for the Production of Ethanol from *Ipomoea batatas (*sweet potato*)*

Deenadayalan Karaiyagowder Govindarajan[1], Yogesan Meganathan[1], Gowthama Prabu Udayakumar[1] and Ram Kothandan[1*]

Bioinformatics Laboratory, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore INDIA, ram.k.bt@kct.ac.in

Abstract: *With the increase in the demand for alternative fuels, bioethanol is found to be suitable alternative for the fossil fuels. In the recent years, the demand for alternative have increased exponentially; in particular bioethanol from agriculture-based raw material is of greater interest. Significant amount of studies has been reported for the production of biofuels; none has reported the techno-economics analysis of the process. In this present study, the techno-economic analysis for the production of bioethanol from sweet potatoes is been discussed; with emphasis on the scale-up of process from lab scale to pilot plant scale with recycle. The entire analysis was performed using Intelligen SuperPro Designer. With the higher demand and emphasis for the environmental pollution - impact analysis was also carried out. The study achieves complete utilization of the raw material with minimal or no environmental risk. In addition, the simulation aims in construction of a self-sustainable plant for the production of ethanol.*

Keywords*: Alternative biofuels, techno-economic analysis, environmental impact analysis, ethanol, self-sustainable.*

I. Introduction

Bioethanol is a renewable form of energy source that is produced from some of the conventional agricultural feedstock such as corn, sugarcane, potato which have a rich source of starch and cellulose molecules. Due to their primary purpose as feedstock and increase in the price, the dependency for alternate source to produce cellulose based ethanol is of great importance. Currently, cassava and sweet potato cultivars are being used as major source and cultivated in large scale in and around the southern India for more than 142000 hectares. More than 400-500 starch processing units are located in TamilNadu accounting for the 90% of the total production. In 2015-16, Orrisa alone contributed to 385.55 metric tonnes of sweet potato as per the reports of National Horticulture Board (NHB).

In the present scenario, these tubers are used in the preparation food-grade starch whereas the ethanol fermentation from these tubers remain untapped. The tubers of the sweet potato yielded a high concentration of carbohydrate upto 80% in the form of sucrose 30% and starch 50%, whereas the tuber of cassava had carbohydrate concentrations of 55% comprising mainly of starch[1]. In addition, the sweet potato based biofuels shows positive energy efficiency and high significance of eutrophication and acidification [2-4].

Sweet potato (*Ipomoea batatas*) is one of the rich source of yield of starch per unit land of cultivation which is a suitable substrate for the fermentation of alcohol from the polysaccharide. Along with starch it also compose other fermentable sugars such as dextran, sucrose, fructose and mainly glucose along with protein[5]. Around 9 different varieties such as Ndou, Serolane, Resisto, Monate, Blesbok, Impilo of sweet potato are cultivated, which have high maltose, fructose and glucose content along with micro elements like vitamins and magnesium, zinc and iron content[6]. Sweet potato pose a easy-to-grow characteristic and can sustain severe condition like drought resistance, high resistance over pathogens and plagues and a farmer friendly properties like high multiplication rate, short grow cycle, protects the soil from being eroded[7].

Large scale ethanol production from the tuber is still at a development stages and complete commercialization of the process and technology is questionable. Utilization of models/ computer simulations to obtain a commercially feasible process is the main aim of the study. This study focusses mainly in the development of a pilot scale plant design and process. The study also emphasises on the
energy consumption and predicts the economical and physical implications of an experiment for the industrial processes. In the later part of the study, details on the material utilization, utility consumptions, the optimal operation conditions, process configurations are discussed.

II. Materials and Methods

Process Simulation:

The entire simulation was carried out used Intelligen Super Pro designer (Academic License).Reports and initial analysis was also done with the same.

A.Raw materials and composition:

The major source of carbohydrate used in the study is sweet potato mainly due to the abundance of source in the southern India. Initial processing includes the pre-treatment of the raw material includes washing, drying, shredding, dryer and pressing. Completely smashed raw material is then liquefied and passed on the fermenters. However, in this simulation completely pressed and stored flour is used a raw material rather than the raw sweet potato. The final composition of the raw material used in the simulation is as follows: water (8%), glucose(2.6%), fructose(2.8%), sucrose(8.6%),starch(76.9%),total proteins(3.4%). Details of the input as feed stream is described in the table 1[8].

TABLE 1: INGREDIENTS, FLOW RATE AND CONCENTRATION OF THE FEED MIXTURE

S.No	MIXTURE	INGREDIENTS	FLOW RATE (kg/batch)	CONCENTRATION (g/L)	MASS COMPOSITION (%)
1	Feed	Glucose	5	75.56	7.14
		Fructose	5	75.56	7.14
		Starch	20	302.22	28.57
		Sucrose	10	151.11	14.28
		Water	30	453.33	42.86
2	Alpha Amylase	Alpha amylase	0.25	1250	100
3	Acid	Sulphuric Acid	0.5	1828.57	100
4	Yeast	*Saccharomyces cerevisiae*	5	1562	100
5	Air	Air	100	1.179	100

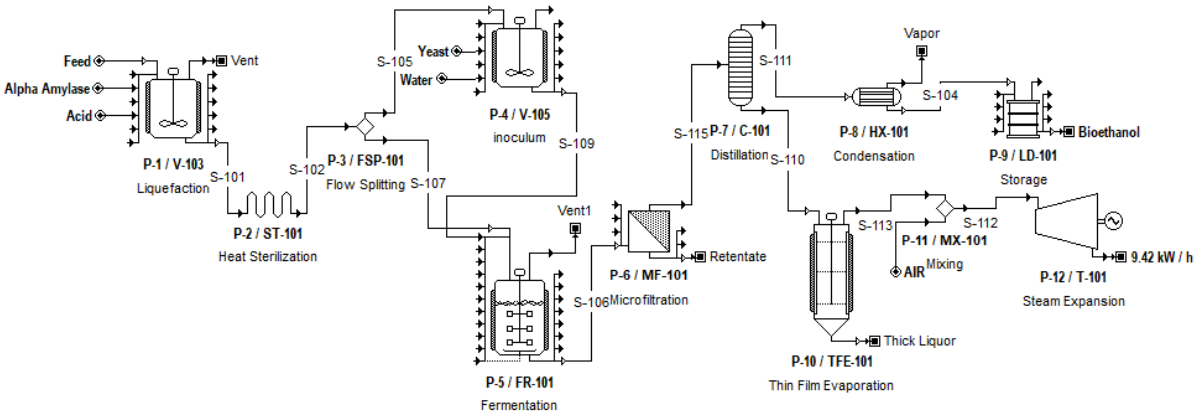
B. Process flow description:

The process diagram shown in fig.l contains various equipment and unit operations involving in bioethanol production. Here, we had not endeavoured to emulate the large-scale bioethanol production unit. This process design contained the basic unit operations required for production of bioethanol from yeast, say *Saccharomyces cerevisiae* species using submerged fermentation in the pilot-scale only.

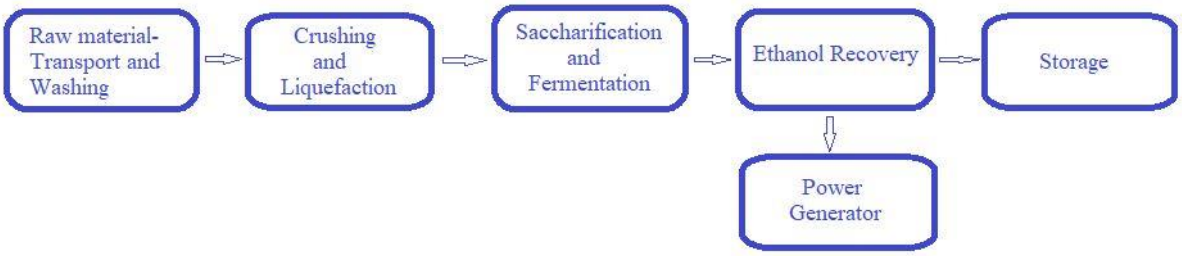
The process flow includes equipments and unit operations/procedures (P) along with the descriptions, streams for transferring feed throughout the process (S), charge points for input feed represented by squares and circles, and arrowheads indicating the direction of flow. The Super-Pro Designer gathers information on input feed (input stream) composition, biomass composition, energy requirements for each and every unit operations and equipment involved in simulation as input data[9]. The process simulator generates output feed (output stream) volume, compositions and other physico-chemical characteristics responsible during each unit operations separately for every step throughout the process. The output for each unit procedures were calculated from the input feed (input stream) based on specific stoichiometric reactions and principles. The stoichiometric equation (1) explains the chemical balance between reactants (input) and products (output).

$$A + B \rightarrow C + D \quad (1)$$

III. Process Design



The equipment were selected in line with the industrial bioethanol production scale directly from the Super-Pro Designer software database[10], The input information such as composition data, physical and chemical properties of the raw material, equipment maximum volumes, coefficients of heat and mass transfer and other specific input and output parameters for each device were entered to the process simulator.



Processing of sweet potato flour

Considering the expense incurred for the transportation and the storage of sweet potatoes in large-scale; partially processed flour were procured and used in the liquefaction process.

Liquefaction and Heat sterilization:

The partially processed flour is discharged to the liquefaction tank (P-7/V-103). Liquefaction is done by the addition of excess water and the pH is adjusted to 5.8 by the addition of 1% sulfuric acid and the pH is monitored. The total amount of water added to the liquefaction tank depends on the ratio of the dry material to that of water[8]. During the process of gelatinization, the alpha-amylase hydrolyzes the starch and there could be noticed a sharp increase in the viscosity of the sweet potato mixture[11].

To overcome the problem of contamination in the process, heat sterilization was done prior to fermentation process[12]. The liquefied raw material were transferred to heat sterilization (P-2/ST-101). Sterilization is carried out for 1 hour were the temperature was maintained at 60°C[13] .

Inoculum preparation and Saccharification(Fermentation):

The yeast cells (*Saccharomyces cerevisiae*) were cultivated in small volumes in a blending tank(P-15/V-105)using the culture medium containing 50% of the sugars used during the fermentation along with magnesium (0.12 g/L) and ammonium salts (0.24 g/L) from heat sterilization(P-2/SF-101) is used as a substrate for inoculum preparation and its incubated at 24.95°C for 1 hour with a agitation time around 15 minutes[14, 15]. Then the culture is transferred to fermenter (P-3/FR-101) where the cells were produced in greater amount.

The conversion of sucrose to glucose and fructose, and the conversion of starch to glucose was considered 100%,according to the experimental data obtained[3, 8]. To the slurry of the sweet potato mixture 3.6mL/kg dry sweet potato a-amylase is added according to dosage experimentally determined[16] and is thoroughly mixed[17, 18].

$$(C_6H_{10}O_5)_n + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose + Water → glucose +fructose

The fermentor (P-3/FR-101) is cleaned well and is prepared to initiate a new process. The saccharification and fermentation can also be considered simultaneously. The fermentation of the sugar was carried out at 30°C, in a time varied between 14–36 hours depending on the raw material. Since we have used fresh sweet potato, for the dilution of 1:5/1:4 about 15 - 20 hours of fermentation would yield more than 90%

conversion of the sugars to ethanol[19, 20]. Several fermentors can also be operated in staggered intervals to give a continuous feed to the continuous distillation system[21].

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2CO_2$$

Microfiltration

Ethanol is a extracellular product which are produced largely during the lag phase of the organism. Hence the cells were initially removed using a micro-filter membrane (MF-101). The cells were removed and the supernatant is transferred out to the distillation column where the further recovery of ethanol will be performed[22].

Distillation for Ethanol Recovery:

The outlet containing the supernatant of the ethanol devoid of the cells is then is fed into the rectifier column which is further fed into the 20 metre high distillation column to remove additional water. Now the ethanol distillate obtained is again recombined with the feed to the rectifying column. The distillate now mainly comprising the ethanol feed can produce pure ethanol upto 95%. The water molecules which are adsorbed inside the micro-porous beads of the molecular sieves, are regenerated by heating and this vapour stream is connected with the stream leading to the condenser.

IV. Results and Discussion:

The commercial production for the synthesis of bioethanol from sweet potato was successfully simulated using the software Super-pro designer. The efficiency of the fermentation design was identified by the consumption of the variety of carbohydrates by the yeast organism. *Saccharomyces cerevisiae* was chosen as the organism for fermentation due to its high productivity and high ethanol tolerance[23] **.**The annual operating cost and feasibility of the production of bioethanol was studied. Fresh and mature sweet potatoes were purchased from the local market and stored at room temperature, with proper aeration not beyond two weeks before they are exploited into the process. The sugars which were majorly present in the sweet potato were broken down from complex sugars to simple sugars through the process carried out in 60 liter the blender. (V-103) The feed flow rate of both the saccharides count to 40 kg as input along with water which is used for acid hydrolysis in the presence of hydrochloric acid by the enzyme, alpha amylase. There can be seen a clear increase in the simple sugars, glucose, which acts as the substrate, by the hydrolysis of the complex sugars. 32 kg of glucose mainly obtained from starch is seen, where the dry mass is liquefied by complete agitation with water. The process of inoculum preparation was done separately by using another blending tank, where the carbon source was provided by the synthesized sugar media which is split using a flow splitter. The total contents from the liquefaction tank were split into the ratio of 2:3 for the yeast culture and the fermentation unit separately. Simultaneously, the fed input given to the Fermentation unit, where the cultured yeast, *Saccharomyces cerevisiae*, acts on the sugar and by aerobic fermentation, ethanol is produced. Glucose acts as the rate limiting step, determining the extent to which the yeast could ferment them to alcohol. The fermenter which ran for 24 hours completely utilized the batch of media which was given. The unused yeast cells, used dry dead cells and other extracellular proteins and other solid, semi-solid substances suspended in the obtained ethanol is clarified using the microfilter membrane with optimal flow rate[24]. The obtained liquid comprising of ethanol, with water and suspended solids are now subjected to distillation unit, while the dry cells are discarded out. The distillation column (C-101), exploiting the volatility of the input mixture, the bioethanol which has a lower boiling point forms the vapor and is sent to the cooling unit at the condenser. Now the purely obtained 20.05 kg/batch of bioethanol is stored in a sterile storage tank at room temperature[25]. The water which leaves the distillation unit after reboiling, is evaporated and pressurized for the power generation unit. Even though the used water is not connected for recycle and usage, it is assumed to utilize at least 90% of the released water is recycled[26-28]. In the turbulence generated evaporation unit, the pure vapor of water is acquired, which helps in reducing the cost consumed in cleaning the sludge removal over the turbines. The steam is pressurized with air in a mixing unit at high pressure and used to run a turbine to generate 9.42 kWh (in a batch) using a Turbine power generator (T-101)[29], which is equivalent to 74,539 kWh annually. This proves the economic potency where the power which can be generated annually equals 5 times the power consumed in a year.

TABLE 2: SELECTED EQUIPMENT OF THE BIOETHANOL PLANT MODEL

S.No	Process	Unit ID	Description	Details
1	Liquefaction and Saccharification	P-1/V-103	Liquefaction Saccharification to simple sugars	Useful volume 90% Max Allowable 80% Rate limiting Compound - Starch

				Residence time 2.4 h
2	Heat Sterilization	P-2/ST-101	Heating Unit	Heating Agent – Steam Sterilization temp – 140° C Outlet Temp – 35° C
3	Inoculum Preparation	P-4/V-105	Growth of the Organism *Saccharomyces cerevisiae*	Temp – 25° C Useful volume 83% Max Allowable 90% Rate reference compound – 90%
4	Fermentation	P-5/FR-101	Fermentation of the sugar	Temp – 37° C Max Allowable 90% Rate reference compound – 90%
5	Microfiltration	P-6/MF-101	Microfiltration for the particle liquid separation	Solid Components removal % - 100% Time – 4 hours Density of water (Component Data) – 1000 kg/m^3
6	Distillation	P-7/C-101	Beer column Rectifying column	Stage efficiency – 80% Component Separation – Bioethanol – 90% Water – 10%
7	Condensation	P-8/HX-101	Condenser	Temp – 20° C Vapor/Liquid split – Bioethanol – 100%
8	Thin Film Evaporation	P-10/TFE-101	Evaporation	Vapor/Liquid Split: Water - 100 % Operation throughput - 0.14 m^3/h
9	Power Generation	P-12/T-101	Steam expansion Straight flow Steam-turbine generator	Isoentropic efficiency – 60% Power shaft efficiency – 90%

TABLE 3: ANNUAL OPERATING COST (IN INR/YEAR) FOR BIOETHANOL PRODUCTION

S.No	Name	Type	Units	Size (Capacity)	Purchase Cost (INR /Unit)
1	V-103	Blending Tank	1	60 L	9,574,400
2	ST-101	Heat Sterilizer	1	0.014 L/s	7,744,000
3	V-105	Blending Tank	1	80.63 L	9,574,400
4	FSP-101	Flow Splitter	1	50.00 kg/h	-
5	FR-101	Fermentor	1	120 L	32,243,200
6	C-101	Distillation Column	1	2,261,950 L	50,476,800
7	HX-101	Condenser	1	0.01	2,252,800
8	LD-101	Liquids Drum	1	30	70,400
9	MF-101	Microfiltration	1	7.42 m^2	2,112,000
10	SG-101	Thin Film Evaporator	1	114.24989 kg/h	6,969,600
11	MX-101	Mixing	1	900 kPa	-
12	T-101	Straight-Flow Steam Turbine-Generator	1	8.35 kW/h	2,041,600

TABLE 4: INPUT AND OUTPUT FLOWRATE AND COMPOSITION OF THE UNIT PROCEDURES INVOLVED

S. No	Process	Unit ID	Input		Input Flow rate (kg/batch)	Output		Output Flow rate (kg/batch)	
1	Saccharification	P-1/V-103	Feed (Total = 70)	Glucose	5	Total = 70.72	Alpha amylase	0.25	
				Fructose	5		Fructose	9.73	
				Starch	20		Glucos	31.95	

							e		
				Sucrose	10		Starch	0	
				Water	30		Sucrose	1	
			Alpha Amylase		0.25		Sulfuric Acid	0.49	
			Acid (Sulfuric acid)		0.5		Water	27.30	
2	Heat Sterilization	P-2/ST-101	Total feed = 70.72	Alpha amylase	0.25	Total = 70.72	Alpha amylase	0.25	
				Fructose	9.73		Fructose	9.73	
				Glucose	31.95		Glucose	31.95	
				Starch	0		Starch	0	
				Sucrose	1		Sucrose	1	
				Sulfuric Acid	0.49		Sulfuric Acid	0.49	
				Water	27.30		Water	27.30	
3	Flow splitting	P-3/FS P-101	Total feed = 70.72	Alpha amylase	0.25	To Inoculum tank (V-101)	Alpha amylase	0.1	
							Fructose	3.89	
				Fructose	9.73		Glucose	12.78	
							Starch	0	
				Glucose	31.95		Sucrose	0.4	
							Sulfuric Acid	0.19	
				Starch	0		Water	10.92	
						To Saccharification tank (FR-101)	Alpha amylase	0.15	
				Sucrose	1		Fructose	5.84	
							Glucose	19.18	

				Sulfuric Acid	0.49		Starch	0	
							Sucrose	0.6	
				Water	27.30		Sulfuric Acid	0.29	
							Water	16.38	
4	Inoculum Preparation	P-3/V-105	Total feed = 70.72	Alpha amylase	0.1	Total = 133.085	Alpha amylase	0.1	
				Fructose	3.89		Biethanol	0.19	
				Glucose	12.78		Biomass	13.43	
				Starch	0		Fructose	3.5	
				Sucrose	0.4		Glucose	1.27	
				Sulfuric Acid	0.19		Sucrose	0.016	
				Water	10.92		Sulfuric Acid	0.199	
			Yeast		5		Water	110.15	
			Water		100		Yeast	4.23	
5	Fermentation	P-4/FR-101	From Flow splitting (FSP-101)	Alpha amylase	0.15	Total = 166.56	Alpha amylase	0.25	
				Fructose	5.84				
				Glucose	19.18		Bioethanol	12.18	
				Starch	0		Biomass	8.31	
				Sucrose	0.6		Dry cells	2.53	
				Sulfuric Acid	0.29				
				Water	16.38		Fructose	9.35	
			From Inoculum tank	Alpha amylase	0.1				
				Biethan	0.19		Glucos	2.04	

			(V-105)	ol			e		
				Biomass	13.43				
				Fructose	3.5		Sulfuric Acid	0.49	
				Glucose	1.27				
				Sucrose	0.016		Water	126.53	
				Sulfuric Acid	0.199				
				Water	110.15		Yeast	4.23	
				Yeast	4.23				
6	Microfiltration	P-5/MX-101	Total = 166.56	Alpha amylase	0.25	Total = 149.6	Alpha amylase	0.24	
				Bioethanol	12.18		Bioethanol	11.11	
				Biomass	8.31		Biomass	7.58	
				Dry cells	2.53		Fructose	8.53	
				Fructose	9.35		Glucose	1.87	
				Glucose	2.04		Sucrose	0.56	
				Sulfuric Acid	0.49		Sulfuric Acid	0.45	
				Water	126.53		Water	115.4	
				Yeast	4.23		Yeast	3.86	
						Dry Cells	Alpha Amylase	0.02	
							Bioethanol	1.07	
							Biomass	0.73	
							Dry cells	2.53	

							Fructose	0.82	
							Glucose	0.17	
							Sucrose	0.054	
							Sulfuric Acid	0.04	
							Water	11.13	
							Yeast	0.37	
7	Distillation	P-6/C-101	Total feed = 149 .6	Alpha amylase	0.24	Total = 137.33	Alpha amylase	0.22	
				Bioethanol	20.06		Bioethanol	0.001	
				Biomass	7.58		Biomass	7.58	
				Fructose	8.53		Fructose	8.53	
				Glucose	1.87		Glucose	1.86	
				Sucrose	0.56		Sucrose	0.56	
				Sulfuric Acid	0.45		Sulfuric Acid	0.45	
				Water	115.4		Water	114.24	
				Yeast	3.86		Yeast	3.86	
						Bioethanol		11.11	
						Water		1.154	
8	Condensation	P-8/HX-101	Total feed = 12.26	Bioethanol	20.06	Total = 20.06	Bioethanol	20.06	
				Water	1.154				
9	Storage	P-9/LD-101	Total feed = 20.06	Bioethanol	20.06	Total = 20.06	Bioethanol	20.06	
10	Thin Film Evaporator	P-10/TFE-101	Total feed = 137.33	Alpha amylase	0.22	Total = 114.24	Water	114.24	
				Bioetha	0.001				

				nol					
				Biomass	7.58				
				Fructose	8.53				
				Glucose	1.86				
				Sucrose	0.56				
				Sulfuric Acid	0.45				
				Water	114.24				
				Yeast	3.86				
11	Mixing	P-11/MX-101	Total feed = 114.24	Water	114.24	Total = 214.25	Hydrogen	76.71	
			Air	Air	100		Nitrogen	23.28	
							Water	114.24	
12	Power Generation	P-12/T-101	Total = 214.25	Hydrogen	76.71	Total = 214.25	Hydrogen	76.71	
				Nitrogen	23.28		Nitrogen	23.28	
				Water	114.24		Water	114.24	

V. CONCLUSION

A self-sustained model for the production of an alternative biofuel, bioethanol from the sweet potato tuber was designed and simulated using the Intelligen Superpro Designer software[30]. There could be witnessed a complete utilization of the tuber and a maximum production of the alternative fuel. Techno-economic analysis by comprehensive study over the individual unit procedures were studied thoroughly.

6.REFERENCE

1. Ziska, L.H., et al., *An evaluation of cassava, sweet potato and field corn as potential carbohydrate sources for bioethanol production in Alabama and Maryland.* biomass and bioenergy, 2009. **33**(11): p. 1503-1508.
2. Wang, M., et al., *Life-cycle energy efficiency and environmental impacts of bioethanol production from sweet potato.* Bioresource technology, 2013. **133**: p. 285-292.
3. Subramanian, K. and K. Ram, *Biochemical conversion of rice straw into bioethanol-an exploratory investigation.* Journal of Biofuels, 2011. **2**(1): p. 33-41.
4. Muthusaravanan, S., et al., *Phytoremediation of heavy metals: mechanisms, methods and enhancements.* Environmental Chemistry Letters, 2018: p. 1-21.
5. Duvernay, W.H., M.S. Chinn, and G.C. Yencho, *Hydrolysis and fermentation of sweetpotatoes for production of fermentable sugars and ethanol.* Industrial crops and products, 2013. **42**: p. 527-537.
6. Laurie, S.M., *Agronomic performance, consumer acceptability and nutrient content of new sweet potato varieties in South Africa*. 2010, University of the Free State.
7. Cao, Y., et al., *Simultaneous saccharification and fermentation of sweet potato powder for the production of ethanol under conditions of very high gravity.* Frontiers of Chemical Science and Engineering, 2011. **5**(3): p. 318-324.
8. Ferrari, M.D., M. Guigou, and C. Lareo, *Energy consumption evaluation of fuel bioethanol production from sweet potato.* Bioresource technology, 2013. **136**: p. 377-384.
9. Kwiatkowski, J.R., et al., *Modeling the process and costs of fuel ethanol production by the corn dry-grind process.* Industrial crops and products, 2006. **23**(3): p. 288-296.
10. Harrison, R.G., et al., *Bioseparations science and engineering*. 2015: Topics in Chemical Engineering.
11. Van Der Maarel, M.J., et al., *Properties and applications of starch-converting enzymes of the α-amylase family.* Journal of biotechnology, 2002. **94**(2): p. 137-155.
12. Atlas, R.M., *Handbook of microbiological media*. 2010: CRC press.
13. Wieslander, A., P. Kjellstrand, and B. Rippe, *Heat sterilization of glucose-containing fluids for peritoneal dialysis: biological consequences of chemical alterations.* Peritoneal dialysis international, 1995. **15**(Suppl 7): p. S52-S59.
14. Khan, Z. and A.K. Dwivedi, *Fermentation of Biomass for Production of Ethanol: A Review.* Universal Journal of Environmental Research & Technology, 2013. **3**(1).
15. Hashem, M. and S.M. Darwish, *Production of bioethanol and associated by-products from potato starch residue stream by Saccharomyces cerevisiae.* Biomass and bioenergy, 2010. **34**(7): p. 953-959.
16. Lareo, C., et al., *Evaluation of sweet potato for fuel bioethanol production: hydrolysis and fermentation.* SpringerPlus, 2013. **2**(1): p. 493.
17. Sarkar, N., et al., *Bioethanol production from agricultural wastes: an overview.* Renewable energy, 2012. **37**(1): p. 19-27.
18. Ray, R.C. and S. Behera, *Bio-fuel agro-industrial production system in sweet sorghum.* Sorghum: cultivation, varieties and uses. Edited by TD Pereira. Nova Science Publishers, New York, 2011: p. 65-82.
19. Widodo, Y., S. Wahyuningsih, and A. Ueda, *Sweet potato production for bio-ethanol and food related industry in Indonesia: Challenges for Sustainability.* Procedia Chemistry, 2015. **14**: p. 493-500.
20. Badger, P., *Ethanol from cellulose: a general review.* Trends in new crops and new uses, 2002. **1**: p. 17-21.
21. Bafrncová, P., et al., *Improvement of very high gravity ethanol fermentation by media supplementation using Saccharomyces cerevisiae.* Biotechnology letters, 1999. **21**(4): p. 337-341.
22. Zeman, L.J. and A.L. Zydney, *Microfiltration and ultrafiltration: principles and applications*. 2017: CRC Press.
23. Azhar, S.H.M., et al., *Yeasts in sustainable bioethanol production: A review.* Biochemistry and Biophysics Reports, 2017. **10**: p. 52-61.
24. Cantrell, D.F. and D.J. Winsness, *Method of processing ethanol byproducts and related subsystems*. 2009, Google Patents.
25. Bouaid, A., M. Martinez, and J. Aracil, *Production of biodiesel from bioethanol and Brassica carinata oil: oxidation stability study.* Bioresource Technology, 2009. **100**(7): p. 2234-2239.
26. Paramasivan, T., et al., *Graphene Family Materials for the Removal of Pesticides from Water*, in *A New Generation Material Graphene: Applications in Water Technology*. 2019, Springer. p. 309-327.
27. Sivarajasekar, N., et al. *Comparative modeling of fluoride biosorption onto waste Gossypium hirsutum seed microwave-bichar using response surface methodology and artificial neural networks*. in *Intelligent Computing, Instrumentation and Control Technologies (ICICICT), 2017 International Conference on*. 2017. IEEE.
28. Sivarajasekar, N., et al., *Defluoridation of water using adsorbents-A concise review.* Journal of Environment & Biotechnology Research, 2017. **6**(1): p. 186-198.
29. Rigollot, G., *Power-generating plant using a combined gas-and steam-turbine cycle*. 1973, Google Patents.
30. Klein-Marcuschamer, D., et al., *Technoeconomic analysis of biofuels: A wiki-based platform for lignocellulosic biorefineries.* biomass and bioenergy, 2010. **34**(12): p. 1914-1921.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Hydrogen Production from Distillery Wastewater Using Anaerobic Sequencing Batch Reactor (AnSBR)

[1]S.Sharmiladevi, [2]Paviyadharshini.S.A, [3]Rasha Ali
Department of Environmental Engineering, Park College of Technology, Anna University
Prema Ravi Nagar, Karumathampatti, Coimbatore-641659
Tamil Nadu, India, Paviya663@gmail.com

***Abstract**: Energy, an urging need of the daily life is going on depleting while the demand for is increasing with time biohydrogen technology provides an alternative source of energy and hails as an typical appropriate technology that meets the basic need for cooking fuel in rural India. The aim of the study is to determine the basis for the design of an Anaerobic Sequencing Batch Reactor (AnSBR) operated in mesophilic conditions for the treatment of distillery wastewater. To study the characteristics of raw distillery wastewater and characteristics of sludge were also analyzed. Initially the treatment of distillery effluent by an AnSBR at lab scale was carried mg/l out using a reactor of 5 liter volume. The reactor was designed and the reactor was fed with distillery wastewater and sludge is maintained for two weeks. The AnSBR was operated in 24 hours HRT with various cycles of OLR.. The efficient COD Removal efficiency is 61.77 percentage.*

***Keywords**: Distillery wastewater, Anaerobic Sequencing Batch Reactor (AnSBR), Hydraulic Retention Time ((HRT), Organic Loading Rate (OLR), Chemical Oxygen Demand (COD)*

I. Introduction

The wastewater released from distilleries is known as spent wash which contains huge quantity of dissolved organic matter, dissolved solids, suspended solids, colour along with other pollutants. Distillery spent wash is the unwanted residual liquid waste generated during alcohol production and pollution caused by it is one of the most critical environmental issue.

AnSBR are used all over the world and have been around since the 1920s. With their growing popularity in Europe and China as well as the United States, they are being used successfully to treat both municipal and industrial wastewaters, particularly in areas characterized by low or varying flow patterns. Municipalities, resorts, casinos, and a number of industries, including dairy, pulp and paper, tanneries and textiles, are using SBRs as practical wastewater treatment alternatives.

Improvements in equipment and technology, especially in aeration devices and computer control systems, have made SBRs a viable choice over the conventional activated-sludge system.

These plants are very practical for a number of reasons:

- In areas where there is a limited amount of space, treatment takes place in a single basin instead of multiple basins, allowing for a smaller footprint. Low total-suspended-solid values of less than 10 milligrams per liter (mg/L) can be achieved consistently through the use of effective decanters that eliminate the need for a separate clarifier.
- 3The treatment cycle can be adjusted to undergo aerobic, anaerobic, and anoxic conditions in order to achieve biological nutrient removal, including nitrification, denitrification, and some phosphorus removal. Biochemical oxygen demand (BOD) levels of less than 5 mg/L can be achieved consistently.
- Total nitrogen limits of less than 5 mg/L can also be achieved by aerobic conversion of ammonia to nitrates (nitrification) and anoxic conversion of nitrates to nitrogen gas (denitrification) within the same tank.
- Low phosphorus limits of less than 2 mg/L can be attained by using a combination of biological treatment (anaerobic phosphorus- absorbing organisms) and chemical agents (aluminum or iron salts) within the vessel and treatment cycle.
- Older wastewater treatment facilities can be retrofitted to an SBR because the basins are already present.
- Wastewater discharge permits are becoming more stringent and SBRs offer a cost-effective way to achieve lower effluent limits. Note that discharge limits that require a greater degree

of treatment may necessitate the addition of a tertiary filtration unit following the SBR treatment phase. This consideration should be an important part of the design process.

Advantages OF ANSBR

The advantages of sequencing batch reactor over other reactor is,

- Process is flexible and easy to operation
- Mixed – liquor solids cannot be washed out by hydraulic surges because flow equalization is provided
- Quiescent settling provides low effluent TSS concentration 5 – 8 mg / l TN is achievable

BIOGAS

Biogas typically refers to a gas produced by the breakdown of organic matter in the absence of oxygen. It is a renewable energy source, like solar and wind energy. Additionally, biogas can be produced from regionally available raw materials such as recycled waste and is environmentally friendly. Biogas is produced by anaerobic digestion with anaerobic bacteria or fermentation of biodegradable materials such as manure, sewage, municipal waste, green waste, material, and crops. It is primarily methane (CH_4) and carbon dioxide (CO_2) and may have small amounts of hydrogen sulphide (H_2S), moisture and siloxanes.

ADVANTAGES OF HYDROGEN ENERGY

- ✓ Readily Available
- ✓ No Harmful Emissions
- ✓ Environment Friendly
- ✓ Used as Fuel in Rockets
- ✓ Fuel Efficient
- ✓ Renewable

II. MATERIALS AND EXPERIMENTAL PROGRAM

A.WASTEWATER SAMPLE

Distillery wastewater collected from Sakthi Sugars Private Limited, Appakudal, Erode district. India will use as substrate. The wastewater can be considered as complex in nature due to the presence of organic content and acidity. After collection, the wastewater was transferred immediately to the laboratory and stored at 4 ◦C, and the wastewater was not corrected for trace elements deficiency.

B. DISTILLERY SLUDGE

Distillery sludge contains high amount of nutrient content, amino acids, suspended and dissolved solids. It also contains mineral contents like calcium, phosphorous, potassium, sodium, magnesium, manganese, iron and etc. The proximate composition of distillery sludge contains metabolize energy, crude protein, true protein, ether extract, nitrogen free extract

C. LOADING OF DISTILLERY WASTEWATER

The volume of influent contains 1:3 ratio of wastewater and sludge with 24 h HRT. The substrate distillery wastewater was stored in 4^0c deep freezer, and the required amount for each OLR was fed into the reactor, before feeding the supernatant was removed from the reactor and the same amount of wastewater was added into the reactor to maintain the working volume of the reactor and also it serves food for microorganisms, the loading take place continuously for every cycle and for each OLR until the hydrogen production attains the steady state.

D. FABRICATION DETAILS OF THE REACTOR

- Volume of the reactor = 5 L
- Height of the reactor = 62 cm
- Diameter of the reactor = 10 cm
- Gas escape valve = 2cm
- Effluent removal probe and other probes = 2 cm

III. RESULT AND DISCUSSION

The Distillery wastewater parameters are analyzed in our laboratory as per IS 3025 (With different parts) parameters are listed below.

TABLE 3.1: CHARACTERISTICS OF DISTILLERY WASTE WATER

S.No	PARAMETERS	VALUES (mg/l)
1.	BOD @27^OC	40000
2.	COD	100000
3.	TDS	87500
4.	TSS	7500
5.	TS	95000
6.	TVS	66000

7.	pH@27°C*	3.9
8.	Chloride	7500
9.	Oil & Grease	Nil

TABLE 3.2: CHARECTERISTIC OF DISTILLERY SLUDGE

S.No	PARAMETERS	VALUES (mg/l)
1.	pH*	5.3
2.	TS	107000
3.	VS	55000
4.	TSS	8500
5.	TDS	98500

- **All values are in mg/l expect pH**

HYDROGEN PRODUCTION RATE

TABLE 3.3: HYDROGEN GAS PRODUCTION IN A CYCLIC PERIOD FOR 24h HRT WITHB AN OLROF 2.5kg

Time (min)	Hydrogen Production Rate in AnSBR (ml)
0	0
30	40
60	65
90	95
120	130
150	140
180	180
210	200
240	130
270	85
300	32
330	15

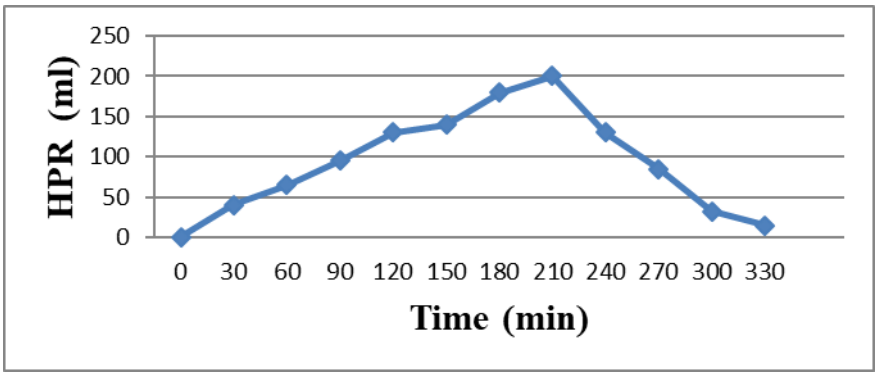


FIG 3.1: RATE OF HYDROGEN GAS PRODUCTION FOR 24h HRT WITH OLR OF 2.5kg COD/m^3.d

In the above graph s discussed about the rate of hydrogen gas production in a cycle period of 24h HRT under organic loading rate of 2.5 kg COD/m^3.d. The graph is plotted between Time (min) and hydrogen gas production in AnSBR(ml) from 24 hour HRT loading. The minimum amount of Hydrogen gas produced in the time of 330 minute and the maximum amount of hydrogen gas produced in the time of 210 minute from 24 hour HRT loading.

TABLE 3.4:HYDROGEN GAS PRODUCTION IN A CYCLIC PERIOD FOR 24h HRT WITH AN OLR OF 3.5kg COD/m^3.d

Time (ml)	Hydrogen Production Rate in AnSBR (ml)
0	0
30	75
60	85
90	155
120	120
150	70
180	100
210	175
240	150
270	110
300	45
330	40

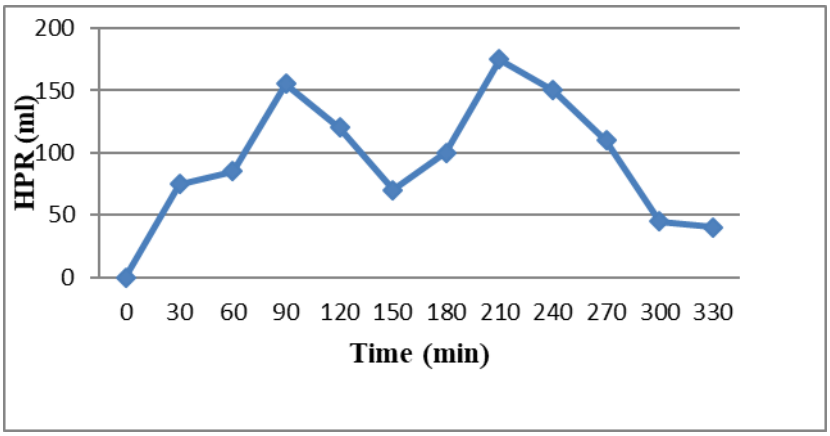


FIG 3.2: RATE OF HYDROGEN GAS PRODUCTION FOR 24h HRT WITH OLR OF 3.5 kg COD/m^3.d

In the above graph s discussed about the rate of hydrogen gas production in a cycle period of 24h HRT under organic loading rate of 3.5 kg COD/m^3.d. The graph is plotted between Time (min) and hydrogen gas production in AnSBR (ml) from 24 hour HRT loading. The minimum amount of Hydrogen gas produced in the time of 330 minute and the maximum amount of hydrogen gas produced in the time of 210 minute from 24 hour HRT loading.

TABLE 3.5: HYDROGEN GAS PRODUCTION FOR A CYCLIC PERIOD OF 24h HRT WITH AN ORGANIC LOADING RATE OF 4.5 kg COD/m^3.d

Time (ml)	Hydrogen Production Rate in AnSBR (ml)
0	0
30	95
60	95
90	80
120	100
150	120
180	150
210	150

240	200
270	170
300	120
330	120

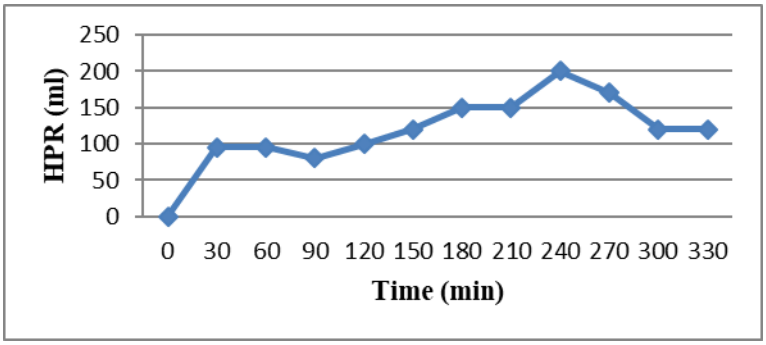


FIG 3.3: RATE OF HYDROGEN GAS PRODUCTION FOR 24h HRT WITH OLR OF 4.5kg COD/m^3.d

In the above graph discussed about the rate of hydrogen gas production in a cycle period of 24h HRT under organic loading rate of 4.5 kg COD/m^3.d. The graph is plotted between Time (min) and hydrogen gas production in AnSBR (ml) from 24 hour HRT loading. The minimum amount of Hydrogen gas produced in the time of 90 minute and the maximum amount of hydrogen gas produced in the time of 240 minute from 24 hour HRT loading.

TABLE 3.6 : VARIATION OF COD REMOVAL EFFICIENCY WITH ORGANIC LOADING RATE 24h HRT

OLR (kg/m^3.d)	pH	Influent (mg/l)	Effluent (mg/l)	COD Removal Efficiency (%)
2.5	5.6	89500	50000	55.86
3.5	5.8	90000	55000	61.11
4.5	6.0	92000	56832	61.77

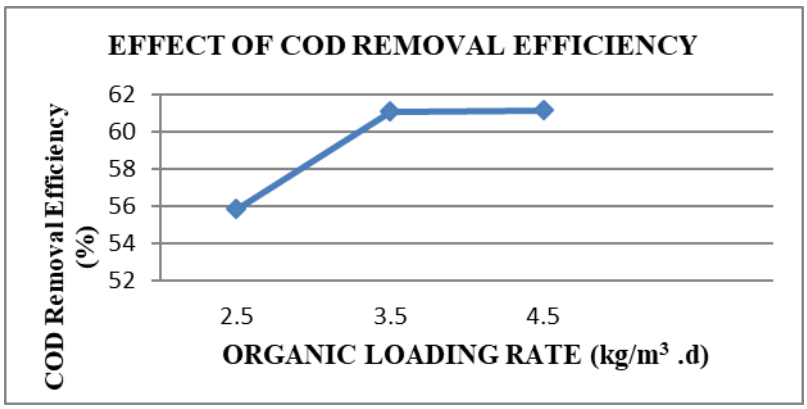


FIG 3.4: COD REMOVAL EFFICIENCY WITH DIFFERENT ORGANIC LOADING RATE 24h HRT

In the above graph discussed about the rate of removal of COD from the different kinds of organic loading. The maximum COD removal efficiency is attained from the 4.5 kg/m^3.d with the percentage of 61.77 %. The minimum COD removal efficiency is attained from the 2.5 kg/m^3.d with the percentage of 55.86 %

IV.CONCLUSION

The following conclusion was based on the above study was the physic – chemical characteristics of distillery wastewater and sludge can be determined. The parameters of the distillery wastewater is pH – 3.9, BOD – 40000 mg/l, COD – 100000 mg/l and the sludge pH – 5.3, TS – 107000 mg/l. The reactor has been designed and fabricated. The diameter of the reactor is 10 cm and height is 62 cm. The different kinds of organic loading (2.5 kg/m^3.d, 3.5 kg/m^3`d , 4.5 kg/m^3.d) are given to the reactor with 24 h HRT. The maximum hydrogen production rate is found and the maximum percentage of COD removal efficiency is determined for that loading. The 4.5 kg/m^3.d OLR gives the maximum percentage of COD removal efficiency 61.77% with 24h HRT.

V. REFERENCES

[1] APHA,1998.Standard method for the examination water and wastewater, 20th American Public Health Association, American Water Works Association, Water pollution Control Federation, Washington, DC.

[2] Bruna Soares Fernandes, Guilherme Peixoto, Fernanda Rui Albrecht,Nora Katia Saavedra del Aguila, Marcelo Zaiat "Potential to produce biohydrogen from various wastewaters" at Energy for Sustainable Development 14 (2010) 143–148

[3] Chiu-Yue Lin, Chyi-How Lay, Biswarup Sen, Chen-Yeon Chu , Gopalakrishnan Kumar, Chin-Chao Chen, Jo-Shu Chang "Fermentative hydrogen production from wastewaters: A review and prognosis" at international journal of hydrogen energy xxx (2012) 1e11.

[4] Dr. Hema Krishna R "Review of research on bio reactors used in wastewater treatment for production of bio hydrogen: future Fuel" IJSIT , 2013, 2(4), 302-310.

[5] Farina.R., (2007) "Pilot Scale Anaerobic Sequencing Batch Reactor for distillery wastewater treatment" ENEA,Agency for New Technologies", Energy & Environment, PROT – 1DR

[6] Gadhe Abhijit, Sonawane Shriram and Varma Mahesh "Kinetic modeling of biohydrogen production from complex wastewater by anaerobic cultures" at Research Journal of Chemistry and Environment Vol.17 (12) December (2013).

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

CRYSTAL VIOLET DYE REMOVAL USING ACTIVATED CARBON *LEUCAENE LEUCOCEPHALA* BY ADSORPTION METHOD

A. Nagaveni[1], M. Anusuya[1, 2], E. Jayanthi[1] and N. Renugadevi[3]
[1]Department of Chemistry, Kongunadu Arts and Science College (Autonomous)
Institution affiliated to Bharathiar University,
G. N Mills post, Coimbatore - 641029, Tamilnadu, INDIA.
[2] Department of Chemistry, Nallamuthu Gounder Mahalingam College affiliated to Bharathiar University,
Palakkad main road, Pollachi - 642001, Tamilnadu, INDIA.
[3]Department of Chemistry, Avinashilingam Institute for Home Science and
Higher Education for Women (Deemed University)
Coimbatore - 641 043, Tamilnadu, INDIA.
E mail: anagaveni89@gmail.com, http://www.kongunaducollege.ac.in

Abstract:

A low- cost and eco- friendly activated carbon was prepared from the pods of Leucaene leucocephala and treated as a carbon material for the removal of Crystal Violet (CV) dye from aqueous solution. Batch adsorption studies were carried out by varying the initial concentration of dye solution, initial pH of the solution, adsorbent dosage and temperature. The results obtained from this study were interpreted in terms of Langmuir adsorption isotherm and Freundlich adsorption isotherm. Scanning Electron Microscopy (SEM) image of activated carbon Leucaene leucocephala was monitored before and after adsorption of CV dye molecules. The percentage removal of CV dye molecules was found to be increases with decreasing initial concentration of dye solution, increasing initial pH of the solution, increasing adsorbent dosage and increasing temperature. Thus, activated carbon Leucaene leucocephala can be employed as a good carbon material for the removal of Crystal Violet dye from an aqueous solution.

Keywords*: adsorbent, activated carbon, Crystal Violet dye, adsorption isotherm.*

I. Introduction

Water is vital for all living creatures and nowadays its gets contaminated due to release of harmful dye molecules from dyeing industries. The industries like textiles, plastics, pharmaceutical, foodstuffs, cosmetics, paints, detergents and leather use synthetic dyes to colour their products [1]. As a result, the effluents coming out from these industries contain dye molecules which cause danger to marine and non- marine organisms. Various methods like flocculation, chemical precipitation, coagulation, ozonation, oxidation, nano-filtration, ionexchange, reverse osmosis and ultra-filtration are used to remove dyes, but they are expensive and create large amount of sludge [2]-[4]. Thus, the most efficient and simplest method followed is the adsorption method [5]. Adsorption process is a surface phenomenon and a promising technique for waste water treatment [6]. Many researchers have used agricultural waste materials as adsorbents such as sawdust [7], [8], rice husk [9]-[11] and fruit shell [12]. In this work, a low- cost adsorbent as activated carbon prepared from the pods of *Leucaene leucocephala* was used to remove Crystal Violet (CV) dye molecules from aqueous solution.

II. Materials and Methods

A. Preparation of the adsorbent

The pods of *Leucaene leucocephala* (White lead tree) shown in the figure 1 were collected from Palakkad district, Kerala and they were made into small pieces. After that dried in sunlight for one week and further desiccated in hot air oven at 60^0 C for 24 hours. The completely dried material was powdered well and chemically activated by treating it with conc H_2SO_4 with constant stirring, kept for 24 hours. The carbonized material thus obtained was washed well with distilled water for several times to remove the excess of acid present and dried at 100° C-120° C in a hot air oven to take away water molecules. The adsorbent thus obtained was ground well and sieved through a 250 mesh, kept in an air tight container for further use.

B. Preparation of Crystal Violet (CV) Dye Solution

Crystal Violet (CV) dye is a cationic triaryl methane dye having molecular formula $C_{25}H_{30}N_3Cl$ was purchased from a local market in Coimbatore and CV dye solution was prepared by dissolving 5 g of CV dye in distilled

water and diluted to 1000 ml. This prepared solution was taken as stock solution which was then diluted to appropriate concentrations. The equipments used were Elico pH meter, photo colorimeter, orbital shaker and incubator orbital shaker.

Figure 1 Pods of *Leucaene leucocephala*



III. Results and Discussion

A. *Effect of Variation of Initial Concentration of CV Dye Solution*

Batch experiments for the adsorption of CV dye with the adsorbent *Leucaene leucocephala* was conducted by varying the initial concentration of CV dye solution.100 ml of dye solution containing 15, 20, 25 and 30 mg of CV dye were prepared from the stock solution and taken in pyrex bottles containing 200 mg of the adsorbent. Then these bottles were agitated at 150 rotation per minute (rpm) on an orbital shaker at room temperature for time intervals (10, 20, 30, 40, 50, 60, 90, 120, 150 and 180 minutes). These solutions were filtered and the dye concentrations of the filtrates were estimated colorimetrically at 590 nm using photo colorimeter.

Figure 2 Effect of Initial Concentration of CV Dye Solution for the Adsorption of CV Dye from an Aqueous Solution onto Adsorbent

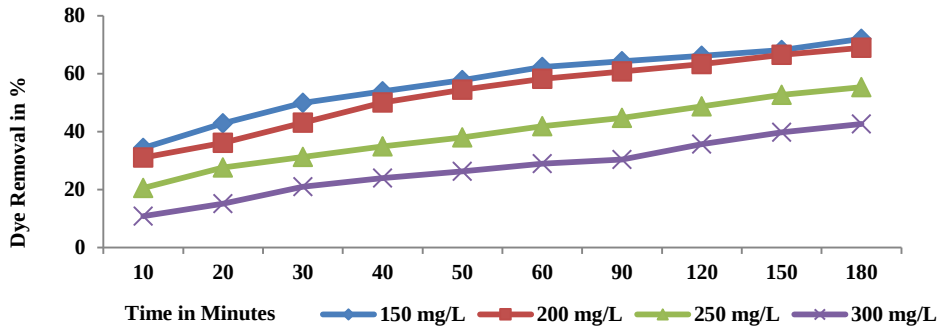


Figure 2 shows the percentage removal of CV dye increases from 42.61 to 72.07 with the adsorbent *Leucaene leucocephala* in 180 minutes of contact time, when the initial concentration of CV dye solution used was varied from 300 to 150 mg/L respectively. The amount of adsorbent kept constant and so the number of adsorption sites is also constant. Therefore, by increasing the amount of CV dye initial concentration, the percentage removal decreases due to limitation of the adsorption sites.

B. *Effect of Variation of pH on the Adsorption of CV Dye*

Batch experiments were performed by varying the initial pH from 5.0 to 9.0 using 0.1 N HCl or 0.1 N NaOH solution and pH measurements were carried out using Elico pH meter. 100 ml of dye solution containing 30 mg of the CV dye were prepared from the stock solution and treated with 200 mg of the adsorbent taken in pyrex bottles. The pH of the above solution was adjusted to desired pH from 5.0 to 9.0 and batch studies were conducted. These solutions were shaken using an orbital shaker at room temperature for various time intervals (10, 20, 30, 40, 50, 60, 90, 120, 150 and 180 minutes).These solutions were filtered and the filtrates obtained were analyzed colorimetrically to find out the percentage amount of CV dye adsorbed.

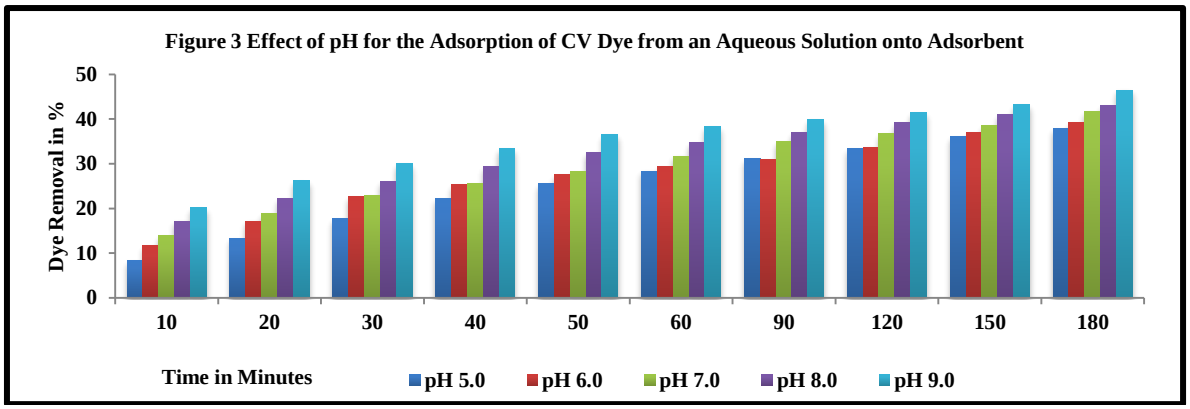


Figure 3 shows the percentage removal of CV dye increased from 37.80 to 46.45 with the adsorbent as the pH varied from 5.0 to 9.0 in 180 minutes of contact time. Generally, at lower pH the percentage removal of cationic dye gets decreased [5]. CV dye is a cationic dye and lower adsorption takes place at acidic pH is due to presence of excess of H^+ ions [8], [13].

C. Effect of Variation of Adsorbent Dosage on the Adsorption of CV Dye

The effect of adsorbent dosage on the adsorption of CV dye was studied at pH 9.0 ± 0.02. 100 ml of dye solution containing 30 mg of the CV dye were taken in pyrex bottles and batch adsorption studies were conducted by varying the adsorbent dosage (200, 300, 400 and 500 mg) of the adsorbent. The pyrex bottles containing the adsorbent and adsorbate were shaken in a orbital shaker for various time intervals and similar procedure have been followed.

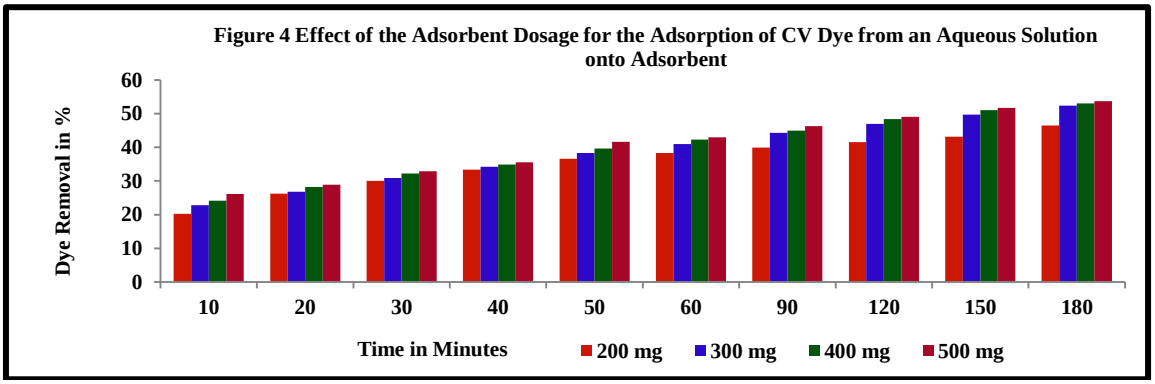


Figure 4 shows the percentage removal of CV dye increased from 46.45 to 53.70, when the adsorbent dosage was varied from 200 to 500 mg due to availability of more adsorption sites in the latter case.

D. Effect of Variation of Temperature on the Adsorption of CV Dye

The effect of temperature on the adsorption process was studied at 295, 305 and 315 K. 100 ml of dye solution containing 30 mg of the CV dye were taken in pyrex bottles and batch adsorption studies were conducted by using the adsorbent dosage 200 mg and at pH 9.0 ± 0.02. Similar procedure has been followed.

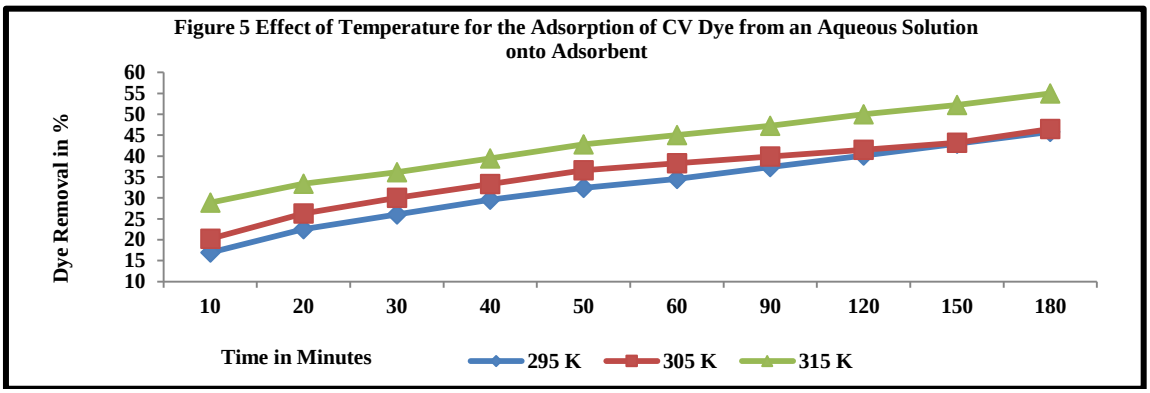
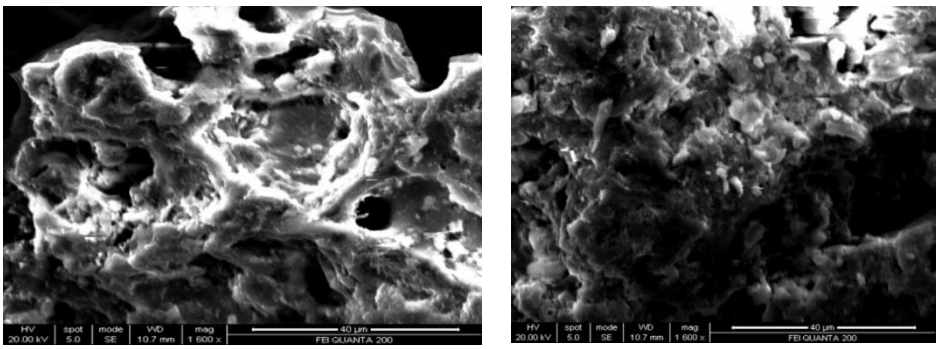


Figure 5 shows the percentage removal of CV dye increased from 45.79 to 55.01 as the temperature was varied from 295 to 315 K using 200 mg of the adsorbent. As temperature increases, there is an increase in mobility of dye molecules and the number of active sites also gets increased. Simultaneously, the pore size gets enlarged due to rise in temperature [5].

E. Characterization of the Adsorbent

Surface morphology of adsorbent *Leucaene leucocephala* was studied using SEM (Scanning Electron Microscopy). Figure 6 shows SEM image of adsorbent *Leucaene leucocephala* before and after adsorption of CV dye molecules respectively. The difference in SEM image clearly indicates that the CV dye molecules get adsorbed to the pores of adsorbent.

Figure 6 SEM Image of Adsorbent *Leucaene leucocephala* before and after Adsorption

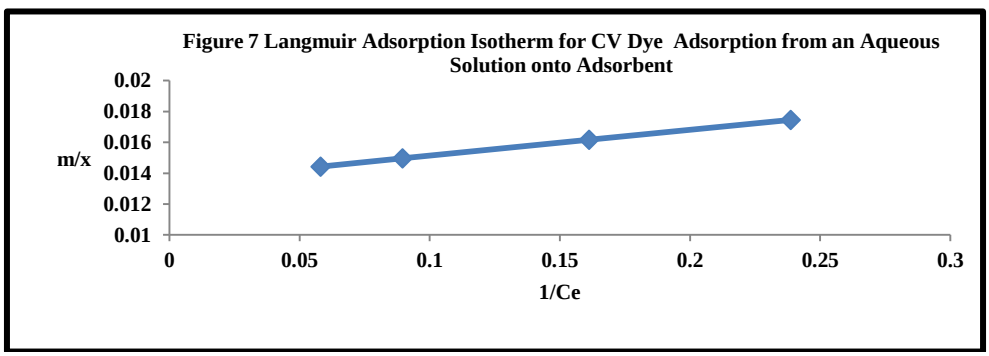


F. Langmuir Adsorption Isotherm

Langmuir adsorption isotherm used to describe the monomolecular adsorption on energetically homogeneous surfaces is given by the equation $\mathbf{(x/m) = k_1^1Ce / (1 + k_1Ce)}$ where the term 'x' denotes the amount of CV dye adsorbed in mg/L, 'm' is weight of adsorbent in mg, 'Ce' is concentration of CV dye at equilibrium, $k_1^1$and k_1 are the Langmuir constants which are the measure of adsorption capacity and energy of adsorption respectively. On rearranging, the above equation becomes $\mathbf{(m/x) = (1/ k_1^1Ce) + (k_1/ k_1^1).}$ The linear plot of 1/Ce Vs m/x shown in the figure 7 indicates that the adsorption followed Langmuir isotherm model for the adsorbent Leucaene leucocephala. The data are given in the table 1 at 180 minutes of contact time.

Table 1 Interpretation of Results of Langmuir Adsorption Isotherm for CV Dye Adsorption from an Aqueous Solution onto Adsorbent

Initial Concentration of CV Dye in mg/L	1/Ce	m/x	Intercept k_1/ k_1^1	Slope $1/ k_1^1$
150	0.2386	0.0174	0.0134	0.0167
200	0.1612	0.0161		
250	0.0895	0.0149		
300	0.0580	0.0144		

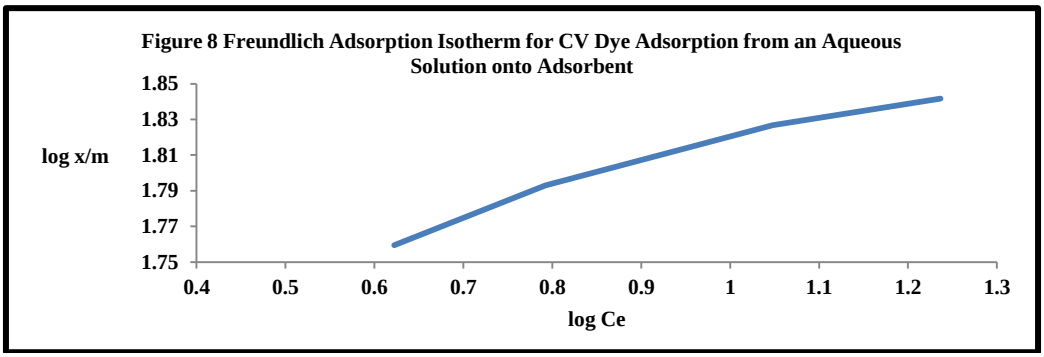


G. Freundlich Adsorption Isotherm

Freundlich adsorption isotherm indicates that the adsorption process occurs on a heterogeneous surface. The linear form of Freundlich adsorption isotherm equation is **log x/m =log K_F+ 1/n log Ce** where the term 'x' denotes the amount of CV dye adsorbed in mg/L, 'm' is weight of adsorbent in mg, 'Ce' is concentration of CV dye at equilibrium, K_F and 1/n are the Freundlich constants related to the adsorption capacity and intensity of adsorption respectively. The value of 1/n (less than 1 obtained in this study) indicates the applicability of Freundlich adsorption isotherm for CV dye adsorption. The data are given in the table 2 at 180 minutes of contact time. Graphically, it is shown in the figure 8.

Table 2 Interpretation of Results of Freundlich Adsorption Isotherm for CV Dye Adsorption from an Aqueous Solution onto Adsorbent

Initial Concentration of CV Dye in mg/L	logCe	logx/m	Intercept	Slope 1/ n
150	0.6223	1.7594	1.6821	0.1331
200	0.7926	1.7931		
250	1.0481	1.8268		
300	1.2365	1.8416		



IV. Conclusion

In this work, batch experiments for the removal of CV dye from an aqueous solution were conducted by varying the parameters such as initial concentration of CV dye, initial pH, adsorbent dosage and temperature. From these studies, we can conclude that activated carbon *Leucaene leucocephala* can be used as a low- cost and eco-friendly carbon material for the removal of CV dye from an aqueous solution.

References

[1] Mona A. Shouman, Soheir A. Khedr and Amina A. Attia, "Basic dye adsorption on low cost biopolymer: kinetic and equilibrium studies," Journal of Applied Chemistry, vol. 2 No. 4, pp. 27-36, 2012.

[2] K. V. Narayana Saibaba, P. King, R. Gopinadh and V. Sreelakshmi, "Response surface optimization of dye removal by using waste Prawn shells," International Journal of Chemical Sciences and Applications. vol. 2, No. 3, pp. 186- 193, 2011.

[3] E. Lorenc-Grabowsk and G. Gryglewic, "Adsorption characteristics of congo red on coal-based mesoporous activated carbon," Dyes Pigment vol. 74, pp. 34–40, 2007.

[4] P. K. Malik and S. K. Saha, "Oxidation of direct dyes with hydrogen peroxide using ferrous ion as catalyst," Sep Puri Technol, vol. 31, pp. 241–250, 2003.

[5] K. S. Bharathi and S. T. Ramesh, "Removal of dyes using agricultural waste as low-cost adsorbents: a review," Appl Water Sci, vol. 3, pp. 773–790, 2013.

[6] Dalia Khalid Mahmoud, Mohamad Amran Mohd Salleh and Wan Azlina Wan Abdul Karim, "Langmuir model application on solid-liquid adsorption using agricultural wastes: Environmental application review," Journal of Purity, Utility Reaction and Environment, vol. 1, No. 4, pp. 200-229, 2012.

[7] M. M. Abd El- Latif, Amal M. Ibrahimand El- Kady, "Adsorption equilibrium, kinetics and thermodynamics of Methylene Blue from aqueous solutions using biopolymer oak sawdust," Journal of American Science, vol. 6, No. 6, pp. 267- 283, 2010.

[8] S. Ansari and Z. Mosayebzadeh, "Removal of Basic dye Methylene Blue from aqueous solutions using sawdust and sawdust coated with polypyrrole," Journal of the Iranian Chemical Society, vol. 7, No. 2, pp. 339- 350, 2010.

[9] Papita Das Saha, Sagnik Chakraborty and Subhabrata Das, "Optimization of hazardous Crystal Violet by chemically treated rice husk: Using central composite response surface methodology," Arch. Environmental Science, vol. 6, pp. 57-61, 2012.

[10] D. K. Singh and B. Srivastava, "Basic dyes removal from wastewater by adsorption on rice husk carbon," Indian Journal of Chemical Technology, vol. 8, pp. 133- 139, 2001.

[11] M. K. Verma and A. K. Mishra, "Kinetic and Isotherm modeling of adsorption of dyes onto rice husk carbon," Global NEST Journal, vol. 12, No. 2, pp. 190-196, 2010.

[12] P. Lalitha, N. Renugadevi and R. Sangeetha, "Kinetics of the adsorption of methylene blue from an industrial dyeing effluent onto activated carbon prepared from the fruits of Mimusops Elengi," Archives of Applied Science Research, vol. 3, No. 3, pp. 492-498, 2011.

[13] S. Wang, Li H, S. Xie, S. Li and L. Xu, "Physical and chemical regeneration of zeolitic adsorbents for dye removal in wastewater treatment," Chemosphere, vol. 65, pp. 82–87, 2006.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Ag/TiO_2 NANOCOMPOSITE: SYNTHESIS, CHARACTERIZATION, PHOTOCATALYTIC AND ANTIMICROBIAL ACTIVITIES

E. Jayanthi[1*], M. Anusuya[1,2] and A. Nagaveni[1]

[1]Department of Chemistry, Kongunadu Arts and Science College (Autonomous) affiliated to Bharathiar University, G. N Mills post, Coimbatore - 641029, Tamilnadu, INDIA.

[2] Department of Chemistry, Nallamuthu Gounder Mahalingam College (Autonomous) affiliated to Bharathiar University, Palakkad Main road, Pollachi - 642001, Tamilnadu, INDIA.

*Corresponding author: E. Jayanthi

E mail: jayakumar.jayanthi@gmail.com, http://www.kongunaducollege.ac.in

Abstract:

In this work, we report the synthesis, photo catalytic and antimicrobial activities of Ag/TiO_2 Nanocomposites prepared by easy, cost effective and less harmful co-precipitation method. The yield of the product is also good when compared to other methods. They were characterized by UV, XRD, FE-SEM and EDAX techniques. The X-ray diffraction (XRD) spectra reveal single phase anatase structure for the samples, formation of uniform spherical Ag doped TiO_2 nanocomposite is ascertained from the surface morphology of FE-SEM. From EDAX, metal composition is observed as Ag-2.33%, 2.72%, 7.90% and Ti-43.62%, 62.30%, 50.97%. The photocatalytic activity of the composite is tested with Rhodamine B dye, Ag-TiO_2 sample degraded the Rhodamine B dye (25ppm) in 150 min indicates good photocatalytic activity. An enhancement in bactericidal activity is also observed against bacterial strains Pseudomonousaeruginosa and serratia with increased Ag substitution. Pseudomonousaeruginosa exhibited good bactericidal effect than serratia.

Keywords: *Nanocomposites, photocatalytic activity, Rhodamine B dye, Pseudomonousaeruginosa, serratia.*

I. Introduction

Water pollution is one of the most leading problems that badly affect the human and aquatic life [1]. Due to Increasing demand and shortage of clean water, the use of clean water and wastewater treatment processes becomes more and more challenged. Water treatment technologies like membrane filtration, advanced oxidation processes (AOPs) and UV irradiation hold great promise to provide alternatives for better protection of public health and the aquatic life [2]. Heterogeneous photo catalyst is recently emerged as an alternative technology of advanced oxidation processes (AOP) for bacteria inactivation [3] and organic pollutants oxidation[4]. Out of the various semiconductor photo catalysts used, TiO_2 (titanium dioxide) (band gap of ~3.2 eV) has been found to be the most suitable because of its nontoxic, insoluble, inexpensive, stable and high production of oxidative hydroxyl radicals (•OH)[5]. TiO_2 exhibits remarkable properties such as hydrophilicity as well as antibacterial and self-cleaning activity. Due to its optical activity, TiO_2 is often used in photo catalytic decomposition and more recently as photo catalyst for the reduction of CO_2 to methanol [6-7]. There are many approaches to synthesize TiO_2 and modified TiO_2 including hydrothermal, electrochemical, microwave, co-precipitation and sol-gel methods [8-9]. Among these methods, the co-precipitation method offers simple methodology, high control of surface area, average crystallite size, phase structure and morphology [10-11]. But the rapid recombination of electron-hole pair limits the efficiency of TiO_2 and it can be prevented by methods like doping, functionalization of the surface with metal particles, and reduction of particle size to the nanoscale.

Considerable research has been carried out to improve the electro catalytic and photo catalytic activity of TiO_2, with metal ion doping [12]. Transition metals doped on TiO_2 play the role of charge carrier trap, which allows for light absorption in the visible range and enhances surface electron excitation by visible-light excited plasmon resonance. TiO_2 doped with noble metal silver (Ag) have shown improved photo catalytic activity compared with its undoped counterpart [13-14]. This is due to the fact that Ag can trap the excited electrons and therefore reducing the recombination rate of photo induced electron-hole pairs. In this work, Ag doped-TiO_2 ($AgTiO_2$) in anatase form was prepared via co-precipitation method. The synthesized Ag-TiO_2 was then investigated for enhanced catalytic activity with regards to the oxygen evolution reaction. Photocatalytic degradation of Nanocomposite is tested for the degradation of organic Rhodamine B dye. It has been reported that doping of Ag can enhance the photocatalytic as well as antibacterial activity of TiO_2 under visible light

illumination by promoting electron–hole separation [15-16]. Bactericidal activity is also observed against bacterial strains *Pseudomonousaeruginosa* and *serratia* with increased Ag substitution.

II. Materials and Methods

Materials: Titanium dioxide (97%) and silver nitrate (99%) were purchased from Aldrich. Rhodamine-B was purchased from Eastman and was of analytical grade and used without further purification. Deionized water was used in all experimental preparations. The samples were prepared by co-precipitation method.

Synthesis Of Ag-TiO_2 Nanocomposite: 20 ml of 3M TiO_2 was taken in 100ml beaker. Then 5 ml of 0.025M ammonium per sulfate and silver nitrate was taken in three different concentrations (0.05M, 0.1M and 0.15M) which were mixed with the above solution. 2.5ml of 0.5M NaOH solution was added to the above solution and stirred on a magnetic stirrer for one hour. After stirring, the solution was kept at 70˚C for 15 minutes and dried the sample. This powder was finely ground using mortar–pestle and kept for further use.

III. Results and Discussion

A. *UV- Visible Spectrum:*

UV spectra of the TiO_2 and Ag-modified TiO_2 samples were recorded in Jasco V-630 spectrometer. TiO_2 spectrum consists of a wide absorption band below ~370nm ascribed to electron transitions from the valence band (VB) to the conduction band (CB). Moreover, an interesting light response was found for Ag-loaded samples. Increase in Ag concentration induces a shift in light absorption to the visible range for wavelengths up to 800nm. UV spectrum of the 0.05% Ag loaded TiO_2 sample did not show a significant increase in visible absorption capacity of the material. In addition, 0.10M & 0.15M Ag-loaded samples shown a shoulder-like peak at ~460nm which has been proposed for Ag nanoparticles inducing visible light absorption. Atoms, ions and clusters of Ag show characteristic absorption peaks with irradiation wavelengths from 190nm to 500nm in UV spectra. The UV spectra clearly shows that when Ag is added to the TiO_2 which can shift the absorption range from UV to visible region.

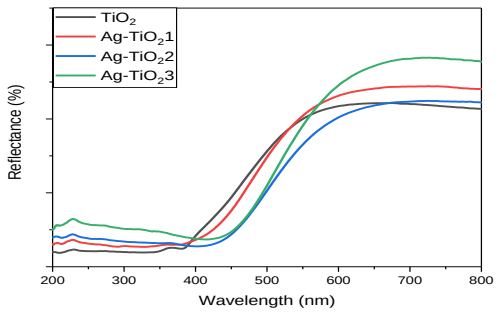


Figure 1. UV Spectra of the TiO_2 and Ag-TiO_2 Samples.

B. *Field Emission Scanning Electron Microscopy Scanning Electron Microscopy (FESEM):*

The figure (2, 3 & 4) shows the FESEM images of freshly synthesized spherical Ag-TiO_2 nanocomposite. It can be observed that the Ag-TiO_2 nanocomposite with pores in the nano range which exist in contact with each other and diameters were also in the nanometer range.

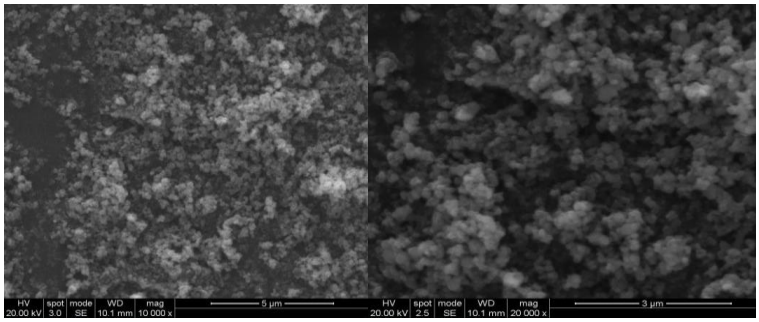


Figure.2 FESEM Images for Ag-TiO_2 (0.05M)

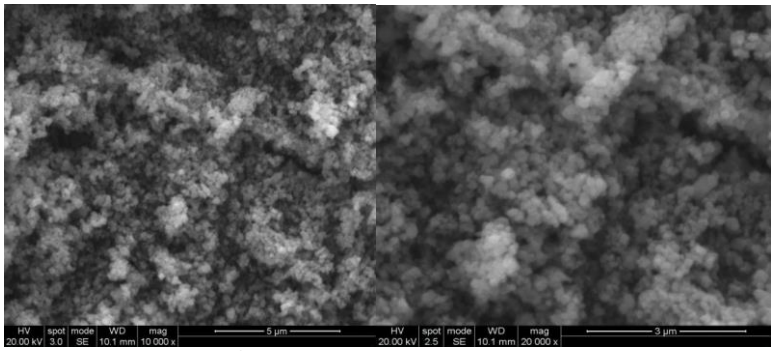


Figure.3 FESEM Images for Ag-TiO_2 (0.10M)

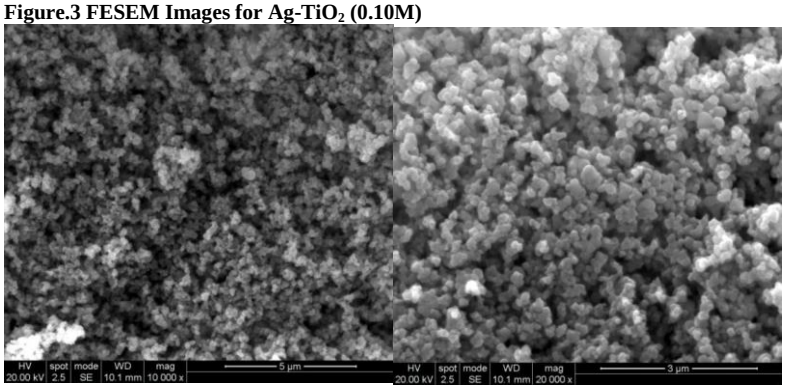


Figure.4 FESEM Images for Ag-TiO_2 (0.15M)

C. Energy Dispersive Analysis Of X-Rays (EDAX)

EDAX spectrum showed the presence of elemental silver and titanium shown in Figure. (5, 6 & 7) and metal composition, i.e., Ag-2.33%, 2.72%, 7.90% and Ti-43.62%, 62.30%, 50.97% were observed. It also showed the absence of any other impurity in the synthesized samples.

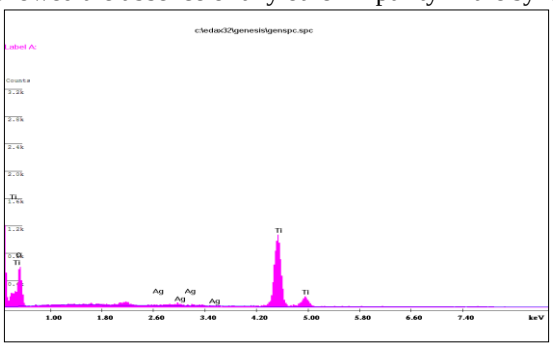


Figure.5 EDAX Images for Ag-TiO_2 (0.05M)

Element	Wight (%)
O	54.05
Ag	2.33
Ti	43.62
Total	100.00

Table 1 EDAX Analysis for Ag-TiO_2 (0.05M)

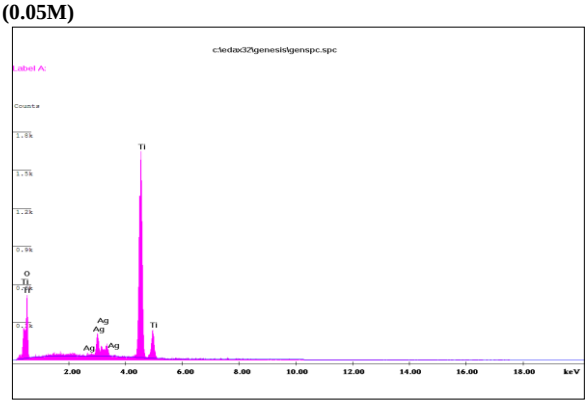


Figure.6 EDAX Images for Ag-TiO_2 (0.10M)

Element	Wight (%)
O	34.98
Ag	2.72
Ti	62.30
Total	100.00

Table. 2 EDAX Analyses for Ag-TiO_2 (0.10M)

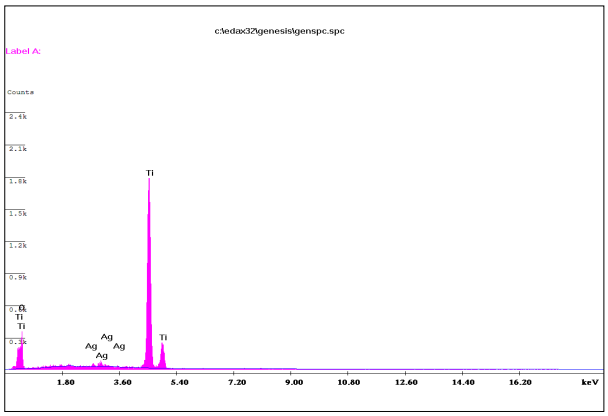


Figure.7 EDAX Images for Ag-TiO_2 (0.15M)

Element	Wight (%)
O	41.14
Ag	7.90
Ti	50.97
Total	100.00

Table. 3 EDAX Analyses for Ag-TiO_2 (0.15M)

D. X-Ray Diffraction (XRD)

The XRD pattern of Ag-TiO_2 contras the formation of dual phases including the anatase phase of TiO_2 and the face centered cubic (FCC) lattice of Ag. The Ag exhibits 5 major peaks at 2θ values of 22.7, 37.8, 48.15, 62.6 and 75.2 degrees, assigned to the corresponding (101), (111), (200), (220) and (311) indices respectively. Five peaks indexed at (101), (111), (200), (220) and (311) matches well with JCPDS card (89-4837) of Ag-TiO_2 nanocomposite. From the Figure (8, 9 & 10) clearly shows there is no other phase in XRD except anatase.

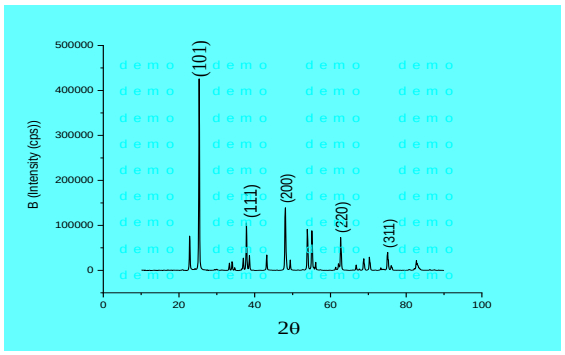


Figure.8 XRD Images for Ag-TiO_2 (0.05M)

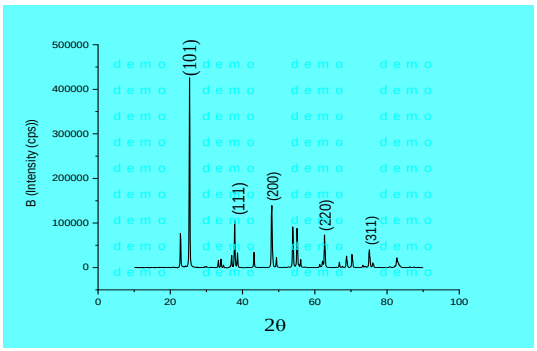


Figure.9 XRD Images for Ag-TiO_2 (0.10M)

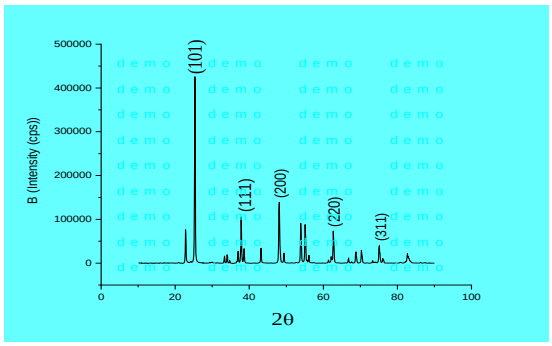


Figure.10 XRD Images for Ag-TiO_2 (0.15M)

PHOTOCATALYTIC ACTIVITY

25 mg of Ag-TiO_2 (0.05 M, 0.10 M and 0.15 M) was taken in 25 ml of Rhodamine-B. Control is taken along with the Ag-TiO_2 naocomposites. After 2 minutes sonication samples were kept in dark room. Then UV was taken for all those samples after 150 minutes degradation and with the time interval of 30 minutes. Figure.11 shows the UV spectrum of Rhodamine-B. Figure 12, 13 and 14 shows the UV-spectra of Ag-TiO_2 loaded

Rhodamin-B. 25 ppm of Rhodamine-B dye was prepared and the reading was noted by using UV-spectrophotometer, which is shown in figure.15, From the figures, it is clear that the Ag-TiO_2 concentration increases, degradation ability also increases Ag-TiO_2 (0.15 M) loaded in Rhodamine-B got degraded completely at 150 minutes time interval. Thus it can act as a very good photocatalyst.

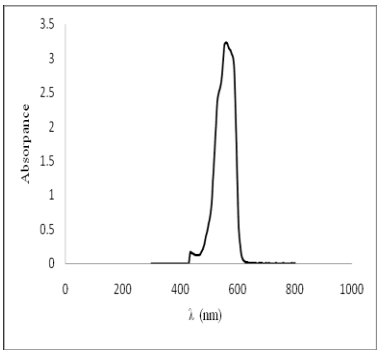


Figure.11Photo Catalytic Activity for Rhodamine-B

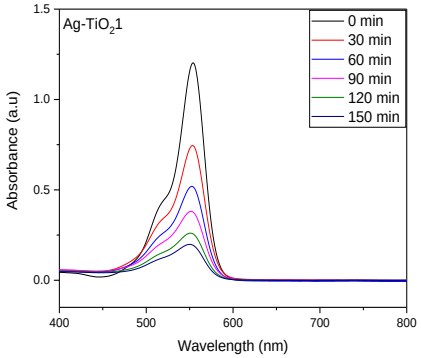


Figure.12 Photo Catalytic Activity for Ag-TiO_2 (0.05M)

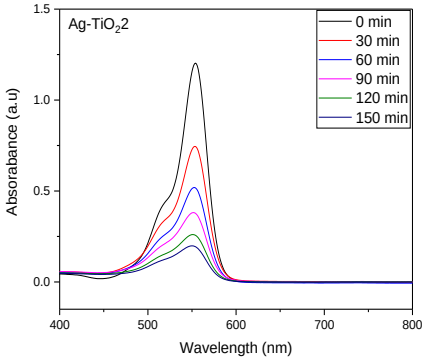


Figure.13 Photo Catalytic Activity for Ag-TiO_2 (0.10M)

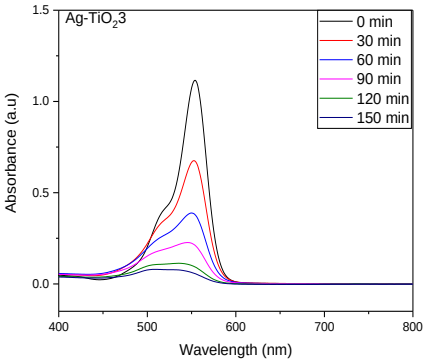


Figure.14 Photo Catalytic Activity for Ag-TiO_2 (0.15M)

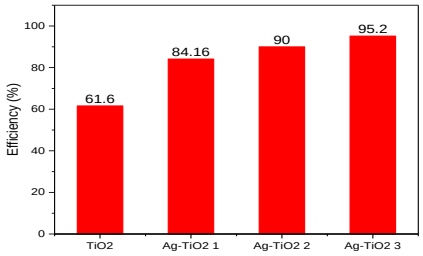


Figure.15 Photo Catalytic Efficiency for Ag-TiO_2 (0.05M, 0.10M and 0.15M)

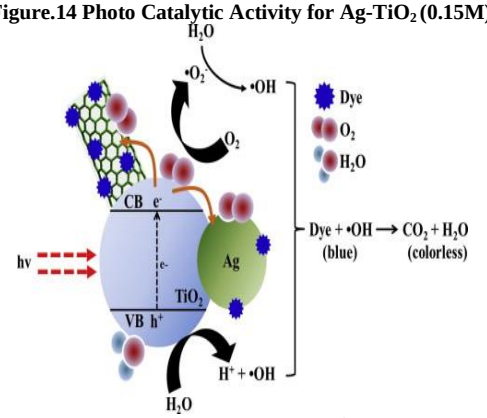


Figure.16. Mechanism of Photo Catalytic Activity.

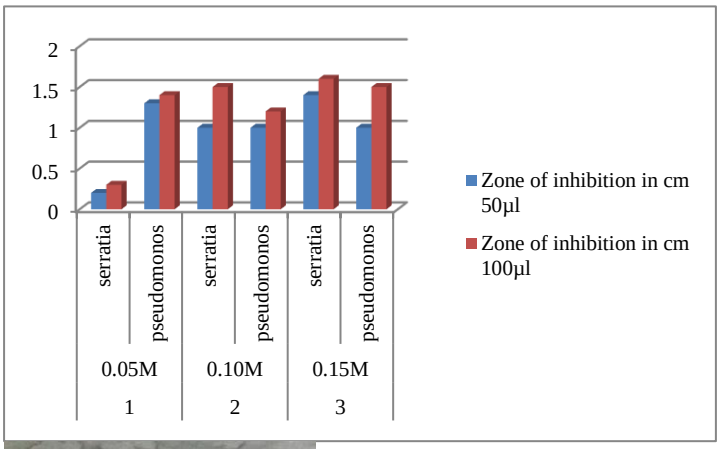
ANTIBACTERIAL ACTIVITY

The antimicrobial activity has been observed to vary as a function of surface area in contact with the microbe, therefore nanocomposite with large surface area ensures a broad range of reactions with the bacterial surface. *Serrtia* and *Pseudomonas aeruginosa* microbial cultures were subjected to analyze their susceptibility/ resistance pattern to test samples by well diffusion method using Mueller Hinton agar medium for bacteria. Sterile medium was dispensed into sterile Petri dishes aseptically. Enriched broth cultures (24 h for bacteria culture, incubated) were used as inoculums. Using sterile cotton swab, the test organisms were swabbed over the surface of the agar plate aseptically. In each of these plates, wells (10 mm) were cut out using sterile cork borer. The samples were dissolved in the acetone and different concentration (50 & 100 g /ml) of the sample was loaded onto the wells. Incubated the plates at 37^0 C (for 24 h for bacteria) upright position of the plates. Solvent was used as control. After the incubation, the diameters of inhibition zones were observed. The inhibition zone

was compared with the control sample. From the result (table 4. ;Figure 17-20), it is observed that prepared Ag-TiO_2 nanocomposites showed good antibacterial activity. Also when concentration of Ag increases antibacterial activity also increases. The antibacterial activity of Ag-TiO_2 nanocomposites against *pseudomonas* is better than the activity against *serratia*.

Sl. No.	Nanocomposite (Ag-TiO_2)	Name of Organism	Zone of inhibition in cm	
			50µl	100µl
1	0.05M	Serratia	0.2	0.3
		Pseudomonas	0.5	0.8
2	0.10M	Serratia	0.8	1.3
		Pseudomonas	1.0	1.4
3	0.15M	Serratia	1.0	1.6
		Pseudomonas	1.5	1.8

Table.4 Antibacterial Activity of Ag- TiO_2 Nanocomposite





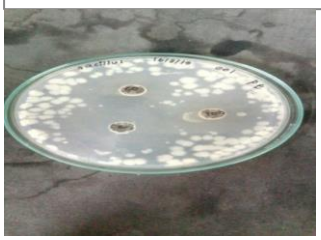


Figure.16 Antibacterial Activity of Ag-TiO_2 (0.05M, 0.10 M an0.15M)

Figure.17 Antibacterial Activity of Ag-TiO_2 (0.05M)

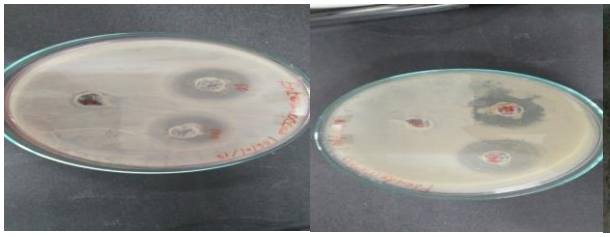


Figure.18 Antibacterial Activity of Ag-TiO_2 (0.10M)



Figure.19 Antibacterial Activity of Ag-TiO_2 (0.15M)

CONCLUSION

In this work we synthesized Ag-TiO_2 nanocomposite by easy, cost effective, less harmful, less timing and good yield co-precipitation method. Ag doped TiO_2 nanocomposites were prepared by varying the concentration of Ag. Nano composites were characterized by UV, XRD, FESEM and EDAX. UV spectrum ascertained the formation of Ag doped TiO_2 nanocomposites which is evident from the shift in energy band gap changed from UV to Visible. The X-ray diffraction (XRD) spectra reveal single phase anatase structure for the samples with

20nm size, formation of uniform spherical Ag doped TiO_2 nanocomposite is ascertained from the surface morphology of FE-SEM. From EDAX, metal composition, i.e., Ag-2.33%, 2.72%, 7.90% and Ti-43.62%, 62.30%, 50.97% were observed. The antibacterial activity of the prepared sample exhibited good antibacterial effect on *Pseudomonosaeruginosa* than *serratia*. From photo catalytic study, it was observed that the prepared Ag-TiO_2 sample degraded the Rhodamine B dye (25ppm) in 150 min. Thus Ag-TiO_2 can act as a good photo catalyst and good antibacterial agent.

REFERENCES

[1] T. Ali, A. Ahmed, U. Alam, I. Uddin, P. Tripathi, M. Muneer "Enhanced photocatalytic and antibacterial activities of Ag-doped TiO_2 nanoparticles under visible light" Materials Chemistry and Physics, Vol. 212, 2018, 325-335,.

[2] V. K. Gupta, I. Ali, T. A. Saleh, A. Nayak, S. Agarwal, "Chemical treatment technologies for waste-water recycling—an overview", RSC Advances, Vol. 2, 2012, 6380–6388.

[3] A. Matilainen M. Sillanpaa "Removal of natural organic matter from drinking water by advanced oxidation processes" Chemosphere Vol. 80, 2010, 351-365.

[4] E. Friedler, Y. Gilboa, "Performance of UV disinfection and the microbial quality of grey water effluent along a reuse system for toilet flushing", Science of The Total Environment, Vol. 408, 2010, 2109– 2117.

[5] O. Carp, C.L.Huisman, A. Reller, "Photoinduced reactivity of titanium dioxide" Progress in Solid State Chemistry, Vol. 32, 2004, 33-177.

[6] S. N. Habisreutinger, L. S. Mende, J. K. Stolarczyk, "Photocatalytic Reduction of CO_2 on TiO_2 and Other Semiconductors" Angewandte Chemie, Wiley Online Library Vol. 52, 2013, 7372-7408.

[7] D. Gumy, A. G. Rincon, R. Hajdu, C. Pulgarin, "Solar photocatalysis for detoxification and disinfection of water: Different types of suspended and fixed TiO_2 catalysts study, Solar Energy, Pure and Applied Chemistry", vol. 80, 2006, 1376-1381.

[8]J. D. Cressler, Silicon heterostructure handbook:" Materials, Fabrication, Devices, Circuits, and Applications of SiGe and Si Stained-Layer Epitaxy", Journal of applied chemistry, 2006.

[9] S. M. Gupta, M. Tripathi "A review on the synthesis of TiO_2 nanoparticles by solution route" Central European Journal of Chemistry, Vol. 10, 2012, 279–294.

[10] K.M. Parida, B. Naik, "Synthesis of mesoporous $TiO_2 - {}_xN_x$ spheres by template free homogeneous co-precipitation method and their photo-catalytic activity under visible light illumination" Journal of Colloid and Interface Science, Vol. 333, 2009, 269-276.

[11]D. Wang,T. Xie, Y. Li, "Nanocrystals: Solution-based synthesis and applications as nanocatalysts" Nano Research,Vol. 2, 2009,30–46.

[12] D. Beydoun, R. Amal, G. Low, S. McEvoy, "Role of Nanoparticles in Photocatalysis" Journal of Nanoparticle Research, Vol.1, 1999, 439–458.

[13] C. He, Y. Yu, X.Hu, "Influence of silver doping on the photocatalytic activity of titania films" Applied Surface Science, Vol. 200, 2002, 239-247.

[14] M. I. Din, R. Khalid,Z. Hussain, "Minireview: Silver-Doped Titanium Dioxide and Silver-Doped Zinc Oxide Photocatalysts" Analytical Letters, Vol. 51, 2018, 892-907.

[15] P. A. Charpentier, C. Chen, K. Azhie, B. Grohe, M. A. Mumin, A.F. Lotus, P. Therrien, S. Mittler "Photocatalytic and antibacterial activities of silver and iron doped titania nanoparticles in solution and polyaspartic coatings" Nanotechnology, Vol. 30, 2008 https://orcid.org/0000-0003-1717-1538.

[16] F. Jing, H. Suo, S. Cui, X. Tang, M. Zhang, X. Shen, B. Lin, G. Jiang, X. Wu, "Facile synthesis of TiO2/Ag composite aerogel with excellent antibacterial properties", Journal of Sol-Gel Science and Technology, Vol. 86, 2018, 590–598.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Optimizing the activation process of red mud for cadmium (II) adsorption - Comparison of the results from response surface methodology and artificial neural network

C. Maheswari, Anjali Dasari

Department of Chemical Engineering, Anil Neerukonda Institute of Technology, Sangivalasa, Visakhapatnam-531162, Andhra Pradesh, India. maheswari.che@anits.edu.in

Abstract:

Response surface methodology (RSM) is the generally used as statistical tool to model and analyze the experimental data. Recently, artificial neural network (ANN) is attracting the attention of researchers to predict the results accurately. In the present work, RSM and ANN were used as modelling tools for evaluating and predicting the results of activation process of red mud. calcination temperature, calcination time, acid concentration and activation time were taken as parameters for activating the red mud. The activated red mud (industrial waste) was used as adsorbent to remove Cadmium (II) from aqueous solution. The effect of these parameters on activation of red mud for adsorption of cadmium was studied through four factor, two level Central Composite Design (CCD). The same set of generated data from CCD was utilized for ANN. Regression co-efficient (R^2), root mean square error (RMSE) of the two methodologies were compared to predict the capability of adsorption. The results show that the ANN model is more accurate in prediction than RSM.

Key words: *RSM, CCD, ANN, red mud, Cadmium (II).*

I.Introduction

Cadmium (II) is one of the highly toxic heavy metal to human, plants and animals. The metal is of special concern because of its non-degradable nature. The main pathway of cadmium into the environment is as a waste from industrial processes like electroplating, smelting, alloy manufacturing, pigments etc. According to WHO's recommendation Cd (II) limit in drinking water is 0.005 mg/l and 0.1 mg/l for discharge water from industries. Many removal techniques like cementation, chemical precipitation, ion exchange, solvent extraction, membrane separation and adsorption are available [3]. Adsorption is one of the most studied techniques among all the available techniques because of its easy availability and low-cost [35]. The main categories of adsorbent are activated carbon [25], agricultural waste [22].

Utilization of industrial wastes for the removal of heavy metals from waste water is an effective way. Among the industrial wastes, red mud has dragged the attention of researchers these days. Red mud is a solid waste residue generated from bayers process of bauxite ores in the production of alumina. In global scenario, annually 90 million tonnes [29] of red mud is being released into the environment. Red mud is highly alkaline with pH ranging from 10 to 13[7]. Due to its high sodium and calcium hydroxide content, red mud is considered to be highly hazardous to the environment. So, the management of red mud is a serious issue. Many of the alumina industries are disposing the red mud in landfills which is not an effective way of management. In recent years, many research studies have been done to utilize the red mud as building material, in metal recovery [30], ceramic production [31, 32], as an adsorbent in waste water treatment and toxic gas cleaning, and also in catalyst development in solid waste and gas catalysis [33]. Out of these applications, studies on adsorption are motivated because of the fact that red mud contains very fine particles of metal ions and organics, which makes it an effective adsorbent for heavy metals.

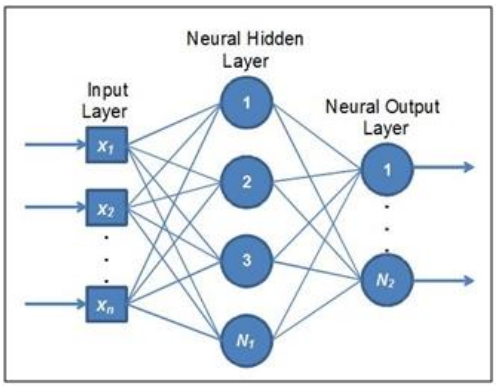
In recent years, the usage of statistical design and analysis of experimental data became popular as it allows one to obtain conditions through a relatively smaller number of systematic experiments. Generally an experimental design includes coefficient estimation from a mathematical model, response prediction and checking of suitability of the model. Response surface methodology is one among the most used modelling tool to design and analyse the experimental data. A central composite design (CCD) is a tool in response surface methodology to design the experimental data and for building a second order (quadratic) model for the response variable without using a complete full factorial experiment. After the design matrix is performed, linear regression is used, sometimes iteratively, to obtain results. The model for the response variable can be written as

$$Y = b_0 + \Sigma b_i X_i$$

Where Y is response, X_i are coded factors, b_i are coefficients of factors and b_0 is the experimental error.

Alternatively in recent years, artificial neural networks (ANN) emerged as a highly effective tool to analyse and predict the experimental data. ANN is a complex network system involving many simple processing elements that can establish complex interrelationships between independent and dependent variables [28]. In general, ANN is a biologically inspired neural net that tries to follow the neurological ability of brain. A basic ANN is a parallel network of inputs (weights), processing transfer function (nodes) and outputs (responses) which makes it an unique computational technique when compared to conventional tools. Typical ANN includes an input layer which is connected to the environment, hidden layer which processes the weights using a non linear function and produces the responses that will be represented by output layer. Each processing element in the hidden layer will receive multiple inputs and produces multiple outputs.

Fig. 1: Typical ANN model



Artificial neural networks have been trained to perform complex functions in various fields including modelling, pattern recognition, identification, classification and control systems. In the past, the similar studies have been done to optimize cadmium adsorption by Shelled Moringa Oleifera Seed [24], Sargassum filipendula species [34]. There are no reports on approach how to model the experimental data of red mud activation for cadmium (II) adsorption using ANN. The present study features the RSM based experimental design as per CCD and the comparison of RSM with ANN results to predict the suitability of models in the activation of red mud for adsorbing cadmium (II).

II. Materials and methods:

Red mud and reagents:

The Red Mud used in the present study (composition: Fe_2O_3- 42%, Al_2O_3-20%, TiO_2-9%, SiO_2-10-12%, Na_2O-4-5%) was obtained from Hindalco Aluminium Industry, Belgaum, India. Cadmium of analytical reagent grade was used. The cadmium concentration was measured using atomic absorption spectrophotometer (AAS). All the chemicals used were of analytical reagent grade. The glass ware used in the study was soaked in dilute HNO_3 and cleaned using distilled water before use.

Stock solution and adsorbent preparation:

1 g of Cadmium was dissolved in a 10 ml 1:1 HNO_3. The solution was then transferred to a 1000 ml standard flask and it was diluted to the mark with distilled water to make 1000 ppm Cd stock solution.

Red mud was washed thoroughly with distilled water and dried at 115 ^oC for 24 hrs and was grinded using ball mill and sieved for 60 mm mesh size. The obtained redmud was acid treated with conc. H_2SO_4 in the ratio of 1:2 (redmud: H_2SO_4) i.e., 25 g of oven dried red mud was taken in a beaker and was suspended in 50 ml of 1N H_2SO_4 solution. The suspension was then stirred for 30 minutes and washed thoroughly with distilled water several times and made to neutral pH. The treated red mud was filtered using whatman no.1 filter paper and the mud cakes were dried at 100^oC over night and stored in sealed covers until using in the experiment.

III.Experimental studies

Batch adsorption studies:

The acid treated redmud was calcinated by varying calcination temperature, calcination time, acid concentration, activation time. Batch adsorption experiments were carried out in 100 ml conical flask containing the aqueous cadmium solution of the desired concentration and the known amount of activated red mud. Initial pH was adjusted to 6 with 1 N Hcl or 1 N NaOH solutions. The solution was agitated at constant speed of 150 rpm and at room temperature till the equilibrium condition was reached. The cadmium solution was then filtered and the cadmium concentration of supernatant was determined by using AAS. Experiments were performed at

different calcination temperatures in the range of 100-800ºC, calcination time 2-7 hr, acid concentration 0.5-2.5 N, activation time 0.5-2.5 hr. All the experiments were conducted for 10 ppm cadmium at pH 6 by adding 1 g/l activated red mud. The percentage adsorption of cadmium on red mud can be found from the equation

$$q = [(C_i - C_e)/C_i]*100$$

Where, C_i is the initial cadmium concentration (mg/l), C_e is the concentration of cadmium when equilibrium is reached.

Effect of Calcination Temperature and Calcination time on activation of red mud

The red mud powder was heated from 200 ºC to 800 ºC to study the effect of heating time on adsorption capacity. The results in Figure 2 showed that increasing the temperature will lead to an increase in the capacity of the adsorbent until 500 ºC. Adsorption capacity reached a critical value around 600 ºC and after that it declined. The internal and external surface area of red mud powder increases when the temperature is increased. However, after reaching the critical value, higher temperatures may break down certain substances in the mineral structure of red mud and the sintering process occurs, which will decrease adsorption capacity. Therefore, the heating temperatures investigation range is from 500 to 600 ºC for CCD studies. The experiments were conducted at constant calcination time 4 hr, acid concentration 1.5 N H_2SO_4 and activation time 1 hr.

Effect of calcinations time in was shown in Figure 3, that the heating times have no considerable influence in the range of 2 to 8 hours. At 4 hours adsorption capacity reach the highest value. The experiments were conducted at constant calcination temperature 500 ºC, acid concentration 1.5 N H_2SO_4 and activation time 1 hr.

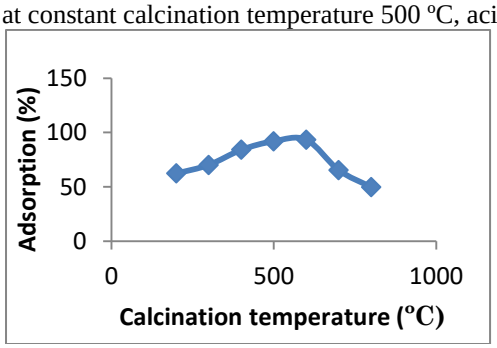


Fig. 2. Effect of calcination temperature.

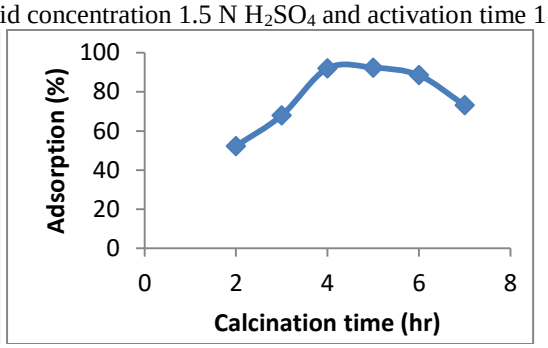


Fig. 3. Effect of Calcination time.

Effect of acid concentration and activation time on activation of red mud

The effect of acid concentration on activation of red mud was shown in Figure 4. The adsorption capacity reached the highest value at 1N of acid concentration and then it decreased. Because when increasing acid concentration as shown in Figure. 4, large amount of aluminium, iron were dissolved by H_2SO_4 and removed from the structure of red mud and specific surface area was increased. But further increasing the value of acid concentration will lead to the decrease of adsorption capacity due to the dissolution of Goethite (α -Fe2O3.nH2O) which adsorbs heavy metals well. The value of acid concentration's study range is 0.5 N – 1.5 N. The experiments were conducted at constant calcination time 4 hr, Calcination temperature 500 °C, and activation time 1 hr.

Effect of increasing the activating time in Figure 5, initially led to rise in adsorption capacity but the highest value of adsorption capacity reached at 1.5 hours then it went down and remained flat. This may be due to most of the ions in red mud reacted with acid and were dissolved, therefore the specific surface area and internal space were increased.

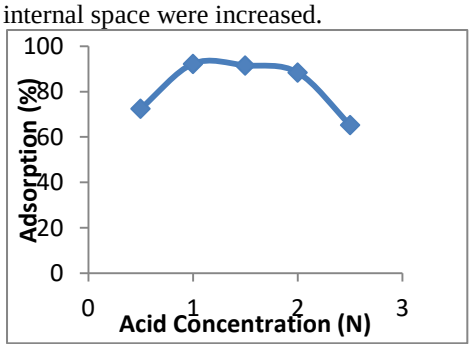


Fig. 4. Effect of Acid Concentration.

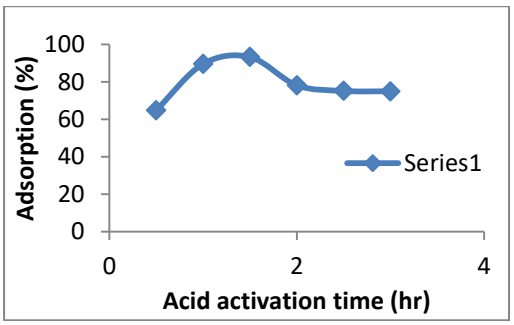


Fig. 5. Effect of acid activation time.

Response surface methodology:

The use of statistical design experiments is advantageous as it allows one to obtain conditions through

a relatively smaller number of systematic experiments. The interaction effects of two or more variables can also be known in RSM, which is not possible in a classical experiment. In order to examine the main factors and their interactions for the cadmium adsorption, Central Composite design (CCD) was used. Therefore set of experiments consisting of 31 sets were designed based on central composite design for four input variables (factors) in two levels (high, low) for adsorption of cadmium on activated red mud using Minitab 14. The range of factors for CCD was fixed by previously performed batch experiments. The factors and levels used in the study are presented in Table 1, gives the conditions for 31 experimental runs designed as per CCD. Thus, the equation giving Cadmium adsorption capacity on red mud, as a function of the factors are presented using a second-order polynomial model with 15 coefficients (b_0,b_1, b_{12}....b_{34})

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4 + b_{11} X^2_1 + b_{22} X^2_2 + b_{33} X^2_3 + b_{44} X^2_4 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{14} X_1 X_4 + b_{23} X_2 X_3 + b_{24} X_2 X_4 + b_{34} X_3 X_4$$

Where X_1, X_2 ,X_3 and X_4 are coded factors studied.

Table 1. Factors and levels used in the factorial design study.

Factors	-1	+1
X_1	300	700
X_2	3	6
X_3	1	2
X_4	1	2

Artificial neural network:

For simulation in Artificial Neural Network, Toolbox 7.10 of MATLAB (R2010a) had been used. The same set of design data that was generated in RSM had been employed here. The input parameters calcination temperature (X_1), calcination time (X_2), acid Concentration (X_3) and activation time (X_4) are introduced into the hidden layer through weights. The neurons in the hidden layer sums up the weighted inputs to neurons including bias as

$$net = \Sigma w_i X_i + b$$

Where w_i are the weights, X_i are the input parameters and b is the bias. The weights are then passed through a transfer function. The transfer function used in the current work for hidden layer is 'tansig'

$$\Phi(net) = 1/[1+\exp(-net)]$$

The response from the hidden layer is now shifted to the output layer where the neurons perform the same work as they done in the hidden layer. The transfer function used in the cuurent work for output layer is 'purelin'. The same function had been employed in previous studies [17, 26, 27]. Percentage of adsorption was used as the target data in ANN. The data was randomly divided as 70% in training set, 15% in validation set and 15% in test set. The network training function was selected as 'trainlm' because of the fact that this function updates weight and bias values according to the Lavenberg-Marquardt(LM) algorithm. The LM algorithm is an iterative technique that locates the minimum of a function that is expressed as the sum of squares of nonlinear functions. It is a standard technique for nonlinear least-squares problems and can be assumed as a combination of steepest descent and the Gauss-Newton method [36]. The Root mean square error (RMSE) is now formulated depending on the output and the actual experiment.

$$RMSE = \sqrt{\left(\sum_{i=1}^{n} (Y_{ex} - Y_p)^2\right)/n}$$

Where Y_p is the predicted response and Y_{ex} is the experimental response.

IV. Results and discussion

Response surface methodology:

31 Experiments shown in Table 3 designed as per CCD matrix were conducted to study the effect of different factors influencing the adsorption capacity as an individual and on interaction with each other. The matrix of four variables Calcination temperature (X_1), Calcination time (X_2), Acid concentration (X_3) and Activation time (X_4) were varied. The higher level of variable was designed as '+' and the lower level was designed as '−'. Cadmium removal percentage obtained from the experiments at the end of 1400 minutes (equilibrium time) for each of the experiments is shown in Table 3. The obtained RSM regression equation is as follows

$$\textbf{\% Adsorption} = 92.6 - 7.6458 X_1 + 5.6458 X_2 - 1.2208 X_3 + 0.0625 X_4 - 13.5448 X^2_1 - 9.8448 X^2_2 - 7.4073$$

X^2_3 - 7.1823X^2_4 + 0.8937 $X_1 X_2$ - 0.1938 $X_1 X_3$ + 0.0313 $X_1 X_4$ + 0.2187 $X_2 X_3$ + 0.5688 $X_2 X_4$ + 0.3313 $X_3 X_4$

Table 2. Estimated regression coefficient and corresponding t- and p- value for Cadmium

Term	Coefficients	Standard error	T-value	P-value
Constant	92.6000	2.280	40.620	0.000
calc. temp.	-7.6458	1.231	-6.210	0.000
calc. time	5.6458	1.231	4.586	0.000
acid conc.	-1.2208	1.231	-0.992	0.336
activating time	0.0625	1.231	0.051	0.960
calc. temp.*calc. temp.	-13.5448	1.128	-12.009	0.000
calc. time*calc. time	-9.8448	1.128	-8.728	0.000
acid conc.*acid conc.	-7.4073	1.128	- 6.567	0.000
activating time*activating time	-7.1823	1.128	- 6.368	0.000
calc. temp.*calc. time	0.8937	1.508	0.593	0.562
calc. temp.*acid conc.	-0.1938	1.508	0.128	0.899
calc. temp.*activating time	0.0313	1.508	0.021	0.984
calc. time*acid conc.	0.2187	1.508	0.145	0.886
calc. time*activating time	0.5688	1.508	0.377	0.711
acid conc.*activating time	0.3313	1.508	0.220	0.829

S = 6.031 R-Sq = 94.9% R-Sq(adj) = 90.5%

Multiple Regression analysis (MRA) of the experimental data was performed based on ANOVA using RSM with MINITAB 14. Multiple regression models relating the factors to the response were developed with the regression coefficients in the form of uncoded factors was shown in Table 2. The MRA model developed was shown in equation. The results of ANOVA on main and interaction effect were shown in Table 2. ANOVA showed that the main effects such as calcination temperature, calcination time, acid concentration and activation time are terms and two-ways interactions were highly significant ($P < 0.05$) and model was applicable very well. The P-value was very important to determine if the model terms are significant or not. The correlation coefficient (0.949) was high showing good fitness of the statistical model. Predicted values of percentage removal for all the 31 experiments are shown in Table 3 and were found to agree well with the experimental values. RSM analysis for adsorption cadmium using red mud indicated that the second-order polynomial model was highly significant and adequate to represent the actual relationship between the response (percentage removal efficiency) and the variables, with very small P -value (0.000) and a high value of coefficient of determination ($R^2 = 0.949$).

Optimization of factors was carried out based on the data using "Response optimizer" tool of MINITAB 14. The optimum values for calcination temperature, Calcination time, acid concentration and activation time are 415.92 ºC, 4.99 h, 1.438 N and 1.336 h respectively.

Artificial neural networks:

Trail and error method was employed to determine the number of neurons in the hidden layer with an initial value of 2 neurons. The model was performed depending on the minimum value of Mean square value. After a certain trails, it was found that model is best at 12 hidden neurons with an MSE of 0.005. The regression analysis was performed on the experimental data and the ANN output. It was found that the model is best with regression coefficient of 0.998, 0.968, 0.995 and 0.989 for training, validation, testing and all data respectively. Predicted values from ANN are shown in Table 3 and they were found to agree well with the experimental values.

Table 3. Validation data

Run	Calcination temperature, ◦C	Calcination time, hr	Acid concentration, H_2SO_4 (N)	activating time, hr	% Removal		
					Experimental	Predicted RSM	Predicted ANN
1	500	4.5	1.5	1.5	92.6	92.6	91.84
2	700	3.0	1.0	1.0	38.5	42.875	34.66
3	700	6.0	1.0	1.0	55.3	54.379	55.16
4	500	4.5	0.5	1.5	72.5	65.412	71.99
5	900	4.5	1.5	1.5	26.4	23.129	24.72

6	300	3.0	2.0	2.0	48.8	56.062	56.24
7	300	6.0	1.0	1.0	64.7	67.558	63.07
8	300	3.0	2.0	1.0	54.7	56.475	55.62
9	500	4.5	1.5	0.5	64.2	63.746	64.21
10	500	4.5	1.5	1.5	92.6	92.6	91.84
11	500	4.5	1.5	2.5	75.4	63.996	73.23
12	700	6.0	1.0	2.0	51.3	55.042	53.68
13	500	1.5	1.5	1.5	48.6	41.929	37.95
14	300	6.0	1.0	2.0	61.5	68.096	62.82
15	300	6.0	2.0	1.0	62.1	65.279	62
16	500	4.5	2.5	1.5	65.3	60.529	74.82
17	700	6.0	2.0	2.0	48.2	53.312	49.45
18	700	6.0	2.0	1.0	50.8	51.325	49.83
19	100	4.5	1.5	1.5	62.3	53.713	60.23
20	500	4.5	1.5	1.5	92.6	92.6	91.84
21	700	3.0	2.0	1.0	39.2	38.946	40.85
22	500	4.5	1.5	1.5	92.6	92.6	91.84
23	300	6.0	2.0	2.0	66.0	67.142	65.01
24	500	7.5	1.5	1.5	69.7	64.813	66.36
25	700	3.0	2.0	2.0	36.0	38.658	31.38
26	300	3.0	1.0	2.0	52.9	57.892	53.34
27	500	4.5	1.5	1.5	92.6	92.6	91.84
28	500	4.5	1.5	1.5	92.6	92.6	91.84
29	500	4.5	1.5	1.5	92.6	92.6	91.84
30	300	3.0	1.0	1.0	58.4	59.629	58.25
31	700	3.0	1.0	2.0	38.1	41.263	37.32

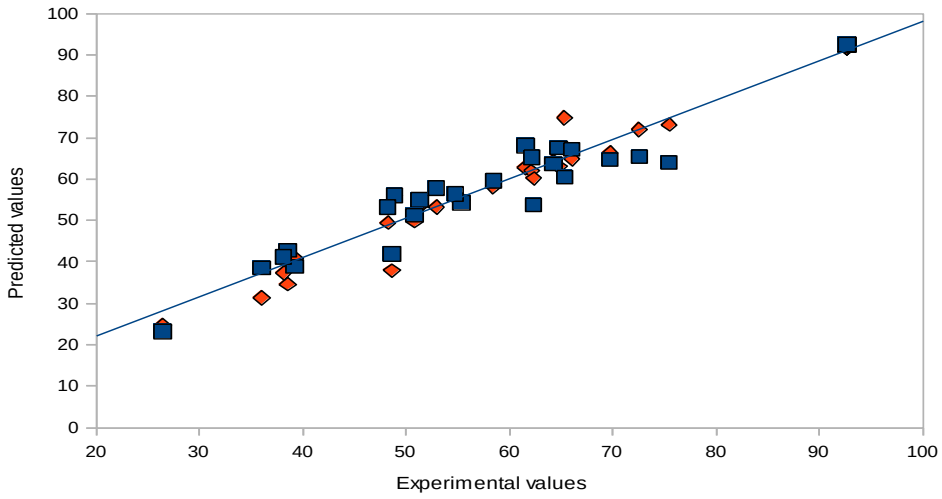
Comparison of RSM and ANN:

RSM and ANN have been compared for percentage adsorption in terms of Regression co-efficient (R^2), root mean square error (RMSE) and the values are presented in the Table 4. Predicted values for both the methodologies were plotted against the experimental values and the deviation is presented in the Figure 6. From the results, it is evident that the ANN is accurate when compared to RSM even for a small set of experimental data.

Table 4. Comparison of RSM and ANN

Parameters	RSM	ANN
R^2	0.949	0.968
RMSE	4.32	3.32

Fig. 6: Predicted values of RSM and ANN against experimental values.

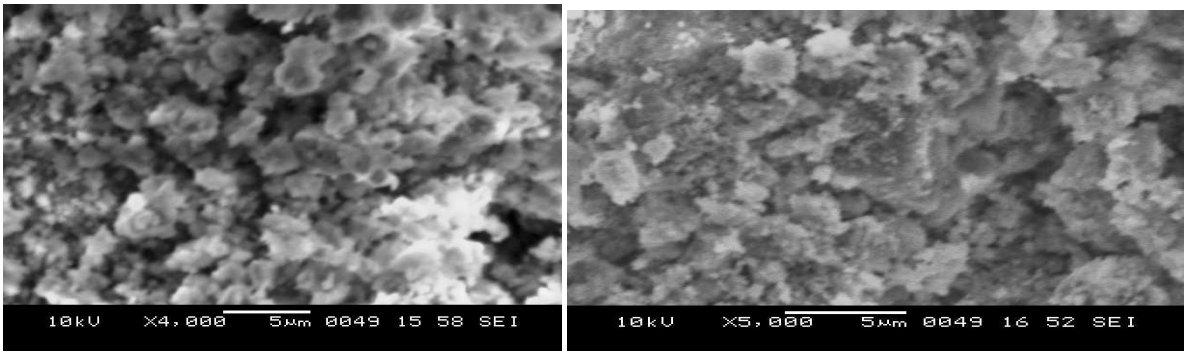


□-RSM ◊-ANN

Characterization studies

Raw red mud is found to be a complex mixture of phases mainly comprised of hematite (Fe_2O_3), Goethite (FeOOH), Gibbasite ($Al(OH)_3$), Anatase (TiO_2) and quartz (SiO_2). SEM EDAX of the surface of the red mud activated by different pretreatment methods was compared with that of raw red mud by scanning electron microscopy. The SEM-EDAX micrograph of different acid treated samples and raw red mud are shown in the Figures 7 and 8 respectively. The SEM analysis shows that the intensity for metals like Al, Si, Ti and Fe in raw red mud are very high. The intensities of elements like Al, Si, Ti and Fe in raw red mud are 60, 25, 10 and 15 respectively. Acid leaching of red mud has resulted in drastically decreased intensities for Fe, Ti, Si and Al.

Similar results were obtained by (Agatzini et al., 2008) and (Uzun et al., 2007).The acid treatment leads to dispersion of dissolved metal oxides as hydroxides which leads to formation of pores. On acid treatment and calcination, the adsorbed salts and exchangeable cations are dissolved first, and then most of the aluminium ions and other metallic ions are removed from the lattice of structure so that many micropores are introduced (Shing et al., 1997). Decreased metal intensities on acid treatment confirm removal of cations from the surface.



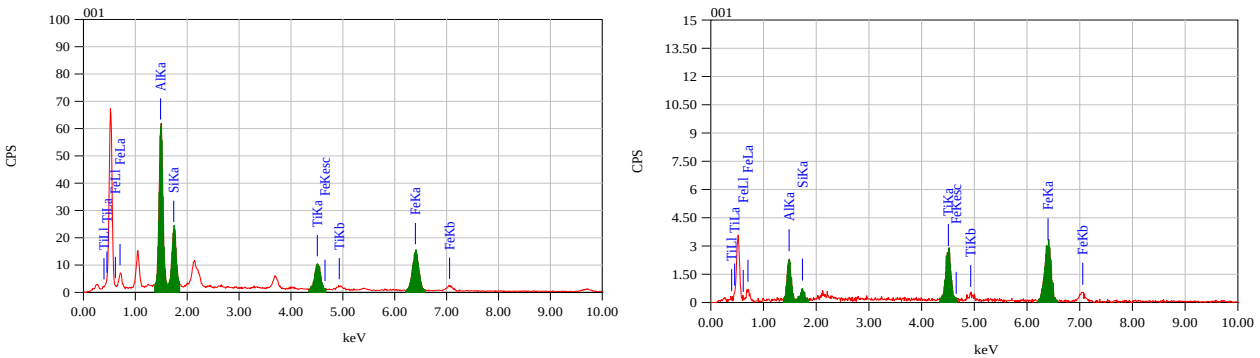


Fig.7. The SEM micrograph of raw red mud Fig.8. The SEM micrograph of red mud treated with H_2SO_4

Table 5. BET analysis of red mud

Sample	Total Surface area, m^2/g	Pore volume, nm
Raw Red mud	20.23	0.092
H_2SO_4 treated-calcinated Red mud	28.2	0.107

Brunauer–Emmett–Teller (BET) analysis was carried out for find out the surface area and pore volume of raw and activated red mud samples. From the BET analysis it can be seen there is a 39.4 % increase in surface area of acid treated-calcinated red mud as the surface areas of raw red mud and acid treated-calcinated are 20.23 and 28.2 respectively. So, the acid treated-calcinated red mud can be used as an effective adsorbent to remove heavy metals in particular cadmium in the present study.

Conclusion:

The effect of Calcination temperature, calcination time, acid concentration and activation time on activation of red mud for adsorption of cadmium was studied through four factor, two level Central Composite Design (CCD) and ANN. The effect of activation conditions of the acid treated-calcinated red mud to use as an adsorbent for Cadmium (II) removal has been optimized. The optimum conditions for the activation of red mud were 415.92 ºC (calcination temperature), 4.99 h (calcination time),1.438 N H_2SO_4 (acid concentration),1.336 h (activation time). The Regression co-efficient (R^2), root mean square error (RMSE) were compared for both the models. The ANN methodology was found to be more accurate when compared to RSM for a small set of experimental data. The surface areas of the original red mud, acid treated-calcinated red mud, samples were determined by BET analyis and it was found that the surface area of the acid treated-calcinated red mud is increased 39.4 % of the original red mud that is 28.2 m^2/g and pore volume was 0.107 nm which is higher than the red mud sample. So this can be used effectively as an adsorbent to remove the heavy metals in particular cadmium (II).

References

1. Upendra Kumar, Manas Bandyopadhyay. Fixed bed column study for Cd(II) removal from wastewater using treated rice husk. Journal of Hazardous Materials B129 (2006) 253–259.
2. JU Shao-hua, LU Shuai-dan, PENG Jin-hui, zhang Li-bo, C. srinivasakannan, GUO Sheng-hui, LI Wei. Removal of cadmium from aqueous solutions using red mud granulated with cement Trans. Nonferrous Met. Soc. China 22(2012) 3140−3146.
3. K.S. Rao, M. Mohapatra, S. Anand, P. Venkateswarlu. Review on cadmium removal from aqueous solutions. International Journal of Engineering, Science and Technology Vol. 2, No. 7, 2010, pp. 81-103.
4. Suchita Rai1, K.L. Wasewar, J. Mukhopadhyay, Chang Kyoo Yoo, Hasan Uslu. Neutralization and utilization of red mud for its better waste management arch. environ. sci. (2012), 6, 13-33 13 .
5. Y. Bai, B. Bartkiewicz. Removal of Cadmium from Wastewater Using Ion Exchange Resin Amberjet 1200H Columns Polish. J. of Environ. Stud. Vol. 18, No. 6 (2009), 1191-1195.
6. Sukru dursun, Dunyamin guclu, Mehmet BAS. Phosphate removal by using activated red mud from Seydisehir Aluminium Factory in Turkey. J. Int. Environmental Application & Science, Vol. 1 (3&4): 98-106 (2006).
7. Yanju Liu, Ravi Naidu, Hui Ming. Red mud as an amendment for pollutants in solid and liquid phases. Geoderma 163 (2011) 1–12.
8. I.D. Pulford, J.S.J. Hargreaves, J. Durisová, B. Kramulova, C. Girard M. Balakrishnan, V.S. Batra, J.L. Rico. Carbonised red mud, A new water treatment product made from a waste material. Journal of Environmental Management 100 (2012) 59e64.
9. G. Lopes, L.R.G. Guilherme,E.T.S. Costa,N. Curi,H.G.V. Penha Increasing arsenic sorption on red mud by phosphogypsum addition. Journal of Hazardous Materials (2012).
10. Huili Luo, Shengsheng Huang, Lin Luo, Yan Liu. Chemical Passivity of Pb in Ore Soil by Using Improved Red-Mud Materials. 2011 Second International Conference on Digital Manufacturing & Automation.
11. Yan Liu. Effects of Granulated Red Mud on Lead-Zinc Waste Soil Remediation and Leeks Growth. 2011 International Conference on Computer Distributed Control and Intelligent Environmental Monitoring.
12. Gavris G., Caraban A., Stanasel O., Badea G.E. Cadmium ions recovery by chemical precipitation method from rezidual solutions of galvanic plating. Journal of sustainable energy vol. 4, no. 1, march, 2013.
13. S.R. Younesi, H. Alimadadi, E. Keshavarz Alamdari, S.P.H. Marashi. Kinetic mechanisms of cementation of cadmium ions by zinc powder from sulphate solutions. Hydrometallurgy 84 (2006) 155–164.
14. Y. Bai, B. Bartkiewicz. Removal of Cadmium from Wastewater Using Ion Exchange Resin Amberjet 1200H Columns Polish. J. of Environ. Stud. Vol. 18, No. 6 (2009), 1191-1195.
15. Ahmad Jusoha, Lam Su Shiung, Nora'aini Alia, M.J.M.M. Noor. A simulation study of the removal efficiency of granular activated carbon on cadmium and lead. Desalination 206 (2007) 9–16.
16. Chunlei Zhua, Zhaokun Luana, Yanqiu Wang, Xingdong Shan. Removal of cadmium from aqueous solutions by adsorption on granular red mud (GRM). Separation and Purification Technology 57 (2007) 161–169.

17. M.Mourabet, A. El Rhilassi, M.Bennani-Ziatni, A. Taitai. Comparative Study of Artificial Neural Network and Response Surface Methodology for Modelling and Optimization the Adsorption Capacity of Fluoride onto Apatitic Tricalcium Phosphate. Universal Journal of Applied Mathematics 2(2), 84-91, 2014.
18. Deniz Bingol, Merve Hercan, Sermin Elevli, Erdal Kilic. Comparison of the results of response surface methodology and artificial neural network for the biosorption of lead using black cumin. Bioresource Technology 112 (2012), 111-115.
19. Kiran M. Desai, Shrikant A. Survase, Parag S. Saudagar, S. S. Lele, Rekha S. Singhal. Comparison of artificial neural network (ANN) and response surface methodology (RSM) in fermentation media optimization: Case study of fermentive production of scleroglucan. Biochemical Engineering Journal 41 (2008), 266-273.
20. Kaan Yetilmezsoy, Sevgi Demirel. Artificial neural network (ANN) approach for modeling of Pb(II) adsorption from aqueous solution by Antep pistachio (Pistacia Vera L.) shells. Journal of Hazardous Materials 153 (2008) 1288–1300.
21. Sh. Youssefi, Z. Emam-Djomeh, and S. M. Mousavi. Comparison of Artificial Neural Network (ANN) and Respone Surface Methodology (RSM) in the prediction of Quality Parameters of Spray-Dried Pomegranate Juice. Drying Technology, 27: 910-917, 2009.
22. K.K. Singh, R. Rastogi, S.H. Hasan. Removal of cadmium from wastewater using agricultural waste 'rice polish'. Journal of Hazardous Materials A121 (2005) 51–58.
23. Azam Ghorbani1, Salumeh Mesgari abbasi, Mohammad Nazarfakhari, Ali Fakhariyan. Optimization of Effective Parameters for Cd (II) Removal from Aqueous Solutions by Red Mud Using Design of Experimental (Box-Behnken). J. Basic. Appl. Sci. Res., 3(1s)406-412, 2013.
24. Abhishek Kardam, Kumar Rohit Raj, Jyoti Kumar Arora, Man Mohan Srivastava, Shalini Srivastava. Artificial Neural Network Modeling for Sorption of Cadmium from Aqueous System by Shelled Moringa Oleifera Seed Powder as an Agricultural Waste. J. Water Resource and Protection, 2010, 2, 339-344.
25. R. Leyva-Ramos, J. R. Rangel-Mendez, J. Mendoza-Barron, L. Fuentes-Rubio and R, M. Guerrero-Coronado. Adsorption of Cadmium(II) from aqueous solution onto activated carbon. Wal. Sci. Tech. Vol. 35, No. 7, pp. 205-211, 1997.
26. E. Oguz , M.Ersoy, Removal of Cu2+ from aqueous solution by adsorption in a fixed bed column and neural network modeling, Chem. Eng. J. ,164,56–62,2010.
27. E.S. Elmolla, M. Chaudhuri, M. M. Eltoukhy, The use of artificial neural network (ANN) for modeling of COD removal from antibiotic aqueous solution by the Fenton process, Journal of Hazardous Materials, 179, 127–134,2010.
28. X. Du, Q. Yuan , J. Zhao, Y. Li, Comparison of general rate model with a new model artificial neural network model in describing chromatographic kinetics of solanesol adsorption in packed column by macroporous resins, Journal of Chromatography A, 1145, 165–174,2007.
29. Kumar S, Kumar R, Bandopadhyay A. Innovative methodologies for the utilization of wastes from metallurgical and allied industries. Resources, Conservation and Recycling. 2006;(48):301–314.
30. Çengeloglu, Y., Kir, E., Ersöz,M., 2001. Recovery and concentration of Al(III), Fe(III), Ti(IV), and Na(I) from red mud. Journal of Colloid and Interface Science 244 (2), 342–346.
31. Sglavo, V.M., et al., 2000a. Bauxite 'red mud' in the ceramic industry. Part 1: thermal behaviour. Journal of the European Ceramic Society 20 (3), 235–244.
32. Sglavo, V.M., et al., 2000b. Bauxite 'red mud' in the ceramic industry. Part 2: production of clay-based ceramics. Journal of the European Ceramic Society 20 (3), 245–252.
33. Shaobin Wang, H.M. Ang, M.O. Tade. Novel applications of red mud as coagulant, adsorbent and catalyst for environmentally benign processes.
34. M.R. Fagundes-Klen a, P. Ferri a, T.D. Martins, C.R.G. Tavares b, E.A. Silva. Equilibrium study of the binary mixture of cadmium–zinc ions biosorption by the Sargassum filipendula species using adsorption isotherms models and neural network. Biochemical Engineering Journal 34 (2007) 136–146.
35. N. Sivarajasekar, R. Baskar. Adsorption of basic red 9 on activated waste Gossypium hirsutum seeds: Process modeling, analysis and optimization using statistical design. Journal of industrial and engineering chemistry,Vol. 20(5), 2014,pp. 2699–2709.
36. A Brief Description of the Levenberg-Marquardt Algorithm Implemened by levmar, 2005.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

EXPERIMENTAL ANALYSIS ON PULP AND PAPER INDUSTRY WASTEWATER BY OZONATION

Dynisha.S [1], J.Thirumal [2], Ashik vivek.A [3]

[1, 2, 3]Department of Civil Engineering, Anna University regional campus, Tirunelveli- 627 007.

dynishaaa@gmail.com

ABSTRACT: *The quality of wastewater that is discharged into the environment is to be improved due to the scarcity of water. Pulp and paper sector currently ranks fourth for its water usage and it is also one of the biggest manufacturers of wastewater. Paper and pulp industry Wastewater is considered as a severe environmental risk when it is untreated or badly treated before discharge into receiving waters because of the presence of toxic substances. Contaminants inherent to paper and pulp wastewater include effluent solids, sediments, absorbable organic halides (AOX), chlorinated organic compounds, chemical oxygen demand (COD), biological oxygen demand (BOD), contaminants, thermal loads, microorganisms such as coliform groups and toxic chemicals. A formal method for handling paper and pulp wastewater includes physical, chemical, and/or biological processes, or in some instances, a combination of these operations is used. Current wastewater treatment technologies are deemed ineffective in the utter removal of pollutants, particularly organic matter. In many instances, these organic compounds are resistant to conventional treatment methods, therefore creating the requirement for modern engineering sciences. This report delineates the effect of ozonation by investigating the removal efficiency of color and COD. The experimentation is conducted in a lab scale with the reaction time of 180 and 120 minutes. The effect of ozonation treatment on SCOD release is optimum at the dosage of 60% at 90 minutes. The maximum COD solubilization was achieved as 65% and also the removal efficiency of color is attained as 100%. This analysis is being proposed to resolve the challenges in removing a wide range of contaminants and for better uses of economic resources.*

KEYWORDS: *Ozonation, Wastewater Treatment, COD, Pulp and Paper Industry, SS reduction.*

I. INTRODUCTION

To meet the human necessities by exploitation of natural resources, the rapid growth of industrial activities increases day by day [12]. One of the biggest consumers of water and a major contributor of pollutants discharge to the environment is pulp and paper industry [1]. It is mostly depicted as energy and water intensive [10]. Approximately, water footprint of 30 cubic meters was produced by this industry for every ton of paper hence makes a significant contribution to the economies of many countries in the world [6]. Around the world, approximately 18millions tons of dry PPMS was produced by 40million tons of paper annually [9]. The wastewater produced by paper and pulp industry is based on the usage of raw materials and by the adopted manufacturing process [11]. The pulp and paper industry effluents contain a variety of toxic organic compounds that may cause deleterious environmental impacts to receiving water bodies if it is discharged untreated [2]. Also have the potential of slime production by microorganisms such as Sphaerotilussp and formation of scum [5].

The wastewaters of high yield pulping processes are characterized by their suspended solids content (including particles of bark), organic substances, chromophoric compounds (mainly labile extractives and lignin fragments), inorganic compounds (nitrogen and phosphorus, as result of the effluent treatment) and salts. Semi chemical processes contain lingosulfonates (derived from lignin). If Kraft pulps are bleached with chlorine compounds, they can present organ chlorine compounds (dioxins and furans) [1]. Among the various sections, the effluents from pulp bleaching are responsible for most of the color, organic matter, and toxicity of the water discharges of this industry [2]. The COD be as high as 11,000 mg/L in paper and pulp wastewater, which is proved in a recent study [4].

In pulp and paper sector the global market for wastewater treatment is expected to increase from $903.9 million in 2012 to $1.569 billion 2020. This is because of rise in water prices as well as tightened environmental standards [8]. To remove aqueous contaminants, conventional technologies including biological, thermal, and physicochemical treatments have been used traditionally. To dispose the separated contaminants, some other techniques such as precipitation, extraction, flocculation, adsorption, and reverse osmosis requires a post-treatments. Conventional treatment processes are not sufficient, since some of the pollutants in industrial

effluents are non biodegradable. These limitations of conventional methods have been an incentive to develop more efficient systems. In order to meet the increasingly stringent discharge limits, mills are forced to adopt unconventional and technologically advanced treatment systems to reduce refractory organic compounds [1]. Hence, treatment with some advanced oxidation processes (AOP's) is needed. It is a highly suggested chemical treatment method for the removal of problematic organic matter growing in the wastewater management industry [3]. As ozone is a powerful oxidant, it is used in wastewater treatment for odor control, effluent disinfection, color removal, and oxidation of organic and inorganic contaminants [13]. The aim of this research is thus to investigate the efficiency and feasibility of ozonation process for the pre treatment of pulp and paper wastewater.

II. COLLECTION OF SAMPLE

The waste water was collected from pulp and paper mill at Erode and it was stored at 4°C. At first, initial characteristics of pulp wastewater is observed and reported in the table 4.1.

SI.NO	PARAMETERS	UNITS	VALUE
1	pH	-	8.4
2	Total Dissolved solids (TDS)	mg/L	4900
3	Total Suspended solids (TSS)	mg/L	1850
4	Total Chemical Oxygen demand (TCOD)	mg/L	3050
5	Soluble Chemical Oxygen demand (SCOD)	mg/L	2200
6	Total Solids (TS)	mg/L	1600
7	Chlorides	mg/L	79.2
8	Alkalinity	mg/L	449.86
10	BOD_5	mg/L	65.19

Table 4.1 Initial Characteristics of Waste Water

III. MATERIALS AND METHODS

A. OZONATION TREATMENT

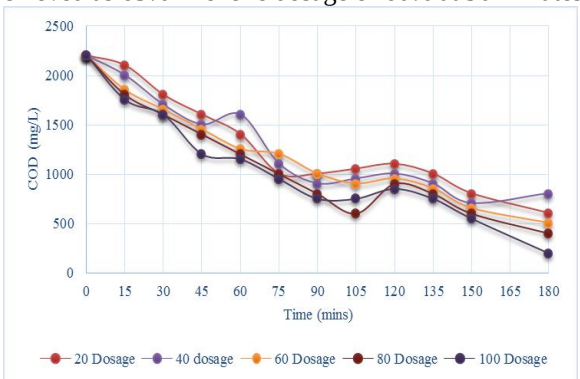
Ozonation treatment was performed using 500ml wastewater in 1L volume of glass reactor. Initially in room temperature wastewater was left free for an hour in the absence of sunlight. 500 ml of wastewater is poured into the closed glass reactor containing three holes. Where, first hole is for ozone, second for sample out, and third for both sample in and out. After that mixture of ozone and oxygen, the tube is inserted to the glass reactor. On the other hole of glass reactor ozone out tube is inserted. To reduce the concentration of ozone the outlet is trapped by 2% KI solution. In the ozonator, initial flow rate of 2 liter is fixed to carry out the process. From the reactor at 15 minutes time interval for 180 minutes samples were collected and stored.

The efficiency of treatment was studied by enumerate COD Solubilization, TSS, color, pH and BOD_5reduction.

IV. RESULT AND DISCUSSION

A. Effect of Ozonation Treatment on SCOD Release and COD Solubilization

The effect of ozonation treatment on SCOD release was investigated and depicted in figure: 1. Due to the effect of ozonation non-biodegradable matters converted into biodegradable matter. The SCOD release gradually increased for 90 minutes beyond which only slight increment was noted. Hence an optimum ozonation dosage was predicted as 60%. Figure: 2 explain the effect of ozonation on COD solubilization. When there is a high ozone dosage then the reaction time also gets increased. The maximum COD solubilization was achieved as 65% in ozone dosage of 60% at 90 minutes time interval.



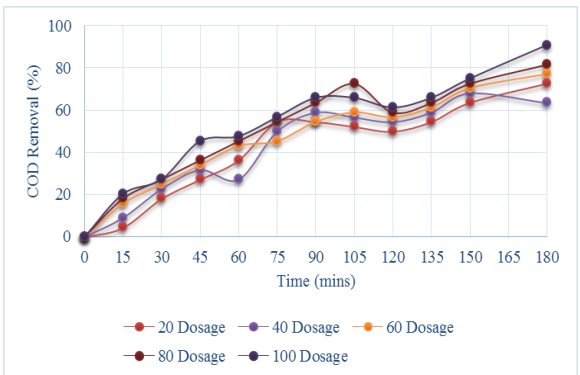


Figure: 1 Effect of ozone on SCOD Release

Figure: 2 Effect of ozone on COD Solubilization

B. Effect of Ozonation Treatment on SS Reduction

Figure: 3 and 4 shows the SS reduction during ozone treatment under different conditions of reaction time and ozone dosage. From the graph, Ozonation dosage of 60% in 90 minutes was found as optimum. At optimum condition the removal efficiency of SS reduction was found to increase from 37.7% to 50.8%.

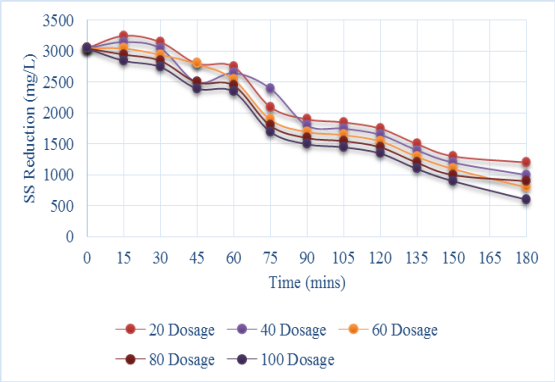


Figure: 3 Effect of ozone on SS Reduction

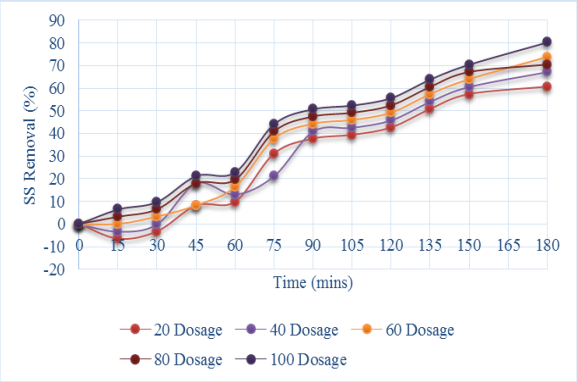


Figure: 4 Effect of ozone on SS concentration

C. Effect of pH and color on Ozone Treatment

At optimum dosage, there occurs a rapid reduction in pH values. Fig: 5 clearly show the effect of pH during ozonation treatment. During ozone treatment color of the waste water gets reduced. It is because that molecular ozone is the selective for the destruction of chromophoric group, decolourization of effluent is favoured by direct molecular ozone attack. In the ozone treatment the color is removed up to 100%. Fig: 6 show the effect of color on ozone treatment.

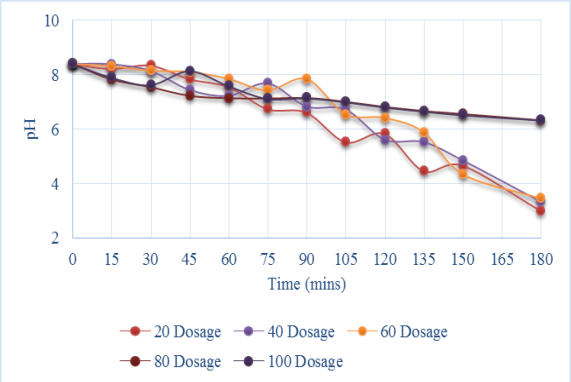


Figure: 5 Effect of pH on ozone Treatment

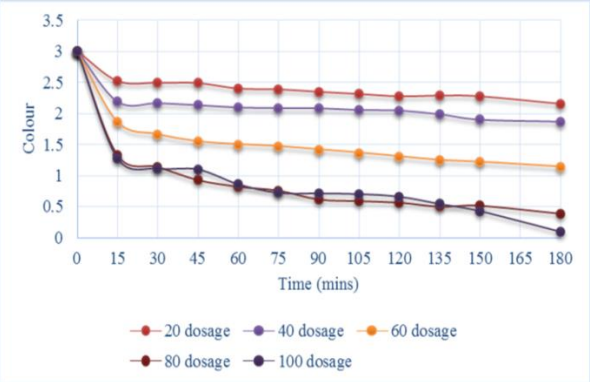
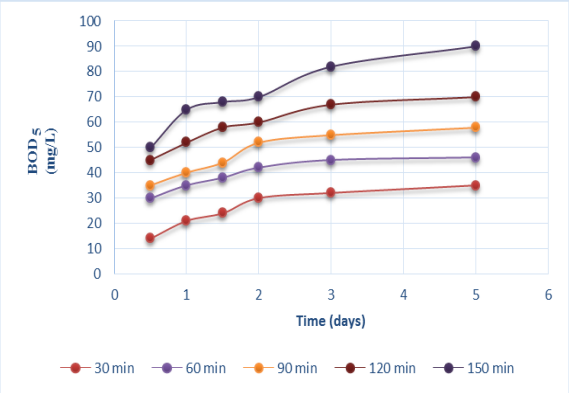


Figure: 6 Effect of Color on ozone Treatment

D. Effect of ozone on biodegradability of waste water treatment

The experiment was carried out in room temperature at pH 8. At the optimum condition of ozonation the BOD and BOD/COD ratio were 90 mg/lit and 0.28. From the graph it was concluded that the treatment removes the non-biodegradable substance and makes less toxic as well as biodegrade effectively. Fig: 7 and 8 describe the variation of BOD with respect to time.



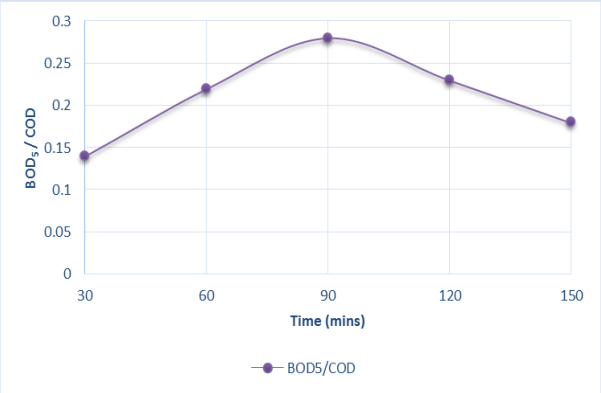


Figure: 7 Variation of BOD with ozonation Time **Figure: 8Variation of BOD/COD Ratio**

V. CONCLUSION

In this research work, the efficiency of ozonation on pulp and paper wastewater is investigated. The effect of ozonation treatment on SCOD release is optimum at the dosage of 60% at 90 minutes. The maximum COD solubilization was achieved as 65% and also the removal efficiency of color is attained as 100% in ozonation. At the optimum condition of ozonation the BOD and BOD/COD ratio were 90 mg/lit and 0.28.This study shows the performance of ozonation and efficient removal of the non-biodegradable substance. Also this analysis is being proposed to resolve the challenges in removing a wide range of contaminants and for better uses of economic resources.

VI. REFERENCES

[1] Laura G. Covinich , Dora I. Bengoechea, Rosa J. Fenoglio, Maria C. Area, 2014, Advanced Oxidation Processes for Wastewater Treatment in the Pulp and Paper Industry: A Review, American Journal of Environmental Engineering 2014, 4(3): 56-70

[2] Parveen Kumar, Satish Kumar, Nishi K. Bhardwaj, Ashutosh Kumar Choudhary, 2011, Advanced Oxidation of Pulp and Paper Industry Effluent, International Conference on Environmental and Agriculture Engineering, IPCBEE vol.15(2011).

[3] S.Krishnan, H.Rawindran, C. M. Sinnathambi, J .W. Lim, 2017,Comparison of various advanced oxidation processes used in remediation of industrial wastewater laden with recalcitrant pollutants,IOP Conference Series: Materials Science and Engineering, 206 (2017) 012089.

[4] Irma Karat, 2013,Advanced Oxidation Processes for Removal of COD from Pulp and Paper Mill Effluents A Technical, Economical and Environmental Evaluation, Master of Science Thesis Stockholm 2013.

[5] Mohammadreza Kamali, ZahraKhodaparast, 2015, Review on recent developments on pulp and paper mill wastewater treatment, Ecotoxicology and Environmental Safety 114(2015)326–342.

[6] Maria Sandberg, G Venkatesh, Karin Granstrpom, 2017, Experimental study and analysis of the functional and life-cycle global warming effect of low-dose chemical pre-treatment of effluent from pulp and paper mills, Journal of Cleaner Production, DOI: 10.1016/j. jclepro.2017.10.214.

[7] Jose Guzman, Rosa Mosteo, Judith Sarasa, Jose A. Alba, Jose L. Ovelleiro, Evaluation of Solar Photo-Fenton and Ozone Based Processes as Citrus Wastewater Pre-treatments, Separation and Purification Technology, DOI: http://dx.doi.org/10.1016/j.seppur.2016.03.025

[8] Renata Toczylowska-Maminska, 2017, Limits and perspectives of pulp and paper industry wastewater treatment – A review, Renewable and Sustainable Energy Reviews, 78 (2017) 764–772.

[9] Yunqin Lin, Jiajin Liang, Chao Zeng, Dehan Wang, Huanjia Lin, 2017, Anaerobic digestion of pulp and paper mill sludge pretreated by microbial consortium OEM1 with simultaneous degradation of lignocelluloses and chlorophenols, Renewable Energy, DOI:10.1016/j.renene.2017.02.049.

[10] C.Veluchamy, Ajay.S. Kalamdhad, 2017, Influence of pretreatment techniques on anaerobic digestion of pulp and paper mill sludge: A review, Bioresource technology, DOI:http//dx.doi.org/10.1016/j.biortech.2017.08.179.

[11] Mohammadreza Kamali, Tania Gameiro, Maria Elisabete, Isabel Capela, 2016, Anaerobic digestion of pulp and paper mill wastes- An overview of the developments and improvement opportunities, Chemical Engineering journal, DOI:http//dx.doi.org/10.1016/j.cej.2016.03.119.

[12] A. Sethupathy, P.Sivashanmugam, 2018, Enhancing biomethane potential of pulp and paper sludge through disperser mediated polyhydroxyalkanoates, Energy Conversion and Management 173 (2018) 179-186.

[13] Keisuke Ikehata, Yuan Li, 2018, Ozone- Based Processes, Advanced oxidation processes for wastewater treatment,DOI:https://doi.org/B978-0-12-810499-6.00005-X.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Experimental Investigation of Solar Bio-mass Integrated Dryer System

Ravinth Kumar A[1], Sam Solomon D P[2], Senthil Kumar K[3] and Rashik M

[1 & 2] Department of Mechanical Engineering, Coimbatore Institute of Technology, Coimbatore, TN, India

[3 & 4] Department of Mechanical Engineering, Adithya Institute of Technology, Coimbatore, TN, India

Abstract*: This paper presents the design, construction and performance of a mixed-mode solar dryer for food preservation. The mixed mode includes a solar collector and biomass burner which can function independently or simultaneously. In the dryer, the heated air from a separate solar collector is passed through a grain bed, and at the same time, the drying cabinet absorbs solar energy directly through the transparent walls and roof. The results obtained during the test period revealed that the temperatures inside the dryer and solar collector were much higher than the ambient temperature during most hours of the day-light. The temperature rise inside the drying cabinet was 74% higher for about three hours immediately after 12.00 (noon). The dryer exhibited sufficient ability to dry food items rapidly to the required moisture level and simultaneously ensures a superior quality of the dried product.*

Keywords*: solar collector, solar dryer, biomass, solar intensity, cabinet.*

I. Introduction

Solar dryer finds its applicatiions in drying a lot of agricultural and food products for preserving them for a considerable long period of time. [1] Mahesh Kumar et al has studied the works done so far in the field of solar dryers. They have presented a variety of dryers fabricated so far such as direct, indirect and hybrid dryers. However, the authors have felt that the area is still open for research in developing countries. [2]Abubakar et al has conducted experiments on a mixed mode solar dryer wherein additional thermal storage materials are used to overcome the non-uniform drying that happens in a multi-tray dryer in Nigeria. The authors have compared the experimental results with that of the numerical results. [3] Hajar Essalhi et al has conducted experiments using a solar air dryer for drying grapes in Morocco wherein the authors have used a water storage tank to heat water in the day time and use the heated water to transfer heat to the grapes during the night time when there is no sunlight. Their experimental results indicate the indirect drying using the solar dryer is efficient than the direct open sun drying. [4] Woranauch has conducted experiments on a solar dryer which is capable of drying meat over a period of two days with a temperature range of about 47 – 50 degree Celsius in Thailand. The author has shown that the quality of drying is comparatively higher in case of the cabinet dryer rather than the open sun drying. [5] Sam Solomon and Ravinth Kumar has conducted experiments to estimate the percentage of heat transfer that takes place through conduction, convection and radiation in a fin. However, in this study, the utilization of solar energy, biomass and solar collector and their corresponding impact on drying is observed. [6] Hamdani et al has studied the effect of utilizing solar energy and the addition of biomass energy in drying fish in the Ache province of Indonesia which relies mainly on the fishing industry. [7] Kabeel and Mohamed have performed numerical analysis on solar dryers with and without rotary dessicant wheels and their corresponding impact on drying. This work shows the advantage of utilizing biomass and solar energy to dry agricultural products effectively using the phenomena of green house effect in addition.

II. Experimental Investigation

Experiments were conducted in the premises of Adithya Institute of Technology in April 2017 during the potential sun shine hours between 9.00 AM and 5.00 PM where the solar intensity is found to be sufficiently high.

A. Experimental Setup

A solar greenhouse drier was designed and fabricated. The drier is shaped in a semi-circle and is covered with UV stabilized polyethylene film.

Figure 1 Experimental Setup



B. Solar Dryer Components

The major components include the following:

1 Solar Collector

The heat absorber (inner box) of the solar air heater was constructed using 2 mm thick aluminium plate, painted black, is mounted in an outer box built from well-seasoned woods. The solar collector assembly consists of air flow channel enclosed by transparent cover (glazing). An absorber mesh screen midway between the glass cover and the absorber back plate provides effective air heating because solar radiation that passes through the transparent cover is then absorbed by both the mesh and back-plate. The glazing is a single layer of 4 mm thick transparent glass sheet.

Figure 2 Solar Collector



Figure 3 Drying Cabinet with Drying Trays



2 Drying Cabinet

The drying cabinet together with the structural frame of the dryer was built from well-seasoned woods which could withstand termite and atmospheric attacks. An outlet vent was provided toward the upper end at the back of the cabinet to facilitate and control the convection flow of air through the dryer. Access door to the drying chamber was also provided at the back of the cabinet. This consists of three removable wooden panels made of 13 mm plywood, which overlapped each other to prevent air leakages when closed. The roof and the two opposite side walls of the cabinet are covered with transparent glass sheets of 4 mm thick, which provided additional heating.

3 Drying Trays

The drying trays are placed inside the drying chamber and were constructed from a double layer of fine chicken wire mesh with a fairly open structure to allow drying air to pass through the food items.

4 Ventilation System

The air flow rate is crucial for the overall performance of the system. Higher air flow consumes excessive fan power and lower rates result in poor thermal performance of the system. Higher the mass flow rates, higher the efficiency of the collector and increased use of electrical energy. However, a certain temperature level is to be maintained in the cabinet for which an optimized usage of electrical energy in running the blower is essential.

C. Experimental Procedure

Air is continuously sent to the solar collector by a blower through the inlet valve of the collector. The temperatures of air at the entry and exit of the collector are measured and recorded. The preheated air from the solar collector is fed into the drying cabinet where the products to be dried are placed in drying trays. The products can be dried by the heat from the hot air that comes from the collector as well as the direct solar radiation that falls inside the cabinet. The set up also includes a biogas drum wherein agricultural wastes and cow dung are stored for about three weeks to generate biogas. This biogas can be burned and the air that enters the cabinet can be heated by the same whenever there is reduced or no solar radiation. Temperatures inside the cabinet and outside the chimney are also noted. Using UV stabilized polyethylene prevents the possible damage that UV rays can cause to the food produce that needs to be dried.

D. Specification

As the cover material is UV stabilized polyethtylene, the ultra violet radiation cannot affect the green house effect inside the drier cabinet.

Table I Specifications

Parameter	Specification
Length x Width x Height	6ft x 6ft x 6ft
Cover Material	UV stabilized polyethylene
Cover thickness	200 microns
Floor Area	36 sq. ft.
Drying capacity	5 kg of green chilies
Maximum Temperature	80°C
Minimum RH	18 %
Drying time	1 day
Drying Items	Chilly, coconut, grapes, potatoes, etc.
No of baffles	2
Per shelf capacity	5 kg of chilies

III. Results and Discussion

A. No Load Condition

Under no load condition, the temperature inside the cabinet was measured without the use of solar collector and without the use of blower wherein removal of moisture due to greenhouse effect alone was observed. The maximum solar radiation was observed to be 610 W/m^2. The relative humidity inside the cabinet was observed to be relatively lesser than that of the ambient. The humidity was observed to reduce with the increase in temperature.

Figure 4. Variation of parameters with respect to time at no load condition

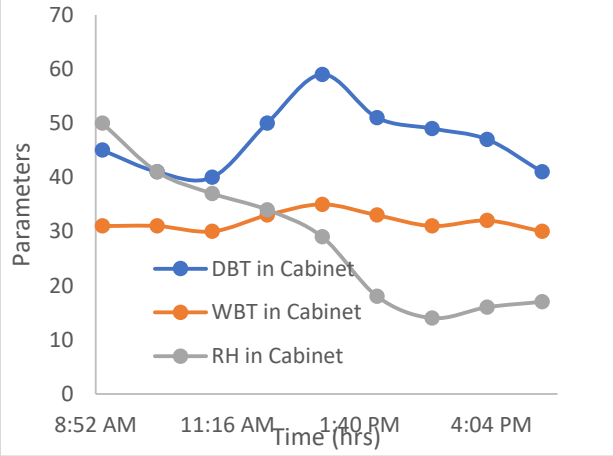
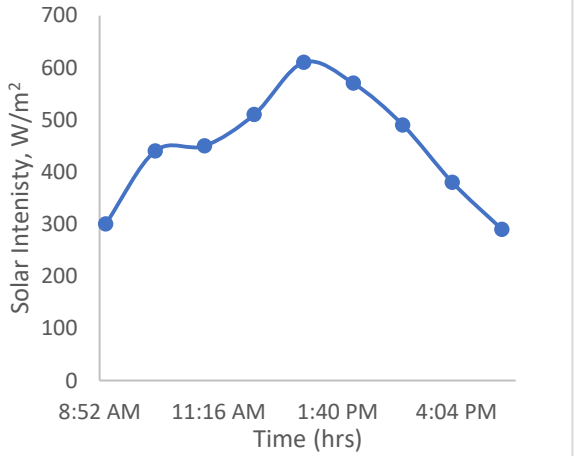


Figure 5. Variation of Solar Intensity with respect to time at no load condition



B. Full Load Condition without Solar Collector

Under full load condition of solar dryer, the maximum intensity of solar radiation was observed to be 448 W/m^2. The same trends were observed in case of DBT, WBT and Relative humidity inside and outside the cabinet. However, with the decrease in solar radiation as in the previous day, reduction in RH is still appreciable owing to the greenhouse effect inside the cabinet.

Figure 6. Variation of parameters with respect to time under full load condition

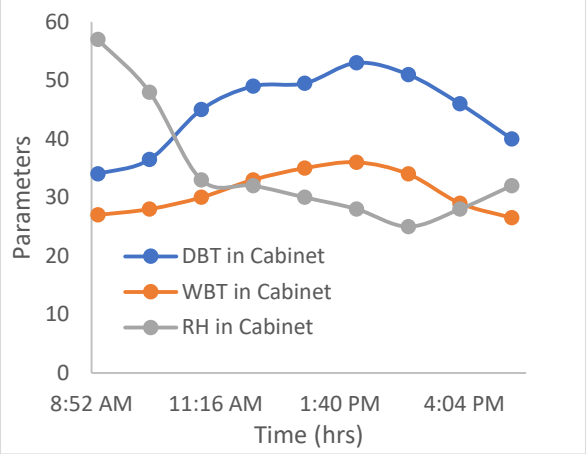
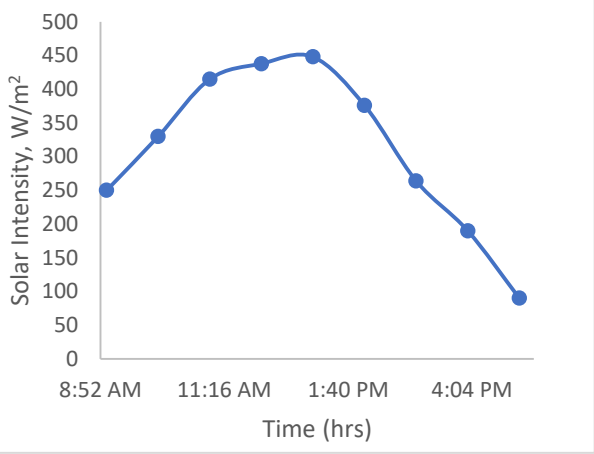


Figure 7. Variation of Solar Intensity with respect to time under full load condition



C. Full Load Condition with Solar Collector

Under full load condition of solar dryer, the maximum intensity of solar radiation was observed to be 390 W/m^2. The same trends were observed in case of DBT, WBT and Relative humidity inside and outside the cabinet. However, with the decrease in solar radiation as in the previous day, reduction in RH is highly appreciable owing to the increased heating effect caused by the solar collector in addition to the greenhouse effect.

Figure 8. Variation of parameters with respect to time under full load condition

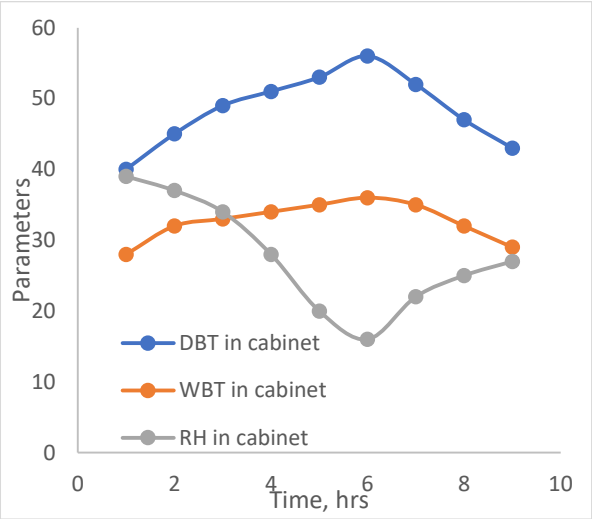
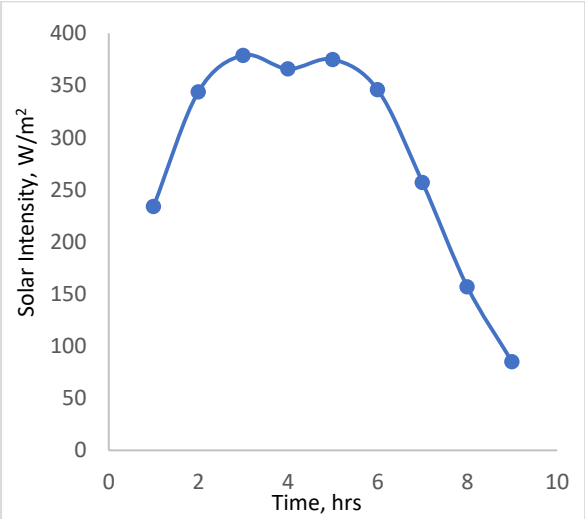


Figure 9. Variation of Solar Intensity with respect to time under full load condition



IV. Application

In this experimental study, experiments were conducted on three different days. Utilization of direct solar radiations have been observed. Utilization of solar energy as direct solar insolation and passage of hot air by forced convection from the solar collector to the cabinet has also been observed. The results show that the compound effect of the later in addition to the greenhouse effect dries the chilies placed in the trays very effectively.

Fig 10 Chilies before drying



Fig 11 Chilies after drying



Use of biogas was not necessary in the above experiments as the solar insolation was sufficient to dry the chilies. However, in days of poor solar radiation, biogas generated from the tank passed through the burner would heat the air which in turn can be utilized for drying. As a scope for future work, amount of biogas used and its impact on DBT, WBT and RH can be studied further.

References

[1] Mahesh Kumar, Sunil Kumar Sansaniwal and Pankaj Khatak, "Progress in Solar dryers for drying various commodities", Renewable and Sustainable Energy Reviews, vol. 55, 2016, pp. 346 - 360.

[2] Abubakar, S., Umaru, S., Kaisan, M.U., Umar, U.A., Ashok, B. and Nandhagopal, K, "Development and Performance comparison of mixed-mode solar crop dryers with and without thermal storage", Renewable Energy, vol. 128, 2018, pp. 285 - 298.

[3] Hajar Essalhi, Mohamed Benchrifa, Rachid Tadili and Bargach, M.N, "Experimental and Theoretical Analysis of drying grapes under an indirect solar dryer and in open sun", Innovative Food Science and Emerging Technologies, vol. 49, pp. 58 – 64.

[4] Woranuch Jangsawang, "Meat Products Drying with a Compact Solar Cabinet Dryer", Proc. International Conference on Alternative Energy in Developing Countries and Emerging Economies, Bangkok, Thailand, 2017, Energy Procedia, pp. 1048 – 1054.

[5] Sam Solomon D P & Ravinth Kumar A, "Experimental Analysis of the combined effect of conduction, convection and radiation in square and cylindrical fins", Proc. International Conference on Industry 4.0, 2019, pp. 21.

[6] Hamdani, Rizal T A and Muhammad Zulfri, "Fabrication and Testing of Hybrid Solar-Biomass dryer for drying fish", Case Studies in Thermal Engineering, https://doi.org/10.1016/j.csite.2018.06.008.

[7] Kabeel A E and Mohamed Abdelgaied, "Peformance of novel solar dryer", Process Safety and Environment Protection, http://dx.doi.org/doi:10.1016/j.psep.2016.03.009.

[8] J. A. Duffie and W. A. Beckman, Solar engineering of thermal processes. Wiley, 2013.

[9] Rai G.D., "Non Conventional Energy Sources", Khanna Publishers, New Delhi, 2007.

[10] Sukhatme S.P., "Solar Energy", Tata McGraw Hill, 2nd Edition, 2007.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Bacterial Leaching of Heavy Metals from Sewage Sludge: Studies on pH Optimization

Venkatesa Prabhu. S [1*], Gizachew Assefa. K[1], Abel W. Ourgessa[1], Ababo Geleta. G[1] and Assefa Alene[1]

[1]Department of Chemical Engineering, College of Biological & Chemical Engineering, Addis Ababa Science & Technology University, Ethiopia.

[*]haiitsvp@gmail.com

Abstract*: Bioleaching is an environmentally-friendly proven method to remove heavy metals from heavy metal contaminated wastes. This process is significantly influenced by medium pH. The objective of this work is to find out an optimum pH for effective removal of heavy metals from sewage sludge through bioleaching. The sludge was collected from municipal waste water accumulated area, Addis Ababa, Ethiopia. The bacteria, Acidithiobacillus ferrooxidans (NCIM 5371), was obtained from National Collection of Industrial Microorganisms, India. The experiments were carried out in 250 mL Erlenmeyer flasks with different initial pH values at temperature 30 °C. The attainment of bioleaching of heavy metals was examined for the period of 20 days. Changes in the pH and ROP were also analyzed. It was observed that the high pH reduction, absence of lag phase and improved heavy metal solubilization were obtained in the experiment conducted with initial pH 3.0. The bioleaching efficiency is distinctly correlated with pH and ORP. Primary heavy metals concentration in the original sludge and residual heavy metals concentration in bioleached sludge were analyzed by EDX. It revealed that the effective removal of heavy metals was achieved at initial pH 3.0. Such a study helps for designing a successful bio-detoxification system.*

Keywords*: Sludge, Acidithiobacillus ferrooxidans, pH, bioleaching, heavy metals***.**

I. Introduction

Urbanization and industrialization are major causes of heavy metal pollution in the soil. Soil pollution instigated by significant amount of toxic waste and controlled solid waste as sludge. Solid wastes stocking, mining, atmospheric deposition, sludge disposal and using of agrochemicals are resulting in the heavy metal pollution in soils. It is major sink for heavy metals released into the environment by aforementioned anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation [1, 2] and their total concentration in soils persists for a long time after their introduction [3, 4]. The pollution of soil by heavy metal is still a thoroughly unsolved environmental problem all over the world. This may pose risk and hazards to humans and the ecosystem.

Therefore, in order to maintain good quality of soil and keep them free from heavy metal contamination, continuous efforts have been made to develop technologies that are easy to use, sustainable and economically feasible. The heavy metal concentration in the wastes sludge can be reduced by several methods such as electro coagulation, alkaline-chlorination-oxidation, reverse osmosis, adsorption, membrane process, evaporative recovery, ion exchange, and electrochemical treatment [5, 6]. The drawback of these approaches are need of chemicals in large amount, high operating cost, difficulties in the working procedure and release of toxic gases in the atmosphere. Thus, suitable remediation methods are required to alter the treatment of wastes for removing heavy metals. Bioleaching is a process, which employs the microbes to transform solid insoluble compounds into soluble and extractable elements. This process has several benefits for extracting the metals such as environmentally friendly, less energy requirement and no need of sophisticated control implements. Although this process is at first developed for metal extraction from its ore, recently, lots of works have been focused for sludge detoxification to remove the toxic heavy metals. In bioleaching, acidophilic sulfur-oxidizing microorganisms are employed for the recovery of metals [7]. The oxidation of elemental sulfur or reduced sulfur compounds leads to generation of H_2SO_4 by the acidophilic bacteria (Eq.1 and Eq.2). Then the bacterially produced H_2SO_4 is responsible for the metal solubilization.

$$S^o + H_2O + 1.5O_2 \rightarrow H_2SO_4 \ldots\ldots\ldots (1)$$

$$\text{Sludge-M} + H_2SO_4 \rightarrow \text{Sludge-2H} + MSO_4 \ldots\ldots\ldots (2)$$

Where, M is a bivalent metal. The effectiveness of bioleaching is largely influenced by different chemical, physical and biological factors of bioleaching system such as concentration of solids, nature of the sludge, temperature, oxidation-reduction potential (ORP), pH, agitation, medium composition, and bacteria [8]. Among these factors initial pH is one of the main factors that influencing the process quite reasonably. It is also required to find the optimal initial pH to accelerate the bioleaching reaction.

Keeping in view the significant role of bacteria in biological leaching, the present work has been designed. The objective of this work is to find out an optimum initial pH value for effective removal of heavy metals from sewage sludge through bioleaching using the bacteria, *Acidithiobacillus ferrooxidans*. Additionally, the bioleaching efficiency is distinctly correlated with medium pH and ORP.

II. Materials and Method

A. Sludge Characterization

Sludge sample was collected from municipal waste water accumulated area, Addis Ababa, Ethiopia. It was collected from different depths (20−80 cm) along the sludge bed. Further, the sludge was then air-dried overnight at room temperature. The total heavy metal present in the sludge was determined by digesting the sludge with $HNO_3/HOCl/H_2SO_4$ at the ratio of 8:1:1. Dissolved heavy metals were estimated by atomic absorption spectrometry (AA200 model; PerkinElmer). The organic substance in the sludge was estimated using standard 1 N $K_2Cr_2O_7$ and Ferroin indicator as described by Walkely–Black technique [9]. Micro–Kjeldahl distillation apparatus was used to analyze the total nitrogen content in the sludge. The ion activity was analyzed using dry sludge/water extract (10:25) through a calibrated pH meter (Eutech Instruments, Singapore). Micro-vanadate-molybdate method was adopted for total available phosphorus content in the sludge. Other metals, Ca, Mg, and K were analyzed using flame photometry (CL378 model; Elico) after extracting the sludge with ammonium acetate. These procedures for sludge characterization were followed as outlined in the American Public Health Association (APHA) standard [10]. Three replicates were used for sludge characterization and the mean values were taken for consideration.

B. Preparation of Sludge Sample

The sludge sample was dried about 24 h in an oven at 150 ºC for removing water. Dried sludge was sieved using standard ASTM sieves to get 125+63 μm of average particle size. This selected size particles were considered for further bioleaching experiments.

C. Bacterial Culture and Acclimatization to Sludge

The bacterial strain, *A. ferrooxidans* (NCIM 5371) was used in this process. It was obtained from National Collection of Industrial Microorganism, India. To prepare working inoculum, the bacterial culture was enriched in 9K medium (pH 4) with S^o as energy source. The medium had: S^o (sterilized by tyndallization), 10 g/L; $MgSO4.7H_2O$, 0.5 g/L; $(NH_4)_2SO_4$, 3 g/L; K_2HPO_4, 0.5 g/L; $Ca(CO_3)_2$, 0.01 g/L; and KCl, 0.1 g/L. The acclimatization of the culture to sludge was carried out for enhancing the resistance against sludge toxicity. For that, 10% (v/v) of culture was inoculated in 90 mL sterilized 9K media containing 0.2% (w/v) of sludge. From this culture, 10 mL was transferred to 90 mL the fresh 9K media containing 0.6% (w/v) of sludge. It was sub-cultured again using the fresh media contained sludge level of 1.0% (w/v). This can be considered as the sludge adapted culture and it was used as inoculums for the further bioleaching experiments.

D. Bioleaching Procedure

The bioleaching were performed in 250 mL Erlenmeyer flasks. Each flask had the 100 mL of working volume which contained 10% (v/v) of inoculum, 90% (v/v) of 9K medium, and 5% (w/v) of sludge loading. To study the effect of initial pH on bioleaching, the experiments were carried out at different initial pH values ranging from 2.0 to 4.0. Initial value of pH of the media was adjusted using the 1N H_2SO_4. The flasks were maintained at 30 °C. A control experiment, without inoculum, was also carried out to compare the leaching efficiency with inoculated experiments. During the bioleaching process, the media pH was measured every day by calibrated pH meter (Eutech Instruments, Singapore). Oxidation reduction potential (ORP) of the medium was also monitored every day using of calibrated ORP meter (Eutech Instruments, Singapore). The samples (5 mL) were periodically collected from the flasks for every two day intervals and subjected to centrifuge at 2000 rpm for 30 minutes. The supernatants were subjected for the determination of dissolved heavy metal concentration by Atomic Absorption Spectrometry (Perkin Elmer, AA200 model). To compensate the volume losses, fresh nutrient solution (9K medium without elemental sulfur) was added. The heavy metal removal efficiency [M (%)] was calculated as the ratio between the solubilized metal and the total metal present in the primary sludge. It can be calculated from Eq.3.

$$M\,(\%) = [(M^t)/\,(M_T)] \times 100 \ldots\ldots\ldots\ldots (3)$$

Here, M^t is solubilized metal concentration at the time t during the bioleaching and M_T is the total metal concentration present in the primary sludge.

III. Results and Discussion

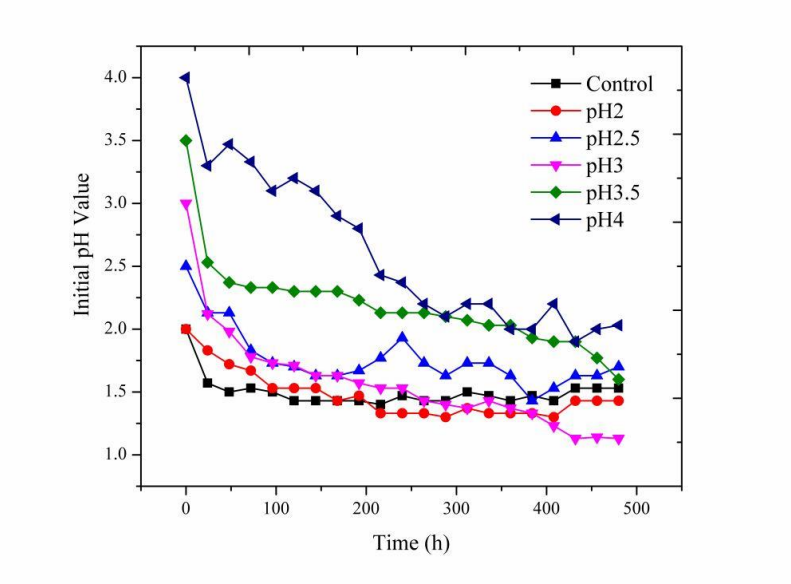
A. Sludge Characteristics

The selective physicochemical properties of collected sludge sample were determined. The characterization of sludge revealed that the sludge was in alkali condition (pH 7.8). The sludge contained 1646 and 1768 mg kg^{-1} of total available Kjeldahl nitrogen and phosphorus, respectively. It contained moderate level of potassium (470 mg kg^{-1}). Magnesium and calcium contents were also found to be 17,810 and 6710 mg kg^{-1}, respectively. Organic carbon present in the sludge was observed to be quite low (0.95 mg kg^{-1}). The heavy metal analysis showed that Cu, Ni, Zn and Pb were found in the sludge and they were found to be 1540, 889, 2457, and 575 mg kg^{-1}, respectively. It indicated that the heavy metals content in the sludge is high and may pose a serious hazard to the environment while it disposing on land without appropriate treatment.

B. Effect of Initial pH on Media pH during Bioleaching

The sulfur oxidation by *A. ferrooxidans* results in production of sulfuric acid which reduces media during bioleaching. This bio-acidification can be assessed by monitoring the pH reduction. The profiles of change in pH during bioleaching at experiment with different initial pH values were depicted in figure 1. In the control, there was no effective change in the pH value was observed. The chemical oxidation of S^o created a minimum drop in pH value (from 2.0 to 1.53) in control experiment. In the inoculated flasks at different initial pH values, significant reduction in the pH value was recorded within two weeks. It revealed that the good adaptation and rapid growth of microorganism. During 20 days of period, the pH value of the media was dropped from the initial pH values 2 to 1.43, 2.5 to 1.47, 3 to 1.13, 3.5 to 1.6, and 4 to 2.03. Therefore, the initial pH value 3.0 would be beneficial to reduce the pH of the media well below which suitable for the effective bioleaching.

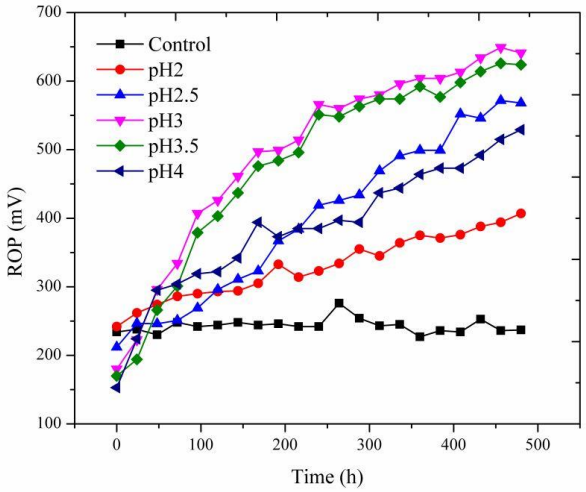
Figure 1 Change in pH value during bileaching at different initial pH values



C. Effect of Initial pH on ORP of the Medium

The change in ORP at different initial pH during bioleaching is presented in figure 2. Due to the absenteeism of bacterial activity in the control experiment, the ORP was found to be constant about 237 mV. The ORP values were attained as 407, 568, 641, 624 and 529 mV in the experiments at 2.0, 2.5, 3, 3.5, and 4.0 initial pH values, respectively, after 20th day. A maximum evolution in ORP from 180 to 641 mV was found in the experiment at 3.0 initial pH. It clearly showed that the initial pH value of 3.0 helps for creating the effective oxidizing environment for the type of sludge and microorganism used. These results are in line with other reports which suggested that 400-500 ORP range is suitable for the improved bioleaching [11].

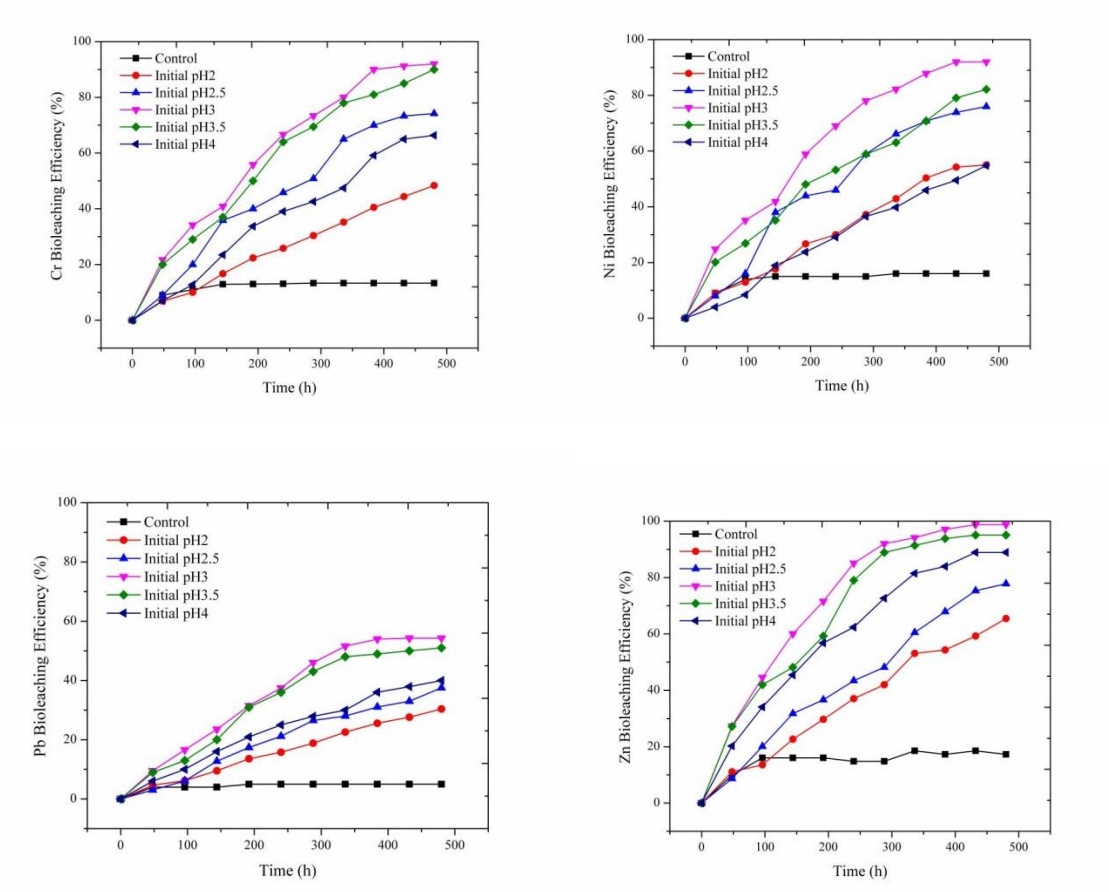
Figure 2 Change in ORP value during bioleaching at different initial pH Values



D. Effect of Initial pH on Heavy Metal Bioleaching

Typical heavy metal removal curves for Zn, Ni, Pb and Cr at different initial pH values are shown in Figure 3. In the control experiments, bioleaching of Zn, Ni, Pb and Cr was 17.28, 16.08, 5 and 13.32%, respectively, at the end bioleaching period. It might be due to solubilization occurring with the addition of sulfuric acid for initializing the pH value to 2.0.

Figure 3 Leaching efficiencies for Cr, Ni, Pb and Zn at different initial pH values.



After 20 days of incubation, the bioleaching efficiencies of Zn were 65.55, 77.7, 98.76, 95.08, and 88.9%; the removal efficiencies of Ni were 55.04, 75.94, 91.96, 82.15, and 54.78%; Pb removal efficiencies were 30.38, 37.52, 54.28, 51.02, and 39.99% and Cr removal efficiencies were 48.33, 74.16, 91.98, 89.98, and 66.44% in the experiments with initial pH values of 2, 2.5, 3, 3.5 and 4, respectively. The highest metal bioleaching was obtained in the experiment with the initial pH value of 3.0. From the study, it is apparent that the leaching efficiency of heavy metals increased by increasing the initial pH up to 3.0. Further increase in initial pH did not yield any higher efficiencies of metal removal. In contrast, reduced metal removal efficiencies were observed in the experiments with initial pH values 3.0 and 4.0. From the studies, it is clear that that the bioleaching efficiency with initial pH 2.5 was optimum for the type of sludge and culture used in this process. It is in line with the published reports suggesting that the initial pH between 2−4 is optimum for *A. ferrooxidans* [12].

Figure 4 EDX-Pattern of (a) primary sludge and (b) leach residue of sludge obtained from experiment at initial pH 3.0.

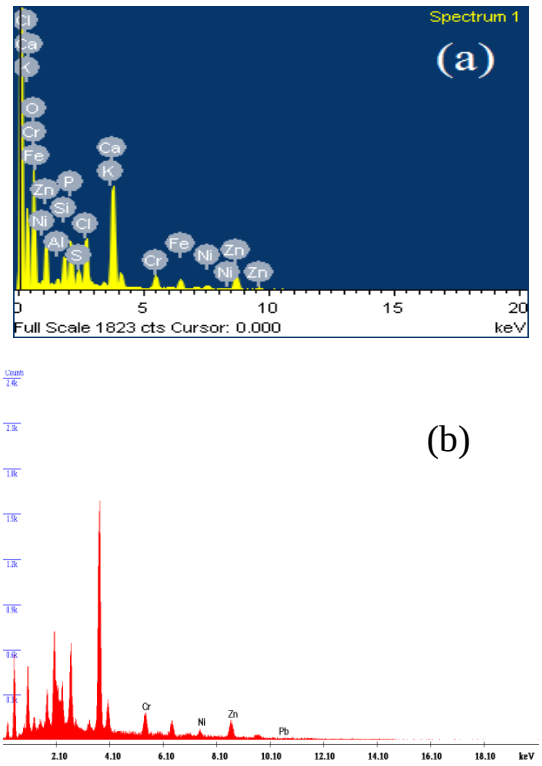
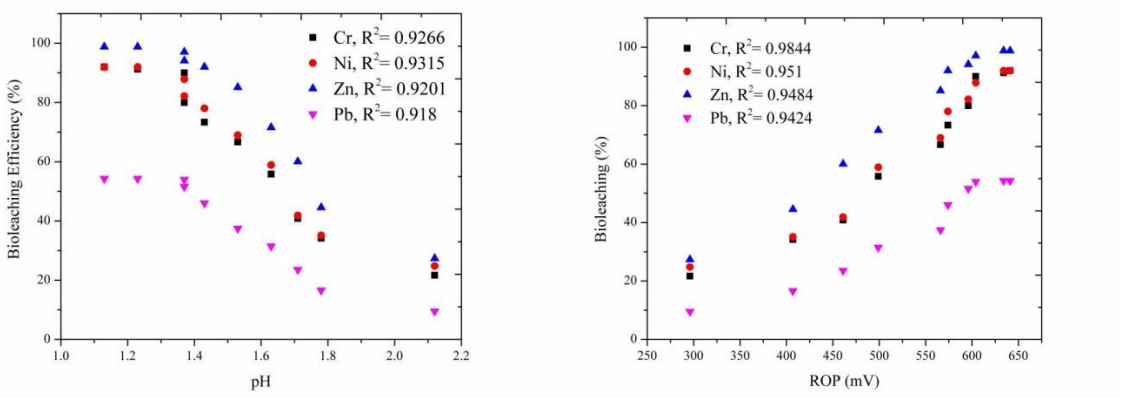


Figure 5 Heavy metal bioleaching relationships with pH and ROP.



The raw sludge and bioleached residue obtained from the experiment with initial pH 3.0 were subjected to elemental analysis using energy-dispersive X-ray spectroscopy (EDX) for confirming the heavy metal reduction. The EDX spectra of raw sludge and leach residue are shown in Fig. 4. Results from the EDX analysis showed that elements O, Na, Mg, Al, Si, S, K, Ca, Cr, Ni, Zn, and Pb were at weight percent of 41.25, 2.85, 0.93, 8.73, 27.96, 0.77, 1.5, 3.7, 1.59, 3.5, 6.74, and 0.45, respectively, for raw sludge and 52.62, 1.85, 0.85, 6.88, 0.47, 24.96, 1.05, 10.47, 0.09, 0.05, 0.65, and 0.05, respectively, for leach residue. It confirms the significant reduction in heavy metal content in leach residue of sludge. Some reports have discussed that the bioleaching efficiency of heavy metals is negatively correlated with medium pH, but positively correlated with ORP [13]. However, the correlations fail to explain the solubilization of heavy metals. As can be seen in Fig. 5, a high correlation was found between the bioleaching efficiency of heavy metal with pH, and ORP. The data presented showed that the bioleaching efficiency of heavy metal had a significant negative correlation with pH and a positive correlation with ORP. Future studies are needed to investigate detailed chemical speciation changes and the bio-oxidation of *A. ferrooxidans* during the bioleaching process.

IV. Conclusion

A study on the removal of heavy metal from the sewage sludge with due importance for understanding the influence of initial pH was carried out. The experiments were performed in 250 mL beakers. The experimental conditions were 100 mL of working volume, 5% (w/v) of sludge loading, and temperature 30 ºC. The results from the experiments proved that the bioleaching efficiencies of heavy metal are effective at the initial pH 3.0. Such a study will be much helpful for understanding and designing the detoxification process with due significance of removal of heavy metal from the harmful sludge before disposal.

V. References

[1] S. Chung, S. Kim, J.O. Kim, and J. Chung, “Feasibility of combining reverse osmosisferrite process for reclamation of metal plating wastewater and recovery of heavy metals,” Industrial & Engineering Chemistry Research, vol. 53, 2014, pp. 15192–15199.

[2] A.F. Moustafa, “ Isothermal reduction process and kinetic of nanomaterials in reducing atmosphere: A review,” Journal of Analytical and Applied Pyrolysis,vol. 127, 2017, pp.126–139.

[3] N. Habbache, N. Alane, S. Djerad, and L. Tifouti, “Leaching of copper oxide with different acid solutions,” Chemical Engineering Journal, vol. 152, 2009, pp. 503-508.

[4] C.J. Chang and J.C. Liu, “Feasibility of copper leaching from an industrial sludge using ammonia solutions,” Journal of Hazardous Materials, vol. 58, 1998, pp.121–132.

[5] F. Akbal, and S. Camc, “Copper, chromium and nickel removal from metal plating wastewater by electrocoagulation,” Desalination, vol. 269, 2011, pp. 214–222.

[6] D. Chena, J. Hou, L.H.Yao, H.M. Jin, G.R. and Z.P. Qian, “Ferrite materials prepared from two industrial wastes: Electroplating sludge and spent pickle liquor,” Separation and Purification Technology, vol. 75(2), 2010, pp. 210–217.

[7] R. Sua, B. Lianga and J. Guana, “Leaching effects of metal from electroplating sludge under phosphate participation in hydrochloric acid medium,” Procedia Environmental Sciences, vol.31, 2016, pp.361–365.

[8] S. Venkatachalam, “Hydrometallurgy1998,” Delhi, India, Narosa Publishing House.

[9] S. Mondal, B. Paul, V. Kumar, D. K. Singh and J.K. Chakravartty, “Parametric optimization for leaching of cobalt from Sukinda ore of lateritic origin – A Taguchi approach,” Separation and Purification Technology, vol.156, 2015, pp.827–834.

[10] O. Levenspiel, “Chemical reaction engineering,” ed. J.W.a. Sons, 1974, Newyork.

[11] D. Bingol and M. Canbazoglu, “Dissolution kinetics of malachite in sulphuric acid,” Hydrometallurgy, vol.72, 2004, pp.159–165. F. Veglio, R. Quaresima, P. Fornari and S. ve Ubaldini, “Recovery of Valuable Metals From Electronic and Galvanic Industrial Wastes By Leaching and Electrowinning,” Waste Management, vol.23, 2003, pp.245–252.

[12] C. E. Okon, D.E. Oku, “Initial Thermal Hydraulic Design of a 1000MWe Water Reactor with an Improved Thermal Efficiency,” American International Journal of Research in Science, Technology, Engineering & Mathematics, vol.9(3), Dec 2014, pp.230-238.

[13] D. Jing-Shi, R. Ren-man, W. Jian-Kang, and S. Xue-nan, “Present situation and prospects in bioleaching of sulphides by moderate thermophiles,” Multipurpose Utilization of Mineral Resources., vol.2, 2002, pp. 33−38.

[14] A. Giaveno, L. Lavalle, P. Chiacchiarini, and E. Donati, “Bioleaching of zinc from low-grade complex sulfide ores in an airlift by isolated Leptospirillum ferrooxidans,” Hydrometallurgy, vol.89, 2007, pp.117−126.

[15] W. Xin, C. Srinivasakannan, D. Xin-hui, P. Jin-hui, Y. Da-jin, and Jushao-hua, “Leaching kinetics of zinc residues augmented with ultrasound,” Sep. Purif. Technol., vol.115, 2013, pp.66−72.

[16] Y. Dong, H. Lin, K. Fu, X. Xu, and S. Zhou, “Bioleaching of two different types of chalcopyrite by Acidithiobacillus ferrooxidans,” Int. J. Min. Met. Mater, vol.20, 2013, pp.119−124.

[17] S.V. Prabhu and R. Baskar, “Effects of sulfur concentration and kinetics on heavy metal bioleaching from electroplating sludge using bubble column,” Research Journal of Chemistry and Environment, Vol.18(12), 2014, pp.70-76.

[18] S.V. Prabhu and R. Baskar, “Kinetics of Heavy Metal Biosolubilization from Electroplating Sludge: Effects of Sulfur Concentration, Journal of the Korean Society for Applied Biological Chemistry, vol. 58(2), 2015, pp. 185–194.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Eco-friendly mosquito repellent fabric using dye derived from *Curcuma aromatica* Salisb.

A.M. Ananda kumar [1]*, K. Anvar[2], R. Rakkimuthu[3], P. Sathishkumar [4], D. Sowmiya[5] and J. Alahupreeti[6]
*Corresponding Author
[1,2,3,4,5 and6] PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi-01 Tamilnadu, India.

anandbiotech2010@gmail.com

Abstract*: In the present study the mosquito repellent activity was evaluated on ecofriendly dyed fabric. The dye was extracted from two different plant parts viz., rhizome of Curcuma aromatica and Fruits of Terminalia chebula using methanol and water respectively. The cloth was dyed using both extracts and tested for mosquito repellent activity in various soaking time intervals and colour fasting ability. The fabrics fixed the dye very efficiently when mordanted with fruit extract of T. chebula. The originality of natural colour was fixed well when the extract accompanied with natural mordant. These fabrics are induced for mosquito repellency test using a simple excito chamber. The results showed the methonolic extract of C. aromatica performed well in mosquito repellency activity at maximum hour of soaking interval i.e 8hrs of soaking period showed 95% repellency when compared with water extract. The dyeing efficiency was also found to be higher in methanolic extract when compared with water extract*.

Keywords*: Curcuma aromatica, fabrics, excito chamber, mordants, repellency test*

I. INTRODUCTION

Mosquitoes are a serious threat to public health, transmitting several dangerous diseases for over two million people in the tropics. Usage of Insecticides and their residual effects in the environment, have turned the researcher's attention towards natural products. [1&2] Many plant species synthesize a variety of secondary metabolites which play a vital role in defense mechanism against insects. Plants are the one of the major alternative resources to repel the disease spreading mosquitoes using the bioactive chemicals. Harmful disease like Malaria, Dengue fever, Chickengunya, etc. are the most common viral diseases caused due to the bite of an infected *Aedes* mosquito affect a wide range of people in the world. They are threatening diseases as there is no proper vaccine or treatment yet found. Avoiding mosquitoes by wearing suitable long sleeved cloths and clearing the rearing places are the only ways to escape from these dreadful diseases [3 and 4]. So it is necessary to identify the plant based chemicals to repel the mosquitoes.

Nowadays environmental protection has become a challenge for the textile industry because it utilizes a lot of chemicals and water for colouration of textile materials. These chemicals are harmful for both human as well as environment. There has been a revival of interest in natural dyes in the global arena due to their non-polluting, non-carcinogenic and eco-friendly nature [5&6] . Environmentalists are always worried about the rampant use of synthetic dyes in textile industry as they cause water pollution and waste disposal problems. Natural dyes are biodegradable and do not cause any health hazards and hence they can be easily used without much environment concerns. Several draw-backs of some synthetic dyes are their carcinogenic and mutagenic nature. Until 19th century natural dyes were the main colorant in textiles. [7]. The interest in the use of natural dyes has been growing rapidly due to stringent environmental standards imposed by many developing countries in response to toxic and allergic reactions associated with synthetic dyes. [8]. With the world becoming more conscious on environmental scenario, there is a greater need to revive the tradition of natural dye [9].

The natural dyes which are non pollutant appear as an ideal choice due to their non-toxic nature and easy handling. While synthetic dyes are reported with many environmental issues, natural dyes comparatively have several advantages over health, safety and ecology. There is an increased awareness and demand for natural dye products in international markets too. Normally natural dyes sorted in three categories. Dyes

obtained from plants, animals and minerals. Naturally the dyes obtained from flowers and animals considered to be safe as compared to others [10]. Hence the present study was carried out on the plant *C. aromatica* Salisb. belongs to the family Zingiberaceae. The mosquito repellent properties of this plant was not explored well when compared with other species. So the study has been attempted to isolate the dye from *C. aromatica* which is potential mosquito repellent on the fabrics. This may lead to manufacture of fabrics with environmental friendly natural dye of mosquito repellent activity too.

III. Materials and Methods

A. Selection of plant material:

The rhizome of the plant, *Curcuma aromatica* L. belongs to family Zingiberaceae was taken for the present study due to its potential dye yielding capacity and mosquito repellent activity. The fruits of *Terminalia chebula* Retz., belongs to family Combretaceae was used as a mordant. Both the plant materials were collected from Pollachi and Coimbatore. The fruits of the tree is particularly valued as an excellent source of tannins, though it also provides a good quality wood, plus it has a wide range of medicinal and edible uses. It is often cultivated on a commercial basis for the tannin in its fruit, and also for its medicinal properties, especially in India

B. Preparation of Extracts

The rhizome of the *C. aromatica* L. plant material was collected from Pollachi and identified at PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi. The collected rhizomes were shade dried and finely powdered using pulverizer. The powder was kept in a clean dry container for further use. The two different solvent extracts *viz*., methanol and water was prepared using soxhlet apparatus. The extract was stored in clean cool dry place until further use. Similarly the extracts of *Terminalia chebula* was prepared and used as a mordant.

C. Preparation of Cloth

Pure cotton cloths will be used as substrate and it will be sufficiently soaked in mordants such as Sodium hydroxide (NaOH) , and extract of Myrobalan too. The mordants will used to fix the colour which runs through it.

D. Test for mosquito repellent activity (Cage method)

The mosquito repellent test will be carried out by preparing a cage (40 cm x 40 cm) stocking 50 mosquito cage was prepared. The effectiveness of the finish is evaluated by depending upon the number of mosquitoes on the samples and the bites. Measuring the antimosquito effectiveness by counting the number of mosquitoes along on the hand during 2 minutes as per. Finished and unfinished samples were testing the best is based on contact bioassays using recommended WHO Pesticide Evaluation Scheme (WHOPES) procedures which state the testing method. The mosquitoes will be collected from the National centre for disease control, field station, Mettupalayam. [11].

$$\text{Repellent rate percentage} = \frac{\text{Mosquitoes under test} - \text{no.of resting mosquitoes}}{\text{No. of mosquitoes under test}} \times 100$$

E. Wash durability test :

Dyed Sample (fabrics) were dipped in 5 % mild detergent solution for 30 min. After that sample were washed with plain water, rubbed vigorously, squeezed and dried in shade. Wash durability of dyed samples for different time were checked up to 25 times of launderings [12]. The variations were observed and photo graphed.

F. Data analysis

Results were averaged and given as mean ± standard deviation, calculated by using MS excel.

IV Results and Discussion

The present study was carried out to prepare a natural dye from C. *aromatica* L. an wild turmeric it is also known as Kasturi manjal in Tamil. The dried rhizome powder was used in traditional rituals from time immemorial. It is also well know for its very important compound curcumin rich in medicinal properties. Positively the rhizome powder is also having good insect repelling activity .

The methanol and water extract of rhizome was prepared using soxhlet apparatus. The processed cotton cloth was soaked in *Curcuma aromatica* extract in various time intervals. Comparatively the methanolic extract fixed the dye considerably when compared with water extract in all time intervals of soaking hours. The importance of mordant was showed in the fabrics which dyed both in mordant and *C. aromatic* dye fix well and the original

colour was obtained. This showed the mordants are most necessary to fix the dye for long lasting. And the plates are represented the effectiveness of the mordant also. The extracts of rhizome part of *Curcuma aromatica* showed good dyeing ability and exhibited effective mosquito repellent activity. The above results of the studies was supported by many workers and explained that the extracts of various plants and the phytochemicals of different parts of the plants are responsible for the mosquito repellent activity [13]. The extracts of *Nerium oleander* [14] in *Lawsonia inermis* extract [15] and *Chromolaena odorata* extract were taken for the study and gave good results on fabric coating and repellency against mosquitoes and [16] in his studied that the plant *Dalbergia sissoo* exhibited good mosquito larvicidal activity apart from dyeing ability. The extracted dyes were prepared various concentrations by dissolving with their respective solvents. The fabrics to be dyed were soaked for several hours and observed and represented in plate 5&6 they showed the conc. of dyes which is 75 and 100% have good dyeing ability for both methanol and water extracts. The results clearly indicated the concentration of dye is highly important for dyeing the fabrics. Comparatively the 100% concentrated dye was found as good dyeing ability and selected it for further studies. The cloth was also tested for colour fastness and induced to normally washed up to 25 times the colour changes were noticed.

The mosquito repellent activity was tested for the above natural dyed cloth. Clothes treated with natural dye of *C. aromatica* were tested for its efficacy to repel the mosquito. The excito chamber was used for mosquito repellent test. The test showed that the repellent was much higher when compared with untreated cloth. The mosquito repellent activity was tested separately for methonolic extract and water extract. The dye stability and repellency was comparatively much higher in methonlic extract comparatively. The maximum repellent activity was observed at 8 hrs soaked fabric with 95% repellency followed by 90 % repellency found in 6 hours of treatment. The minimum repellency was observed as 80% at 2 hrs of soaking time. The repellent activity also tested after washing 92 % of repellent of activity was observed in 8 hrs of soaking fabric in extract and 75% was the lowest repellent activity was observed in 2hrs of soaking. The extracted *C. aromatica* dyes of rhizome parts have good colour fastness on fabrics and with mordant exhibited significant mosquito repellent activity. The similar finding was reported by [17] the natural products especially extracts from roots, stems, leaves, flowers, fruits and seeds mixed with some natural oils showed good repellent activity against mosquitoes. The work was also supported by [12] that the cotton fabric dyed with Pomegranate peel showed good dyeing ability and also effect mosquito repellent activity. They also depict that natural dye extracted from plant are effective and also promising safety form mosquito vector disease. It is also ecofriendly, biodegradable, non toxic to health of all organism and ecosystem too. The results of [18] was similar to that of present studies the citronella oil extracted from the *Cymbopogon citratus* showed high repellent activity after treating with cotton fabric. They also concluded that the extracts such as *Phyllanthus niruri* and *Vetiveria zizanoides* are also exhibited their good mosquito repellent activity on finished fabrics.

The plant showed 96% repellency as that of present studies which exhibited approximately more than 90% of mosquito repellency. The water extract also exhibited the repellent activity. The mosquitoes repelled up to 85 % in 8 hrs of soaking time. The lowest repellency of 70% and 60 % was observed at 2 hrs of soaking time of the fabrics before and after washing respectively. Similarly the maximum repellency was observed as 85 % and 82 % in before and after washing of fabrics. Even though the soaking hours was differed they exhibited more than 70% of mosquito repellent activity before washing and 60 % repellency was found in washed fabrics (Tables 1-2). According to [19] the finished textiles using herbal method have good repellent activity mosquitoes. They recorded the similar activity on rosemary extract also. Hence the present study clearly depict that the plant extracts and associated phytochemicals have good dyeing ability and mosquito repellent activity.

V. Conclusion

Hence the present study clearly showed that the colour fastness and maximum mosquito repellency was found in methanolic extract coated fabrics at high concentrations. The raw materials for making this ecofriendly dye are cheaper when compared to other chemical so the commercial production must be encouraged at industrial level. Mosquitoes are very dreadful insects in spreading many diseases. Nowadays the mosquito repellents and pesticides are manufactured using various chemicals. This may lead to hazardous to human beings and also to environment. Hence the type eco-friendly mosquito repellent textiles are to be manufactured commercially in large scale for the welfare of society to replace the chemical repellents. The development of mosquito repellent fabrics using plant based dye may suggested for developing defence textiles to safeguard our army personnels from communicable disease through mosquitoes in the war fields.

Table 1: Showing the mosquito repellency of naturally dyed cotton fabric with Methonolic extract of *Curcuma aromatica*

S.No.	Dyeing treatment	Soaking hours	Mosquito repellency of cloth (%)	
		Methanolic Extract	Before wash	After wash
1	Untreated cloth	-	20± 1.25	10±1.34
2	Methanol Extract alone	4	82± 4.50	78±1.52
3	*C. aromatica* dyed cloth with mordant	2	80± 3.06	75±3.50
4	*C. aromatica* dyed cloth with mordant	4	85± 2.08	82±2.00
5	*C. aromatica* dyed cloth with mordant	6	90± 2.51	89±2.51
6	*C. aromatica* dyed cloth with mordant	8	95± 2.64	92±4.04

Values are expressed as mean ± standard deviation (SD)

Table 2: Showing the mosquito repellency of naturally dyed cotton fabric with water extract of *Curcuma aromatica*

S.No.	Dyeing treatment	Soaking hours	Mosquito Repellency of cloth (%)	
		Water Extract	Before wash	After wash
1	Untreated cloth	-	20±1.54	15±1.35
2	Water extract alone	4	70±3.60	60±1.15
3	*C. aromatica* dyed cloth with mordant	2	75±3.21	65±3.00
4	*C. aromatica* dyed cloth with mordant	4	78±1.73	69±1.73
5	*C. aromatica* dyed cloth with mordant	6	80±1.00	75±3.05
6	*C. aromatica* dyed cloth with mordant	8	85±2.52	82±1.52

Values are expressed as mean ± standard deviation (SD)

Fig.1. Graph showing mosquito repellent activity of Methonolic extract dyed fabric on before and after wash.

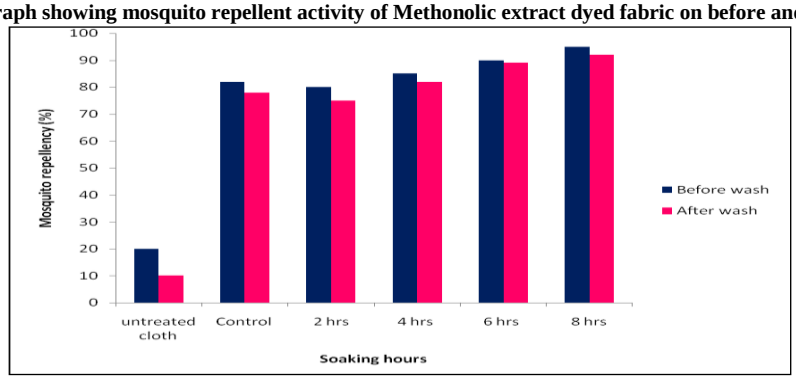
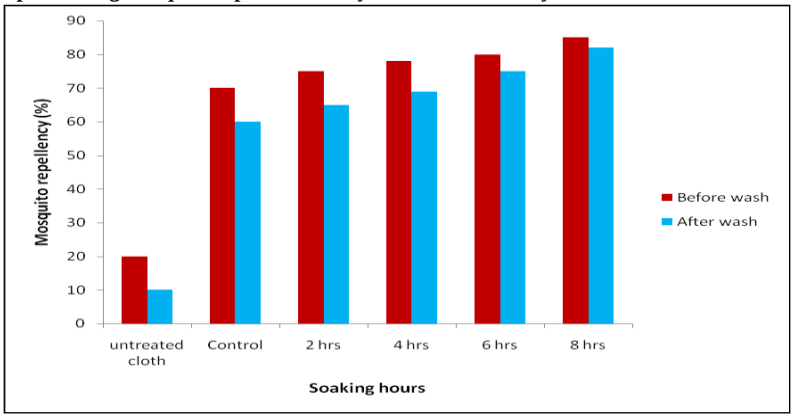


Fig.2. Graph showing mosquito repellent activity of Water extract dyed fabric on before and after wash.



VII Reference

[1] Andualem Getie,. Evaluation of antimalarial activity of seeds of *Dodonaea angustifolia* and leaves of *Entada abyssinica* against plasmodium berghei In swiss albino mice. A Thesis Submitted to the School of Graduate Studies of the Addis Ababa University (Biomedical Science), 2010, pp: 1 – 66.

[2] E. K. Patel, A. Gupta and R. J. Oswal, A Review on: Mosquito repellent methods. *Int. J. of pharm. chem & Bio sci.*, 2012, Vol. 2(3): 310-317.

[3] T.Alan Eaton,. Insect Repellents. Visit our website: *extension.unh.edu*., 2009, pp: 2.

[4] Fikiremariam Haile dessalgne, Solomon Abate Mekonnen and Birhan Abdulkadir IND RIS,. Variability of Pyrethrum (*Chrysanthemum cinerariaefolium*) clones for chemical traits grown at Bekoji and Meraro of south eastern Ethiopia. *Int. J. of Med. & Aromatic Plants*, 2011, Vol. 1(2): 166-174.

[5] Siva, Status of natural dyes and dye yielding plants in India. *Current Sci*., 2007, Vol. 92(7): 916-920.

[6] A.Ali, N. Tabanca, B. Demirci, K. H. C. Baser, J. Ellis, S.Gray, B. R.Lackey, C. Murphy, I. A. Khan and D. E. Wedge, Composition, mosquito larvicidal, biting deterrent and antifungal activity of essential oils of different plant parts of *Cupressus arizonica* var. *glabra* ('Carolina Saphire'). *Nat. Prod. Commun.*, 2013, Vol.8: 257-260.

[7] R. Singh and S. Srivastava, Exploration of Flower Based Natural Dyes - A Review. *Res.J. of Recent Sci.,* 2015, Vol.4(8): 2277-2502.

[8] Thomas Beachtold, Extraction of natural dyes for textile dyeing from coloured plants wastes released from the food and beverage industry. *J. of Sci. of Food & Agri*., 2006, Vol. 86(2): 233–242.

[9] R.Anitha, T. Ramachandran, R. Rajendran and M. Mahalaksmi, Micro encapsulation of lemon grass oil for mosquito repellent finishes in polyester textiles. *Elixir. Bio. Phys.*, 2007, Vol. 40: 5196-5200.

[10] Redwanjihad, Dyeing of silk using natural dyes extracted from local plants. *Int. J. of Sci. & Eng.Res*., 2014, Vol. 5(11): 809-818.

[11] Granch Berhe Tseghai, Mosquito Repellent Finish of Cotton Fabric by Extracting Castor Oil. *Int. J. of Sci. & Eng. Res.*, 2016, Vol. 7(5): 873-876.

[12] Rimpi Pandey and Archana singh, Development of eco friendly mosquito repellent cotton fabrics. *Int. J. of cur. Res.* 2016 Vol.8(7): 33877-33879

[13] R. K. Raveen, T. Kamakshi, M. Deepa, S. Arivoli and Samuel Tennyson, Larvicidal activity of *Nerium oleander* L. (Apocynaceae) flower extracts against *Culex quinque* fasciatus Say (Diptera: Culicidae). *Int. J. Mosq. Res.,* 2014, Vol. 1(1): 8-42.

[14] Dass K. and P. Mariappan, Larvicidal activity of *Lawsonia inermis* and *Murraya exotica* leaves extract on filarial vector, *Culex quinquefasciatus*. *Int. J. Mosq. Res*., 2014, Vol.1(2): 25-27.

[15] J. Sukhthanker, H. Hemanth Kumar, Godinho M.H.S. and Ashwani Kumar, Larvicidal activity of methanolic leaf extracts of plant, *Chromolaena odorata* L. (Asteraceae) against vector mosquitoes. *Int. J. Mosq. Res.,* 2014, Vol. 1(3): 33-38.

[16] S. S.Cheng, H. T. Chang, S. T. Chang, K. H. Tsai and W. J.Chen. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae, *Biores. Technol.*, 2003, Vol. 89(1): 99-102.

[17] M. Isman, Growth inhibitory and antifeedant effects of azadiractin on six noctuids of regional economic importance. *Pesticide Sci.*, 1993, Vol. pp: 381: 57-63.

[18] M. Anish Sharmila, P. Senthil Priya, S. Boobalan and Karthikeyan L. M.. Development of mosquito repellent finished cotton fabric using eco friendly Cymbopogan citros oil. *Int. J. of Sci. Tech. & Manag*., 2015 Vol. 4(2): 96-101.

[19] J. Banupriya and V. Maheshwari. Effects of mosquito repellent finishes by herbal method on textiles. *Int. J. of Pharm. & Life Sci.*, 2013, Vol. 4(11): 3133-3134.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Ruthenium(II) complexes of benzothiazolamine Schiff bases: Synthesis, characterization and in vitro cytotoxicity

R. Valarmathi[1], K. Sampath[2] and C. Jayabalakrishnan[3,*]

[1]Department of Chemistry, Hindustan College of Engineering and Technology, Coimbatore – 641 032, Tamil Nadu, INDIA.

[2]Department of Chemistry, Kumaraguru College of Technology, Coimbatore – 641 049, Tamil Nadu, INDIA.

[3]PPG College of Arts and Science, Coimbatore – 641 035, Tamil Nadu, INDIA.

drcjbstar@gmail.com

Abstract*: Four new Ruthenium(II) complexes were synthesized from the metal precursors of the type [RuHCl(CO)(EPh3)3] (where E= P/As) with monobasic tridentate benzothiazolyl Schiff base ligands. Structural features of the ligands and its ruthenium(II) complexes were determined by various physico-chemical methods and spectral (IR, UV-Vis and NMR) techniques. The in vitro cytotoxic activity of the ligands and complexes were screened by the panel of bacteria and the selected cancer cell line. The growth inhibition activity of the ligands and complexes reveals that the complexes possesses higher cytotoxic activity than the respective ligands.*

Keywords*: Ruthenium; benzothiazolyl ligands; Schiff bases; MCF-7; Bacteria*

I. Introduction

Ruthenium metal plays a key roles in proteins and enzymes, and also in processing nucleic acids. In recent years, researchers have shown their interest on metal-containing drugs [1]. The metal complexes interact with DNA may lead to number of active anticancer drugs, most prominent being platinum-based; drugs among them being cisplatin which has turned testicular cancer from a deadly disease into a curable one [4]. Instead of their significant activity against cancers, platinum compounds are not sufficiently effective against some of the most frequent human cancers like breast tumor. Moreover, the administrated cisplatin often develop acquired cellular resistance and cause serious ill effects. Thus both the advantages and drawbacks of platinum chemotherapy have motivated to develop alternative transition metal complexes which are less toxic and more effective for chemotherapeutic applications. In this view, ruthenium compounds, because of their favourable properties like kinetic ligand-exchange, variable oxidation states and coordination numbers under physiological conditions and lower toxicity toward normal cells are in the forefront of these investigations [2].

Schiff bases have shown a wide spectrum of chemotherapeutic properties. The broad range of biological activities of benzothiazolyl Schiff bases and their metal complexes includes antitumor, antibacterial and antiviral properties [3]. In addition, the biological properties of Schiff bases are further altered by condensation with an aldehyde. With this forefront investigation, we synthesized ruthenium(II) complexes containing mixed ligands. The biological properties of the benzothiazolyl Schiff bases and its complexes have been characterized using various spectroscopic techniques and the cytotoxicity of the ligands and its ruthenium complexes were investigated by a panel of bacteria and MCF-7 cancer cell line.

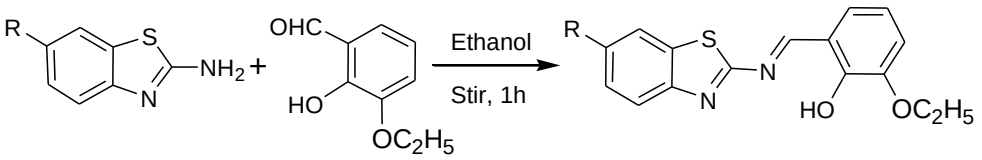
II. Experimental

All the chemicals and solvents were used are chemically pure and AR grade. The cancer cell line, MCF-7, were obtained from National centre for cell science (NCCS), Pune, India. Solvents were purified and dried according to the standard procedure. The metal precursors $[RuHCl(CO)(PPh_3)_3]$ and $[RuHCl(CO)(AsPh_3)_3]$ were prepared by reported literature methods [4,5]. IR spectra were recorded as KBr pellets in the 400-4000 cm^{-1} region using a Perkin Elmer FT-IR 8000 spectrophotometer. Electronic spectra were recorded in DMSO solution with a Systronics double beam UV-Vis spectrophotometer 2202 in the range 200-800 nm. NMR spectra were recorded using TMS and orthophosphoric acid as internal reference, respectively. Anticancer study was carried out at the Kovai Medical Centre and Hospital Pharmacy College, Coimbatore, Tamil Nadu. Melting points were recorded with Veego VMP-DS heating table.

Synthesis of ligand

A solution of 3-ethoxysalicylaldehyde (0.01 mol) in ethanol was slowly added with stirring to a solution of 2-aminobenzothiazole/2-amino-6-methylbenzothiazole (0.01 mol) in methanol over a period of 1 h. The formed

yellow colored precipitate was filtered off and purity was checked by TLC. The outline of synthesis of Schiff base ligands is given in Scheme 1.



Ligand	R
HL^1	H
HL^2	CH_3

Scheme 1 Synthesis of Schiff base ligands

Synthesis of complexes

A solution of respective metal precursor (0.5 mmol) in benzene was slowly added with stirring to a solution of the respective ligand (0.5 mmol) in methanol and refluxed for about 6 h. The resulting colored solution was kept aside. The formed precipitate is filtered off and subjected to purification by TLC.

Antibacterial activity

The antibacterial activity was screened against benzothiazolyl ligands and complexes by agar diffusion method. In the petri plates, pour 10 mL of Mueller Hinton Agar and allowed to solidify. Uniformly spread 0.1 mL of standardized bacterial suspension (2x106 cells/mL) to this plates and then the well (6mm) was cut at the center. The sample solutions were added and incubated the each well for about 24 h at 37 °C. The diameter of the inhibition zone was observed around the well and the data were collected as triplicate runs.

In vitro anticancer activity

Cytotoxicity of the complexes was carried out on human cervical cancer cell line (MCF-7) and cell viability was carried out using the MTT assay method [6]. The HeLa cells were grown in eagles minimum essential medium (EMEM) containing 10 % fetal bovine serum (FBS). For the screening experiment, the cells were seeded onto 96-well plates at plating density of 10,000 cells/ well and incubated to allow for cell attachment at 37 °C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to the addition of the complexes. The compounds were dissolved in DMSO and diluted in the respective medium containing 1 % FBS. After 24 h the medium was replaced with the respective medium with 1 % FBS containing the compounds at various concentrations and incubated at 37 °C under conditions of 5 % CO_2, 95 % air and 100 % relative humidity for 48 h. Triplication was maintained and the medium not containing the compounds served as control. After 48 h, 15 mm3 of MTT (5 mg / cm3) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then removed and the formed formazan crystals were dissolved in 100 mm^3 of DMSO. The absorbance was then measured at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula and a graph was plotted with the percentage of cell inhibition versus concentration. From this, the IC_{50} was calculated: % cell Inhibition = [mean OD of untreated cells (control)/mean OD of treated cells (control)] x 100.

III. Results and Discussion

The analytical (Table 1) and spectroscopic results reveal that the existence of 1:1 metal to ligand stoichiometry in all the four ruthenium(II) complexes.

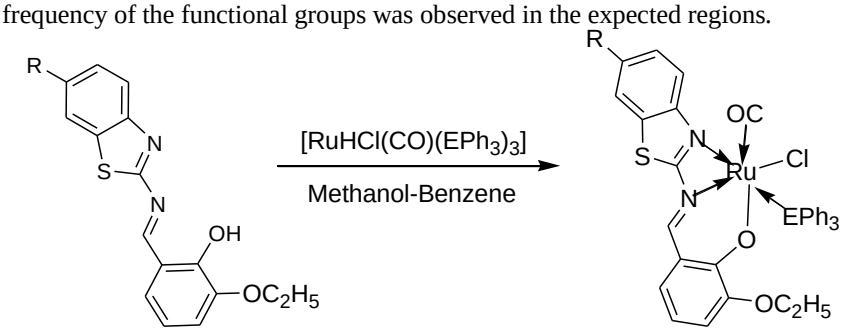
Table 1. Physico-chemical data of the ligands and complexes

Ligands and Complexes	Color	M.Pt., °C	Elemental analysis calculated (Found) %			
			C	H	N	S
HL^1	Yellow	170	64.41 (64.01)	4.73 (5.01)	9.39 (9.55)	10.75 (10.88)
HL^2	Orange	181	65.36 (64.99)	5.16 (4.98)	8.97 (9.03)	10.26 (10.52)
$[Ru(CO)(PPh_3)L^1]$	Green	218	57.97 (58.11)	4.03 (3.86)	3.86 (3.95)	4.42 (4.44)
$[Ru(CO)(AsPh_3)L^1]$	Brown	230	54.66 (54.78)	3.80 (3.81)	3.64 (3.70)	4.17 (4.22)
$[Ru(CO)(PPh_3)L^2]$	Yellow	244	58.49 (58. 21)	4.23 (4.11)	3.79 (3.79)	4.34 (4.33)
$[Ru(CO)(AsPh_3)L^2]$	Dark Brown	163	55.21 (55.01)	3.99 (4.01)	3.58 (3.58)	4.09 (3.99)

All the complexes are stable in air. The benzothiazolyl ruthenium(II) complexes are soluble in most common solvents. The synthesis and proposed structure of the complexes are shown in Scheme 2.

The FT-IR spectral data of the compounds are shown in Table 2. The infrared spectrum of the ligands showed an azomethine frequency in the range of 1621-1625 cm^{-1} which has been decreased in the complexes in the range 1600-1613 cm^{-1}. This confirms the coordination of benzothiazolyl ligand to the ruthenium metal [3]. The phenolic CO group of ligands show a stretching frequency in the range of 1232-1240 cm^{-1} which increased in the spectra of the complexes confirms the other coordination is through O atom. The thiazolyl C=N stretching frequency of the ligands was appeared at 1588-1590 cm^{-1}. It has been decreased in the complexes spectrum in the range of

1570-1580 cm^{-1} which confirms the third coordination is through thiazolyl N atom [3]. In all the complexes, the other stretching frequency of the functional groups was observed in the expected regions.



Scheme 2. The synthesis and proposed structure of the complexes, R=H/CH_3; E=P/As

The UV-Visible spectrum of the ligands and benzothiazolyl complexes (Table 2) are recorded in the DMSO solvent. The free ligands showed three bands at 274-424 nm due to ligand centered transitions, π-π* and n-π*. These bands were shifted in the spectrum of the complexes at 302-413 nm. It confirms the coordination of benzothiazolyl ligands to the ruthenium metal [7]. In the visible region, a new band formed at 512-534 nm due to charge transfer bands. The pattern of the spectrum of the ligands and complexes are in good agreement with the reported ruthenium complexes [3,8].

Table 2. FT-IR and Electronic spectral data of the ligands and complexes

Ligands and Complexes	FT-IR, cm^{-1}					UV-Vis, λ_{max} (nm)
	ν(C=N)	ν(Ph-OH)	ν(Ph-CO)	ν(C=O)	ν(C=N) thiazole	
HL^1	1621	3386	1240	-	1588	305, 363, 422
HL^2	1625	3390	1232	-	1590	274, 375, 424
$[RuCl(CO)(PPh_3)L^1]$	1612	-	1284	1946	1571	310, 365, 413, 532
$[RuCl(CO)(AsPh_3)L^1]$	1613	-	1290	1950	1570	307, 364, 390, 534
$[RuCl(CO)(PPh_3)L^2]$	1600	-	1276	1961	1573	314, 356, 395, 512
$[RuCl(CO)(AsPh_3)L^2]$	1605	-	1274	1944	1580	302, 355, 384, 521

The NMR spectra of the ligands and complexes are recorded using deutrated DMSO solvent and tabulated in Table 3. In the ^1H NMR, the phenolic OH group of the benzothizolyl ligands appeared at δ 11.21-11.27. This peak was disappeared in the spectrum of the complexes confirms the coordination through phenolic oxygen atom. The azomethine proton signal showed at δ 9.89-9.91 in the complexes which was downfielded than the respective ligands (δ 9.39-9.45) [8]. It reveals the second coordination through azomethine N atom. The aromatic protons of the ligands and complexes are displayed δ 6.70-8.10 where the other protons (ethoxy CH_2, ethoxy CH_3 and CH_3) are appeared in the appropriate regions.

Table 3. ^1H NMR spectral data of ligands and complexes

Ligands and Complexes	^1H NMR data, δ					
	Ph–OH	HC=N	Aromatic	Ethoxy OCH_2	Ethoxy CH_3	CH_3
HL^1	11.21	9.45	6.94-8.10	4.14	1.40	-
HL^2	11.27	9.39	6.88-7.85	4.13	1.39	2.44
$[RuCl(CO)(PPh_3)L^1]$	-	9.89	6.70-7.64	4.05	1.39	-
$[RuCl(CO)(AsPh_3)L^1]$	-	9.90	7.11-7.88	4.05	1.36	-
$[RuCl(CO)(PPh_3)L^2]$	-	9.91	6.70-7.64	4.05	1.35	2.51
$[RuCl(CO)(AsPh_3)L^2]$	-	9.89	7.05-7.79	4.04	1.36	2.51

The ^{13}C NMR signals of the ligands and complexes are shown in Table 4. In the uncoordinated ligands, the carbon signals of C=N and thiazolyl C=N were showed at δ 151 and δ 166 respectively. Upon coordination, theses peaks were shifted in the region δ 163-167 and δ 170-173 respectively which supports the coordination of benzothiazolyl ligand to ruthenium atom [3]. The ethoxy CH_3 and CH_2 carbon signals were appeared at δ 14-15 and δ 64-66 respectively. All other carbon signals are observed in the expected regions.

In the complexes, $[RuCl(CO)(PPh_3)L^1]$ and $[RuCl(CO)(PPh_3)L^2]$, the phosphorous signal were appeared as a singlet at δ 25. In general, all the analytical and spectroscopic studies clearly reveals that the benzothiazolyl ligands act as tridentate (ONN) ligand to the ruthenium atom.

Table 3. ^{13}C and P^{31} NMR spectral data of ligands and complexes

Ligands and Complexes	^{13}C NMR data, δ							^{31}P NMR data, δ PPh_3
	Aromatic Carbon	C=N imine	C=O	Ethoxy CH_2	Ethoxy CH_3	CH_3	C=N thiazole	
HL^1	118-147	151	-	64	15	-	166	-
HL^2	117-149	151	-	64	14	21	166	-
$[RuCl(CO)(PPh_3)L^1]$	128-134	164	192	64	15	-	170	25.52
$[RuCl(CO)(AsPh_3)L^1]$	116-139	167	193	64	15	-	171	-
$[RuCl(CO)(PPh_3)L^2]$	128-140	164	194	64	15	21	173	25.50
$[RuCl(CO)(AsPh_3)L^2]$	128-139	163	192	66	15	22	172	-

Biological activity

The antibacterial activity of the ligands and complexes are shown in Table 5. All the complexes have shown significant cytotoxic activity than the respective benzothiazolyl ligands which reveals that the coordination of the Schiff base ligand to the metal can considerably increases the cytotoxic activity [9]. Though the complexes have shown potential activity against the selected bacteria, none of them could reach the standard drug, Ciprofloxacin. Among the selected bacterial species, the complexes showed higher cytotoxic activity against *Vibrio chlorae.*

Table 5, Antibacterial activity

Ligands and Complexes	Diameter of inhibition zone (mm)[a]		
	Escherichia coli	*Pseudomonas aeruginosa*	*Vibrio chlorae*
HL^1	15	15	16
HL^2	17	18	20
$[RuCl(CO)(PPh_3)L^1]$	18	19	21
$[RuCl(CO)(AsPh_3)L^1]$	20	20	23
$[RuCl(CO)(PPh_3)L^2]$	21	20	21
$[RuCl(CO)(AsPh_3)L^2]$	22	22	24
Ciprofloxacin	23	26	28
DMSO	No activity		

[a] Values are an average of triplicate runs.

The antibacterial activity of the ligands and complexes motivated us to explore their *in vitro* anticancer studies by MTT assay against MCF-7. The IC_{50} value of the ligands and complexes are shown in Table 6. The benzothiazolyl ligands and complexes exhibited potent *in vitro* antitumor activity. The ruthenium complexes have shown higher cytotoxic activity than the respective free ligands [10]. Among the complexes, the complexes with additional methyl group are more toxic towards the selected cancer cells than the other.

Table 6. *In vitro* anticancer activity of the ligands and complexes against MCF-7 cancer cell line

Ligands and Complexes	IC_{50}
HL^1	103
HL^2	53
$[RuCl(CO)(PPh_3)L^1]$	44
$[RuCl(CO)(AsPh_3)L^1]$	21
$[RuCl(CO)(PPh_3)L^2]$	29
$[RuCl(CO)(AsPh_3)L^2]$	24

IV. Conclusion

The potential antitumor ruthenium(II) complexes were synthesized by the complexation newly synthesized benzothiazolyl ligands with metal precursors. All the ligands and complexes were characterized by micro analysis and various analytical and spectrotroscopic *viz.*, FT-IR, UV-Vis, ^1H, ^{13}C & ^{31}P NMR, techniques. Both the

ligands are coordinated with ruthenium atom *via* ONN tridentate. An Octahedral geometry has been tentatively proposed for all the complexes. The cytotoxicity of the ligands and complexes were screened against a panel of bacteria and the selected cancer line. The in vitro studies clearly shows that the complexes are more toxic than the ligands. In particular, the complexes with additional methyl group showed higher activity than the other complexes.

V. References

[1] A. Kumar Singh, D. Shankar Pandey, Q. Xua et al., "Recent advances in supramolecular and biological aspects of arene ruthenium(II) complexes," Coord. Chem. Rev., vol. 270-271, pp. 31-56, 2014.

[2] B. T. Paterson, J. G. Collins, F. M. Foley et al., "Dinuclear ruthenium(II) complexes as probes for DNA bulge sites" J. Chem. Soc. Dalton Trans., pp. 4343-4350, 2002.

[3] K. Sampath, M. Mohanraj, C. Jayabalakrishnan, "DNA interaction and antioxidant studies of ruthenium (II) complexes containing mixed ligands," Inorg. Nano Met. Chem., vol. 47, pp. 1049-1056, 2017.

[4] K. Natarajan, U. Agarwala, "β-Diketonato(Triphenylphosphine) complexes of ruthenium(II)," Inorg. Nucl. Chem. Lett., vol. 14, pp. 7-10, 1978.

[5] R. A. Sanchez-Delgado, W. Y. Lee, S. R. Choi et al., "The chemistry and catalytic properties of ruthenium and osmium complexes. part 5. synthesis of new compounds arsine ligands and catalytic activity in the homogeneous hydrogenation of aldehydes," Trans. Met. Chem., vol. 16, pp. 241-244, 1991.

[6] T. Mossman, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," J. Immnol. Methods, vol. 65, pp. 55-63, 1983.

[7] S. Sathiyaraj, K. Sampath, C. Jayabalakrishnan, "Synthesis, Spectral Characterization, DNA Binding, DNA Cleavage, and Antioxidant Studies of Ruthenium(III) Heterocyclic Thiosemicarbazone Complexes," Synth. React. Inorg. Met. Org. Chem., vol. 44, pp. 1261-1271, 2014.

[8] B. Ghosh, P. Adak, S. Naskar et al., "Ruthenium(II) complexes of thiosemicarbazones: synthesis, X-ray crystal structures, spectroscopy, electrochemistry, DFT studies and fluoride sensing properties," Inorg. Chim. Acta, vol. 459, pp. 1-14, 2017

[9] S. Kannan, M. Sivagamasundari, R. Ramesh et al., "Ruthenium(II) carbonyl complexes of dehydroacetic acid thiosemicarbazone: Synthesis, structure, light emission and biological activity," J. Organomet. Chem., vol. 693, pp. 2251-2257, 2008.

[10] G. Kalaiarasi, S. R. Jeya Rajkumar, S. Dharani et al, "Biological evaluation of new organoruthenium(II) metallates containing 3-acetyl-8-methoxy-2H-chromen-2-one appended CNS donor Schiff bases," J. Organomet. Chem., vol. 866, pp. 223-242, 2018.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Mathematical Modeling of Commensal Relation between *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

Sivaprakash. B
Department of Chemical Engineering , Annamalai University, Annamalainagar
Email: shiv_bhaskar@yahoo.com

Abstract : *In mixed culture microbial systems the interaction among the existing species plays a vital role in overall performance of the process. Though the interactions can be classified as negative and positive types in perspective of the single species' survival or growth, all interactions are viewed as beneficial because they act to maintain ecological balance in global context. However in specific usage of microbial processes, interaction pattern need to be studied thoroughly for maximum output in terms of maximum degradation in waste treatment and maximum yield in production processes. The present investigation deals with mathematical analysis of commensal interaction between Streptococcus thermophilus (1) and Lactobacillus bulgaricus (2) to design a chemostat for continuous process. It was observed that the two microorganisms follows logistic type growth in pure culture and the species 1 experiences linear interaction pattern in mixed culture. The growth and interaction parameters were computed using MATLAB software and the optimum operating condition for maximum growth of the organisms in the chemostat was simulated using numerical integration technique.*

Key words: *Chemostat, microbial interaction, dilution rate, commensalism*

I. Introduction

The importance of mixed populations of microorganisms can be traced to the beginnings of life on earth. In natural systems mixed populations of microorganisms are the rule rather than the exception. The natural cycles of carbon, nitrogen, oxygen and numerous other elements on our planet all require the active participation of many different microorganisms [1]. Among commercial processes, biological waste water treatment, cheese manufacture, production of ethanol, hydrogen, etc are few examples where multiple microbial species are required. Distinct microbial populations frequently interact with each other which form the driving force in the evolution of community structure [2]. Six patterns of interactions have been observed namely, neutralism, amensalism, competition, commensalism, mutualism and predation (also parasitism) [3]. Interactions between microbial populations can be recognized as negative interactions (competition and amensalism), positive interactions (commensalism and mutualism) or interactions that are positive for one but negative for the other population (predation / parasitism). In simple communities, one or more of these interactions can be observed and studied. Within a complex natural biological community, all these possible interactions will probably occur between different populations[4]. The performance and behavior of the mixed culture systems greatly depends on the interaction patterns among the various species involved[5], [6],[7].

The commensal pattern of interaction is a common phenomenon occurring in mixed culture systems concerned with food processing operations[8]. Such kind of interaction is observed between *Streptococcus thermophilus* and *Lactobacillus bulgaricus*[9]. These two organisms are often present in association in dairy processing operations. Manufacture of cultured dairy products depends at some point on controlled production of lactic acid. Generally, selected strains of organisms are added to milk at some stage of manufacture to bring about these changes. Single species seldom are sufficient, so mixtures of cultures possessing desirable capabilities are most often used. These organisms usually have complex nutritional requirements for growth and activity. The objectives of the present study are to predict the kinetic parameters for the growth rate of both these species in pure culture using logistic equation and to develop suitable equations that account the interaction effects between them and determination of the interaction constants. Also an attempt has been made to estimate the dilution rates for stable operating conditions for coexistence of both the species in a chemostat.

II. Experimental

The associative growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, find applications in the manufacture of yogurt and Italian and Swiss chesses. The report of Moon and Reinbold [9] on the investigation of this system at 37ºC with the ratio of numbers of lactobacilli to streptococci of 2:1 at inoculation has been taken in this modelling study. They used Lee's agar for enumeration of coccus species in mixed and pure culture, but modified to increase recovery efficiency by addition of 0.3% carboxylmethyl cellulose, o.1% Tween 80 and 0.05M phosphate buffer. *S. thermophilus* colonies were counted after 30 h incubation at 32 ºC. At this time and temperature, *L. bulgaricus* will not form colonies on this medium. Viable numbers of *L. bulgaricus* in mixed and pure culture were determined by colony formation on LBS agar. *S.thermophilus* did not form colonies on this agar. Their study indicated that when cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* produced more acid in mixed cultures than in single strain culture. Growth of *S. thermophilus* in mixed culture was enhanced during the exponential phase and reached higher numbers in the stationary phase than when grown alone. *Lactobacillus bulgaricus* liberated Seitz-filterable compounds during its growth that stimulated growth and acid production of *Streptococcus thermophilus.* These compounds are believed to be responsible for the commensal response observed in mixed cultures. Though there is commensal effect for the coccal species, its growth rate after 5 h decreased. The reason for decline during 5 to 15 h remains unexplored whereas after 15 h, pH reduction due to acid production is found to create an unfavourable environment that suppresses the growth of coccal species. The salient feature of this pair include not only the commensal effect for streptococcus by bacillus species but also the influence of competition for the nutrient factors on the system wherein due to the rapid growth, coccal species behaved as a competitor for the bacillus.

A. Mathematical modelling

The model equations for *Streptococcus thermophilus* (species 1) and *Lactobacillus bulgaricus* (species 2) is given by

$$\frac{dx_1}{dt} = k_1 x_1 (1 - \beta_1 x_1) + a_{12} x_2 \qquad (1)$$

$$\frac{dx_2}{dt} = k_2 x_2 (1 - \beta_2 x_2) \qquad (2)$$

where x_1 and x_2 are the microbial abundance (numbers) of species 1 and 2 respectively. k_1, k_2 (h^{-1}), β_1 and β_2 (ml/numbers) are the constants of the logistic model for species 1 and 2 respectively and a_{12} (1/h) is the interaction parameter.

The equations for continuous operation in a chemostat are given by

$$\frac{dx_1}{dt} = -Dx_1 + k_1 x_1 (1 - \beta_1 x_1) + a_{12} x_2 \qquad (3)$$

$$\frac{dx_2}{dt} = -Dx_2 + k_2 x_2 (1 - \beta_2 x_2) \qquad (4)$$

where D is the dilution rate in h^{-1}.

These two equations under steady state conditions get reduced to

$$Dx_{1s} = k_1 x_{1s} (1 - \beta_1 x_{1s}) + a_{12} x_{2s} \qquad (5)$$

$$Dx_{2s} = k_2 x_{2s} (1 - \beta_2 x_{2s}) \qquad (6)$$

where x_{1s} and x_{2s} are the steady state concentrations of the two species that would be obtained in a chemostat under stable operating conditions.

From equation (6), x_{2s} can be solved for a given dilution rate and is given by

$$x_{2s} = \left(1 - \frac{D}{k_2}\right)\frac{1}{\beta_2} \qquad (7)$$

From the above equation it can be inferred that the essential condition for x_{2s} to be greater than zero is that $D < k_2$. Now x_{1s} can be obtained by substituting for x_{2s} from equation (7) into (6) which is given as follows.

$$x_{1s} = \frac{-(D-k_1)+\sqrt{(D-k_1)^2+4k_1\beta_1(a_{12}x_{2s})}}{2k_1\beta_1} \qquad (8)$$

III. Results and Discussions

A. Batch culture

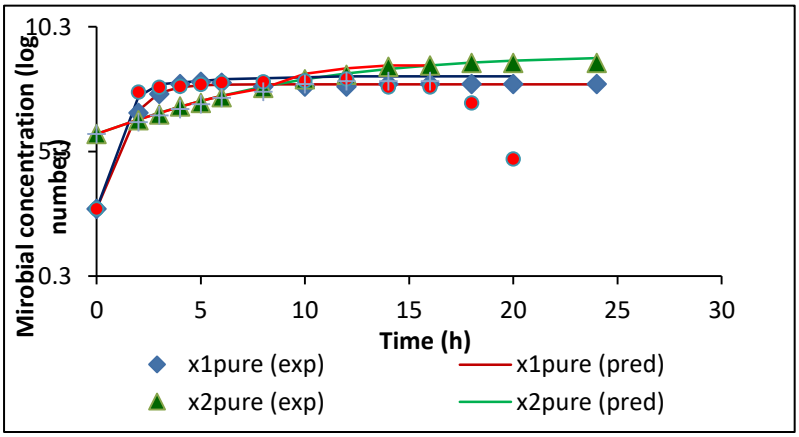
The logistic model is found to fit the experimental data in an appreciable manner with the regression coefficient of 0.9892 and 0.9885 for species 1 and 2 respectively. The values of the logistic coefficients, along with the correlation coefficients obtained using cftool kit available in MATLAB 7.1 version is presented in Table 1. The growth rate of coccus species is found to be more than that of bacillus species. The maximum growth is attained at 5 h itself for the former species whereas it took around 18 h for the latter. Though with respect to growth rate streptococcus dominates, the maximum growth is attained for the lactobacillus. This is evident from the stationary phase numbers obtained in each case (for coccus species it is 1.26 x 10^8 and for bacillus species it is 7.08 x 10^8). This trend is also explained from the values of the logistic constants k for the two species. The logistic model explains the growth pattern of both the species in pure culture with reasonable accuracy. The error for the species 1 is 12. 8 % whereas for second species it is 13.4 %

Table 1 Values of the logistic constants

Species	k (h^{-1})	β (ml/number)	R^2
Streptococcus thermophilus	**4.224**	**1.00 x 10^{-8}**	**0.9892**
Lactobacillus bulgaricus	**0.52**	**1.41 x 10^{-9}**	**0.9885**

The model for associated culture growth is found to fit the experimental growth of the coccal species with reasonable accuracy till 5 hours and beyond this time the model cannot represent the experimental data. This is because of the reason that the environmental condition that prevails after 5 h changes in terms of pH that is detrimental to the coccal species' growth. This effect has not been incorporated into the model as it is too complicate. The commensal effect is evident from the equation (3) (positive interaction) for coccus species. The interaction parameter a_{12} is calculated as 10.281 (1/h). The model for bacillus species is able to predict the mixed culture with an error of 17.36% and the comparison between experimental and predicted data for pure and mixed culture growths is shown in figure 1.

Fig. 1 Experimental and predicted growths under pure and mixed culture operations

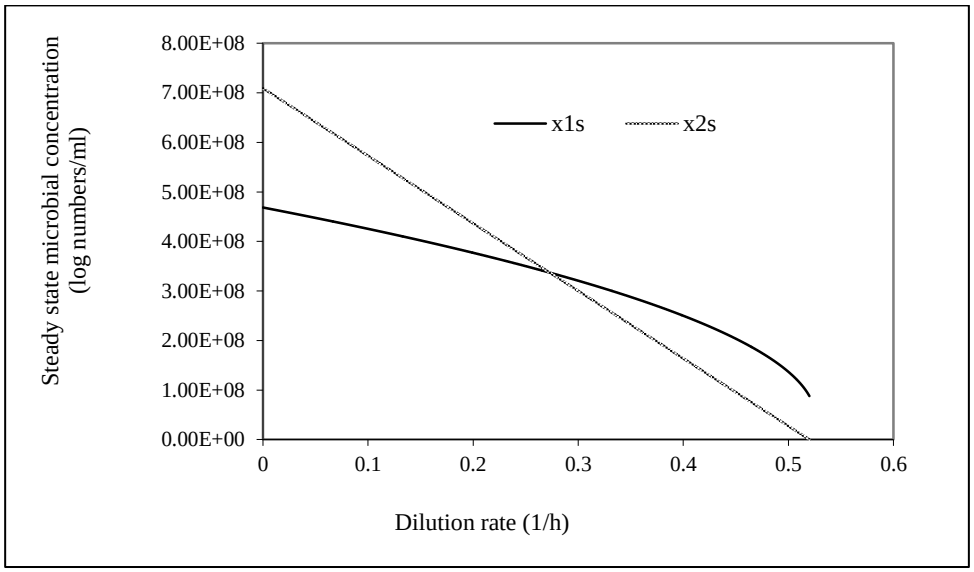


B. Continuous culture - Chemostat Model

Since most of the industrial operations are generally carried out in continuous mode, the model has been extended to a chemostat given by equations (3) and (4). Based on the constraint observed from equation (7), it is obvious that the dilution rate to be maintained in the chemostat should not exceed 0.52 h^{-1}. Because dilution rates higher than this value will result in the extinction of the second species. The equations (7) and (8) are solved to simulate the dilution rate and the respective steady state concentrations of both the species and the result is presented in figure 2 as a plot of the dilution rate versus the steady state microbial abundance of the two species. The simulated data shows the strong dependence of the steady state microbial abundance of both the species in mixed culture on the dilution rate of the chemostat. Though the dilution rate affects the steady state yield of both the organisms, the bacillus species is affected more when compared to the bacillus species' yield.

Also the coccal species experiences a sharp decline in its steady state concentration, whereas the decline is gradual for the bacillus species. Equal amounts of the microbial concentrations of the two species are found to be obtained for the dilution rate of 0.26 h^{-1}.

Fig.2. Dilution rate Vs steady state microbial concentrations



It is implied from the figure 2 that when the dilution rate is maintained below 0.26 h^{-1} the yield of bacillus species is predominant and in the range $0.26 > D > 0.52$ the coccal species' yield will be predominant. Thus when the species 1 or the product from it is desired the chemostat should be operated in the dilution rate below 0.26 h^{-1} and if the second species is desired in more amount the dilution rate greater than 0.26 h^{-1} should be used. One important point to be noted while carrying out this mixed culture growth in the chemostat is that the pH needs proper monitoring and control that is to be maintained that favours the effective growth of both the species as the lactic acid produced by bacillus species decreases the pH causing unfavourable environment for the coccal species.

IV. Conclusion

The present investigation deals with mathematical analysis of commensal interaction between Streptococcus thermophilus (1) and Lactobacillus bulgaricus (2) to design a chemostat for continuous process. It was observed that the two microorganisms follows logistic type growth in pure culture and the species 1 experiences linear interaction pattern in mixed culture. The growth and interaction parameters were computed using MATLAB software and the optimum operating condition for maximum growth of the organisms in the chemostat was simulated using numerical integration technique.

References

[1] Grootjen DRJ, Meijlink HHM, van der Lans RGJM, Luyben KChAM. Cofermentation of glucose and xylose with immobilized *Pichia stipitis* and *Saccharomyces cerevisiae*. Enzyme Microbiol Technol 1990;12:860–4.

[2] Kondo T, Kondo M. Efficient production of acetic acid from glucose in a mixed culture of *Zymomonas mobilis* and *Acetobacter* sp. J Ferment Bioeng 1996;81:42–6.

[3] Bailey, E. James and David F. Ollis, Biochemical Engineering Fundamentals, McGraw-Hill, Newdelhi, 1986.

[4] Picon, A., and M. Nuñez, Growth stimulation of a proteinase positive *Lactococcus lactis* strain by a proteinase negative *Lactococcus lactis* strain, International Journal of Food Microbiology, 2007, 119, 308–313.

[5] Vasiliadou, I. A., S. Pavlou and D. V. Vayenas, Dynamics of a chemostat with three competitive hydrogen oxidizing denitrifying microbial populations and their efficiency for denitrification, Ecological Modelling, 2009, 220, 1169–1180.

[6] Sivaprakash, B, T.Karunanithi and S.Jayalakshmi, Modeling of Microbial Interactions using Software and Simulation of Stable Operating Conditions in a Chemostat, Proceedings published by International Journal of Computer Applications, 2011, 15-21.

[7] Sivaprakash, B, T.Karunanithi and S.Jayalakshmi, Application of Software in Mathematical Bioscience for Modeling and Simulation of the Behavior of Multiple Interactive Microbial Populations, CCIS, Springer,2011, Vol.145, 128 – 137.

[8] Tohyama M, Patarinska T, Qiang Z, Shimizu K. Modeling of the mixed culture and periodic control for PHB production. Biochem Eng J 2002;10:157–73.

[9] Moon, J. Nancy and G. W. Reinbold, Commensalism and Competition in mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, Journal of Milk Food Technology, 1976, 39, 337–341.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Heat Transfer Studies and Development of Nusselt Number Correlation Using Al_2o_3–CMC and SA-Based Nanofluid on Plate Type Heat Exchanger

A.Raj Kumar, M. Thirumarimurugan, E. Sudalai Manikandan
Department of Chemical Engineering, Coimbatore Institute of Technology
Civil Aerodrome post, Avinashi road, Coimbatore, Tamilnadu,India- 641014.
Email: **rajkumarcit2013@gmail.com**

Abstract: *Thermal performance studies on PHE was conducted using different non-newtonian nanofluid pertains Al_2O_3 as nanoparticles in two different base fluid such as aqueous carboxymethyl cellulose (CMC) and sodium alginate (SA). The current work implies that there is a significant enhancement in heat transfer between hot utility (water) and two different base fluids in a plate type heat exchanger (PHE) using nanoparticle. Non-newtonian nanofluid was prepared using Al_2O_3 in the concentration range of 0.01 and 0.05 weight%. Non-newtonian nanofluid (cold utility) and water were operated in both parallel and countercurrent flow pattern in PHE. The thermal analysis was carried out to determine the effectiveness of PHE and cold fluid Reynolds number, for the different flow rate of cold fluid (2 to 8 lpm) and different concentration of base fluid as 0.01%, 0.05% and 0.1 weight%. The result shows that, effectiveness of PHE increases with increase in the nanofluid concentration and cold fluid flow rate, but it decreases with increase in the concentration of non-newtonian fluid. It was found that the SA-based nanofluid shown better heat transfer enhancement compared to CMC-based nanofluid. The Nusselt number correlations was developed using the least square regression analysis for the estimation of heat transfer coefficient in non-newtonian nanofluid. The correlation found to have a low RMS value of 2.032 for CMC system and 2.58 for SA system.*

Keywords: *Carboxymethyl cellulose, Sodium alginate, Non-newtonian, Nano particle, Alumina.*

I. Introduction

In industry and engineering applications, a plate type heat exchanger has played a vital role, since it is used in industry such as; gas liquefiers, oil and gas processing, automobile radiators, waste heat recovery, aeronautical, cryogenic systems, power plant, air-conditioning, petrochemical industry, food industry, refrigeration and process industries. The advantage of using PHE are their excellent heat transfer performance, compact design and provides facility of alteration of the heat transfer area simply by addition or removal of the plates. A PHE consists of a number of thin plates and has port holes providing a passage for flowing fluids. The corners of the plates are sealed with gasket to support the inter plate channels directing fluids into alternate channels. The plates of a PHE are held together between a fixed frame plate and a moving pressure plate. They are clamped together by compression with a bolt which compresses the gaskets and provides a perfect seal. The transferring of heat takes place through the plate between the channels (e.g., [1], [2]).

The efficacy of heat transfer in heat exchanging equipment greatly depends on the thermo physical properties of working fluids as in [3]. Due to inherent properties of high thermal conductivities, enhanced heat transfer coefficient (HTC) and a small penalty of Pressure drop, nanofluids have been widely used as coolant for the last two decades (e.g.,[4], [5], [6], [7]). Main reason for higher thermal conductivity of nanofluids is due to brownian motion and better effective mixing. It should be noted that the size of nanoparticles plays an important role in the enhancement of thermal conductivity of nanofluid. Nanofluid is prepared by adding metals, metal oxides, carbon nanotubes or any other solid nano material's to a base fluid like water, ethylene glycol or engine oil as in [8], [9]. The study on heat transfer enhancement using nanofluids in PHE has been done by a many researchers (e.g., [10], [11], [12]). Alumina/water and ZnO/water nanofluids were employed as a coolant in a PHE to study the heat transfer rate and pressure drop by Kwon et al., (2011) [13]. Their outcomes revealed that nanofluid is not beneficial for augmenting the heat transfer enhancement. Pantzali *et al.*, (2009) [14] employed CuO/water nanofluids (4 vol. %) to analyze the performance in a PHE and suggested that effectiveness of heat exchanger is affected by flow nature of coolant inside the PHE. Vajjha and Das (2009) [15] studied the various nanofluids, such as, alumina, silica and copper oxide with mixture of EG/Water (60/40) as a base fluid and concluded that, as the nanoparticle concentration increases, the specific heat capacity decreases. Studies on determination of viscosity and thermal conductivity of nanofluids a function of temperature has been performed

by many authors (e.g., [16], [17], [18]). Their result shows that nanofluid enhances the viscosity and thermal conductivity of working fluids.

Several researches have carried out in newtonian fluids like water and ethylene glycol to study the performance of PHE, whereas very few researchers have carried out by using non-newtonian nanofluids. Reilly et al. (1965) [19] studied the natural convection heat transfer from heated vertical plate to a non-newtonian fluid. Non-newtonian nanofluids are widely encountered in many industrials and technological applications, such as melts of polymers, biological solutions, paints, tars, asphalts, and glues. High heat transfer capacity and low pumping power of some non-newtonian fluids makes them attractive as a coolant for various applications such as micro channel heat exchangers. In this study, heat transfer characteristics of alumina nanoparticles dispersed in non-newtonian nanofluid and their enhancement in heat transfer are investigated.

Many researchers have developed the correlation to find the convective heat transfer coefficients for PHE by considering the limited number of factors in the use of non-newtonian nanofluid. Afonso et al. (2003) [20] conducted an experimental investigation to arrive a correlation for the determination of convective heat transfer coefficient in a PHE using yogurt as test fluid. Fernandes et al. (2005) [21], [22], [23], [24] has demonstrated numerical studies on PHE with yogurt as test fluid. Similar numerical studies in a PHE using egg yolk as test fluid were conducted by Gut et al. (2003) [25]. Muthamizhi et al. (2015) [26] developed the Nusselt number correlation to find the heat transfer coefficients in a PHE for various concentrations of CMC. We have developed the Nusselt number correlation in terms of entire process parameters to look at the heat transfer coefficient in a PHE for various concentrations of SA and CMC with Al_2O_3 nanoparticles. The correlation provides a combined relation between Nu, Re and Pr by investigating several non-newtonian fluids practiced for the heat transfer enhancement. Lastly, by using the experimental data the developed correlation has been validated, and also compared with the correlations found in the literature.

II. Materials and methods

i. Sample Preparation

In the present study, Al_2O_3 nanoparticles purchased from SD fine chemicals Limited were dispersed in distilled water by the two-step method. Firstly, water based nanofluids of desired concentrations (i.e. 0.01, 0.05 wt. %) were prepared. Average sizes of nanoparticles used were 20-30 nm. The solution was subjected to mechanical agitation using mechanical stirrer by taking an appropriate amounts of CMC / SA solution was prepared separately and were added to the suspension and stirred well by the ultrasonicator (for 60min, 20 kHz and 100W) to achieve the homogenous nanofluids of desired concentration. Base fluids used in this investigation are CMC and SA, exhibits shear thinning behavior ($n<1$). In order to test the stability of nanofluids, density was tested before and after the experimental tests. However, no significant change in density was observed. In order to minimize problems caused by potential clustering and sedimentation of nanoparticles, during each test a fresh nanofluid was prepared and used immediately.

ii. Experimental setup

The plate type heat exchanger consists of 5 plates and 2 end plates made of aluminium with dimensions of 150mm x 150mm (inner) and 250 x 250 mm (outer). The experiments were carried out in both parallel and counter flow patterns by taking carboxymethyl cellulose/sodium alginate in cold side and water as hot side fluid. Two centrifugal pump of 0.25 HP, two instant water heaters (3KW each), two rotameters, four thermocouples and two storage vessels of 100 liter capacity were installed for carrying out the experiment.

The experimental setup of plate type heat exchanger with all accessories is shown in the figure 1. The experimental studies involved the determination of outlet temperature of both cold and hot fluid for various flow rates in parallel and countercurrent flow pattern.

iii. Experimental procedure

The experimental set-up was washed thoroughly with water before starting the experiment. The stored cold fluid (non-newtonian fluid) was pressurized using a centrifugal pump and control was achieved by rotameters connected between the pump and the heat exchanger. A control valve ensures the definite volume flow of water in to the heater. Cold fluid at an inlet temperature of 31 ± 3˚C and hot fluid (Water) an inlet temperature of 60 ± 3˚C was pumped into the heat exchanger. Constant hot water flow rate of 4 lpm was maintained throughout the experiment. Flow rate of cold fluid was varied from 2 to 8 lpm and the steady state temperature of hot and cold fluids were noted (T_1, t_1, T_2, t_2). A step increment in cold fluid flow rate was given and respective steady state temperatures were taken. The above procedure was repeated for different flow rates of cold fluid with different non-newtonian base fluid compositions (0.01%, 0.05%, and 0.1%) and with different nanoparticle composition (0.01%, 0.05%). Time taken for filling definite volume was noted at the exit to reduce uncertainty in flow measurements. For each test fluids, the experiments are repeated for few readings to minimize the uncertainty in measured experimental parameters and reproducibility was found to be within ± 2%. After completing a run

with different flow rates, the procedure was repeated for different compositions of CMC – water and SA – water systems.

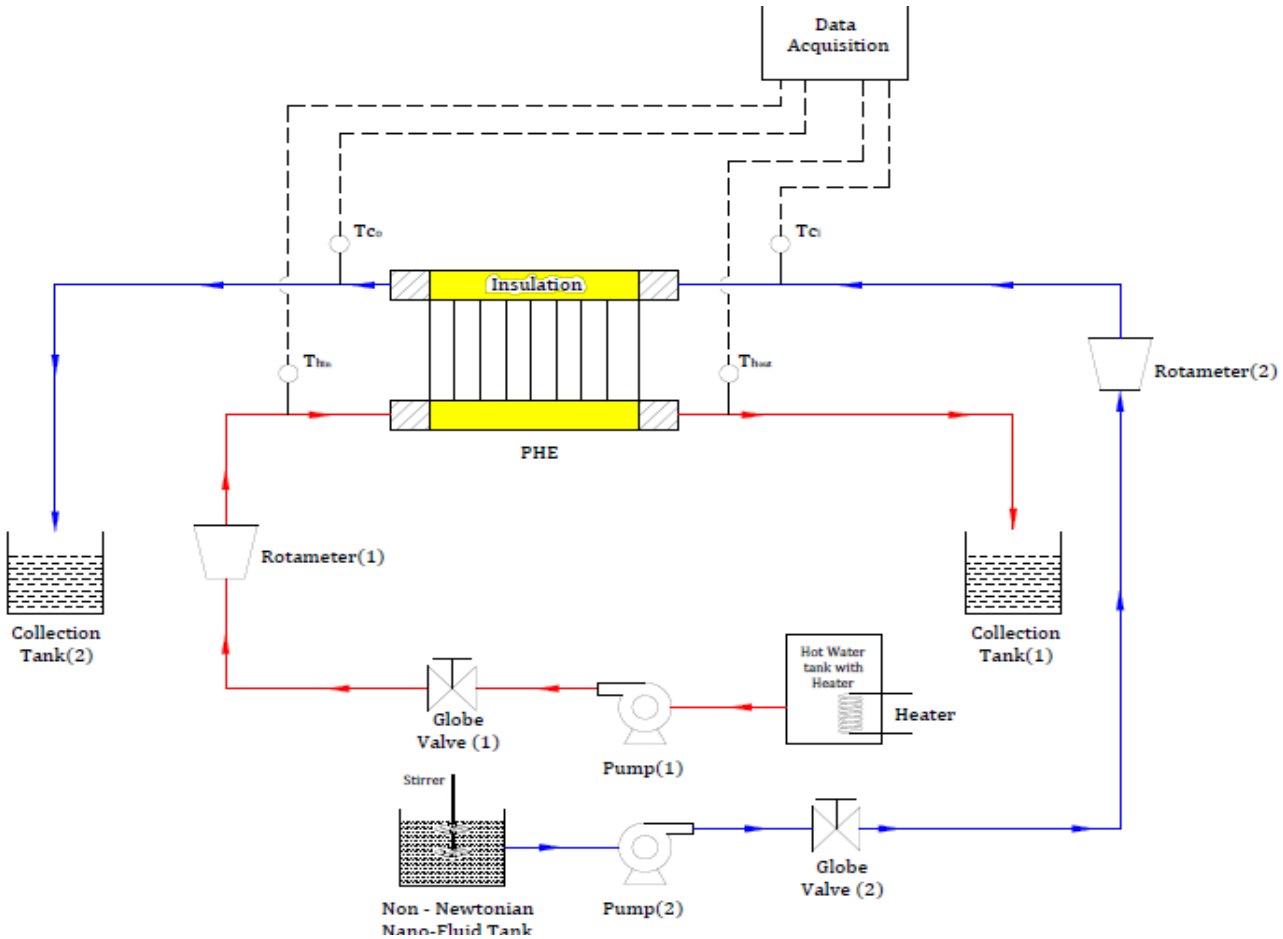


Figure 1 Schematic diagram of Experimental setup

iv. Data analysis

The effective physical properties of non-newtonian nanofluids were evaluated using the reported empirical correlations in the literature for two phase fluids. The density, thermal conductivity, specific heat and viscosity for various volume concentrations calculated using the following correlation as reported in [27], [28].

$$\rho_{nf} = \phi\rho_p + (1-\phi)\rho_{bf} \dots\dots\dots\dots (1)$$

$$(\rho C_p)_{nf} = \phi(\rho C_p)_p + (1-\phi)(\rho C_p)_{bf} \dots\dots\dots\dots (2)$$

$$\mu_{nf} = \mu_{bf}(123\,\phi^2 + 7.3\phi^2 + 1) \dots\dots\dots\dots (3)$$

The CMC, SA base fluid and nanofluid exhibit the power law model due to its rheological property, it is expressed as

$$\tau = K\gamma^n \dots\dots\dots\dots (4)$$

Dimensionless number for purely non-newtonian fluid (pseudoplastic), are defined as follows (e.g., [29])

$$\mathrm{Re} = \frac{\rho u^{2-n} D^n}{K} \quad Pr = \frac{C_p\, K\, (\frac{u}{D})^{n-1}}{k} \dots\dots\dots\dots (5)$$

Convective heat transfer coefficient for nanofluids is calculated as follows

$$h_{nf} = \frac{(C_p\rho)_{nf} UA(T_{b2} - T_{b1})}{\Pi DL.LMTD} \dots\dots\dots\dots (6)$$

Correlation generally used to predict the heat transfer coefficients found in literature is Dittus-boelter equation given as follows.

$$Nnu = 0.023\, Nre^{0.8} Npr^{0.4} \dots\dots\dots\dots (7)$$

v. Development of Nusselt number correlation based on dimensional analysis

A physical model was developed before to characterize the PHE performance using limited parameters alone. Equations predicting heat transfer coefficients generally suggested in literature could not be used for non-newtonian fluids. Flow behavior index (n) and consistency index (k, Pa s^n) shall be included to show the better flow behavior of non-newtonian fluid into the correlation. In order to predict the heat transfer coefficient of non-newtonian nanofluids, the experimental data were fitted to following correlation (e.g., [26]).

$$Nu = f\left(\frac{v}{D_h^{\frac{n}{n-2}}\rho^{\frac{1}{n-2}}}\right) * \left(\frac{C_p}{D_h^{\frac{2-2n}{n-2}}\rho^{\frac{1-n}{n-2}}K}\right) * \left(\frac{\Delta T.\beta g}{D_h^{\frac{n+2}{n-2}}\rho^{\frac{2}{n-2}}}\right) \dots\dots\dots\dots (8)$$

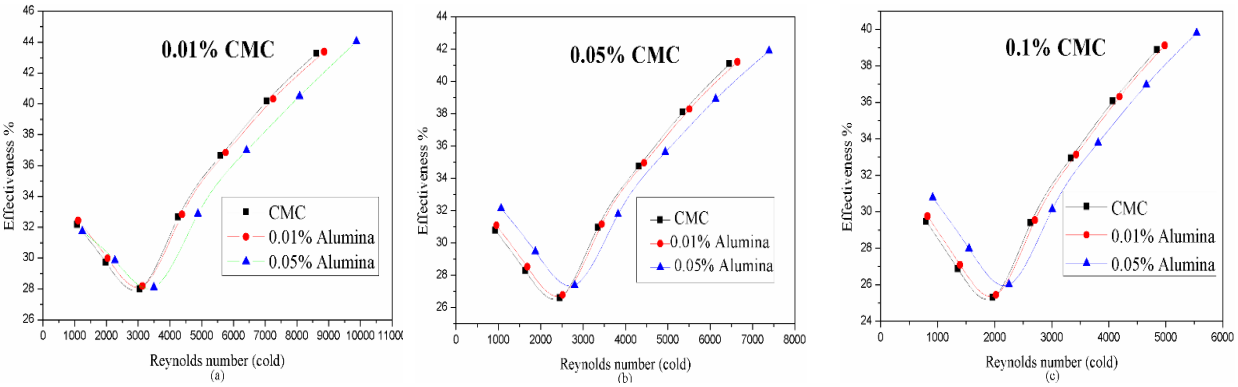
Hence, this chapter aimed to investigate the effect of non-newtonian nanofluid flow rate of CMC and SA based for PHE of specific configuration, and develops a correlation based on dimensional analysis using experimental data.

III. Results and discussion

i. Experimental Heat transfer study

To evaluate the reliability and accuracy of the measurements, experimental system was tested with distilled water before charging the non-newtonian nanofluids. Plots between effectiveness and Reynolds number clearly shows enhancement of heat transfer due to addition of nanoparticles addition in non-newtonian fluids. Variation in effectiveness of PHE plotted against cold Reynolds number shown in the figure 2 for counter flow and figure 3 for parallel flow. Increase in concentration of nanoparticles increases the brownian motion of nanoparticles and increase in the thermal conductivity is an effective reason for the enhancement of effectiveness. However, enhancement is attributed to various other properties like density and specific heat. But increase in non-newtonian fluid concentration decreases the effect of nanoparticles as it leads to increase in apparent viscosity leading to decrease the effectiveness. Effectiveness is higher for lower concentration of non-newtonian fluid and decreases with increase in concentration of non-newtonian fluid. For example, Reynolds number of 3044, effectiveness for non-newtonian fluid containing CMC of concentration 0.01% counter flow is 27.99% whereas for CMC of concentration 0.05%, effectiveness is 26.60% and for the concentration of 0.1%, effectiveness decreases to 25.29%., Whereas effectiveness increases with increase in nanoparticle concentration. For Reynolds number of 3132 effectiveness of 0.01%alumina nanofluids is 28.19% and for 0.05% alumina nanofluid effectiveness is 28.10%. An increase in thermal conductivity is an effective reason for increase in the effectiveness of PHE.

In SA system, for Reynolds number of 3053, effectiveness for non- newtonian fluid containing SA of concentration 0.01% in counter flow is 28.07% whereas for SA of concentration 0.05%, effectiveness is 26.65% and for the concentration 0.1%, effectiveness decreases to 25.29%., Whereas effectiveness increases with increase in nanoparticle concentration. For Reynolds number 3141, effectiveness of 0.01%alumina nanofluids is increased to 28.26% and for 0.05% alumina nanofluids effectiveness is 28.08%. Sodium alginate shows better thermal efficiency compared to that of carboxyl methyl cellulose system and also effectiveness increases as the flow rate of cold fluid increases.



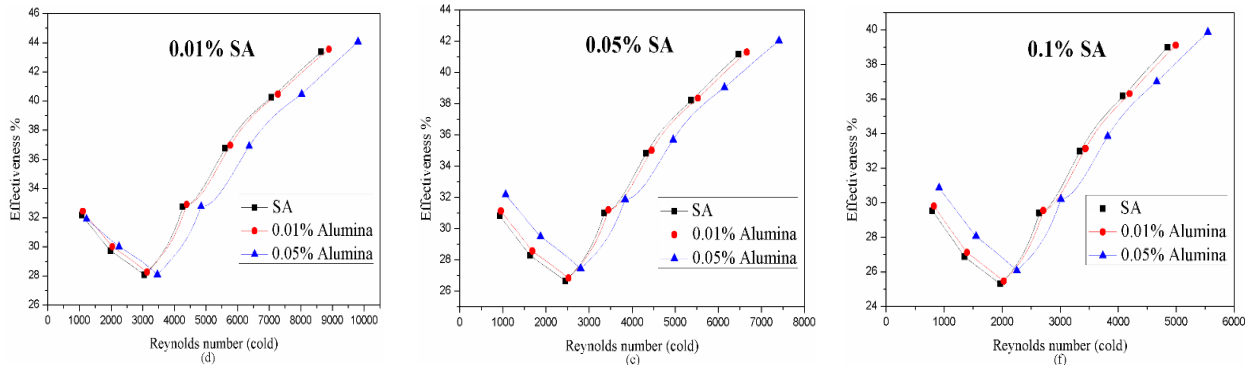


Figure.2. Effectiveness as a function of Reynolds number for various concentration of base fluid (CMC/SA) and nanoparticle in a counter current flow

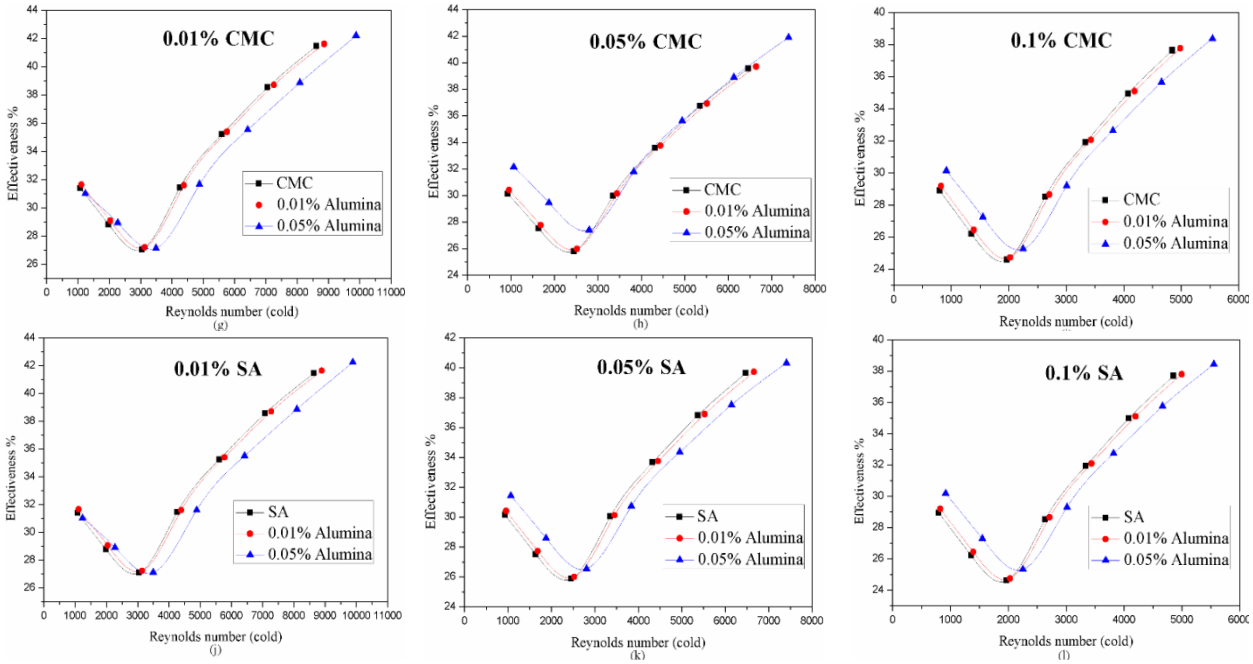


Figure.3. Effectiveness as a function of Reynolds number for various concentrations of base fluid (CMC/SA) and nanoparticle in a parallel flow

ii. Estimation of powers in the Nusselt number correlation using experimental data

The Nusselt number correlation was developed by using dimensional analysis can be written in the standard form as follows

$$Nu = Z\left(\left(\frac{v}{D_h^{\frac{n}{n-2}}\rho^{\frac{1}{n-2}}}\right)^{a} * \left(\frac{C_p}{D_h^{\frac{2-2n}{n-2}}\rho^{\frac{1-n}{n-2}}K}\right)^{b} * \left(\frac{\Delta T.\beta g}{D_h^{\frac{n+2}{n-2}}\rho^{\frac{2}{n-2}}}\right)^{c}\right) \ldots\ldots\ldots\ldots (9)$$

Where z, a, b and c are constants are evaluated using experimental data, based on linear regression method.

Let Y is the function of x_1, x_2 and x_3 as

$$Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 \ldots\ldots\ldots\ldots (10)$$

Wherea_0,a_1,$a_2$and a_3 are constants, evaluated using linear regression method.A set of equations say 'n' where n = number of constants, (n = 4) are formed by multiplying the equation by the variables (here$x_1, x_2 and x_3$).

By solving the linear regression equations, the values of constants $a_0, a_1, a_2 and a_3$ are found, this gives the coefficients of Nusselt number correlation i.e. $z, a, b and c$.

The correlation for the carboxymethyl cellulose system with and without addition of Al_2O_3 nanoparticle is

$$Nu = 0.00004539\left(\left(\frac{v}{D_h^{\frac{n}{n-2}}\rho^{\frac{1}{n-2}}}\right)^{0.843} * \left(\frac{C_p}{D_h^{\frac{2-2n}{n-2}}\rho^{\frac{1-n}{n-2}}K}\right)^{1.4218} * \left(\frac{\Delta T.\beta g}{D_h^{\frac{n+2}{n-2}}\rho^{\frac{2}{n-2}}}\right)^{0.2153}\right) \ldots\ldots (11)$$

The correlation for the sodium alginate system with and without addition of Al_2O_3 nanoparticle is

$$Nu = 0.00684\left(\left(\frac{v}{D_h^{\frac{n}{n-2}}\rho^{\frac{1}{n-2}}}\right)^{0.9278} * \left(\frac{C_p}{D_h^{\frac{2-2n}{n-2}}\rho^{\frac{1-n}{n-2}}K}\right)^{0.8227} * \left(\frac{\Delta T.\beta g}{D_h^{\frac{n+2}{n-2}}\rho^{\frac{2}{n-2}}}\right)^{0.0816}\right) \ldots\ldots\ldots (12)$$

Root means square (RMS) value of the developed correlation has been calculated. Afonso et al. (2003) [23] and Fernandes et al. (2005) [24], [25] developed Nusselt number correlation for PHE system by using yoghurt as a non-newtonian fluid, and the correlation has been used for calculating Nusselt number value for the different concentration of non-newtonian nanofluid. RMS value (Table 1) of correlation found in the literature and developed correlations were compared, it was found that the developed correlation shows good agreement with the system to examine heat transfer coefficient.

Table.1 RMS value of Nusselt number correlations

RMS	CMC System	SA System
Developed correlation	2.032	2.58
Afonso et al	68.31	68.32
Fernandes et.al	81.29	81.31

iii. **Comparison of Experimental and developed Nusselt number correlation**

The Nusselt number value calculated for the different concentration of non-newtonian nanofluid**.** Figure 4 and 5 shows the change in calculated Nusselt number for both experimental and developed correlation against Reynolds Number. It clearly understands that Nusselt number value calculated by the correlation is almost near to the experimental value. So the developed correlation of Nusselt number equation using dimensional analysis is valid for the analysis of non-newtonian nanofluid in a PHE system.

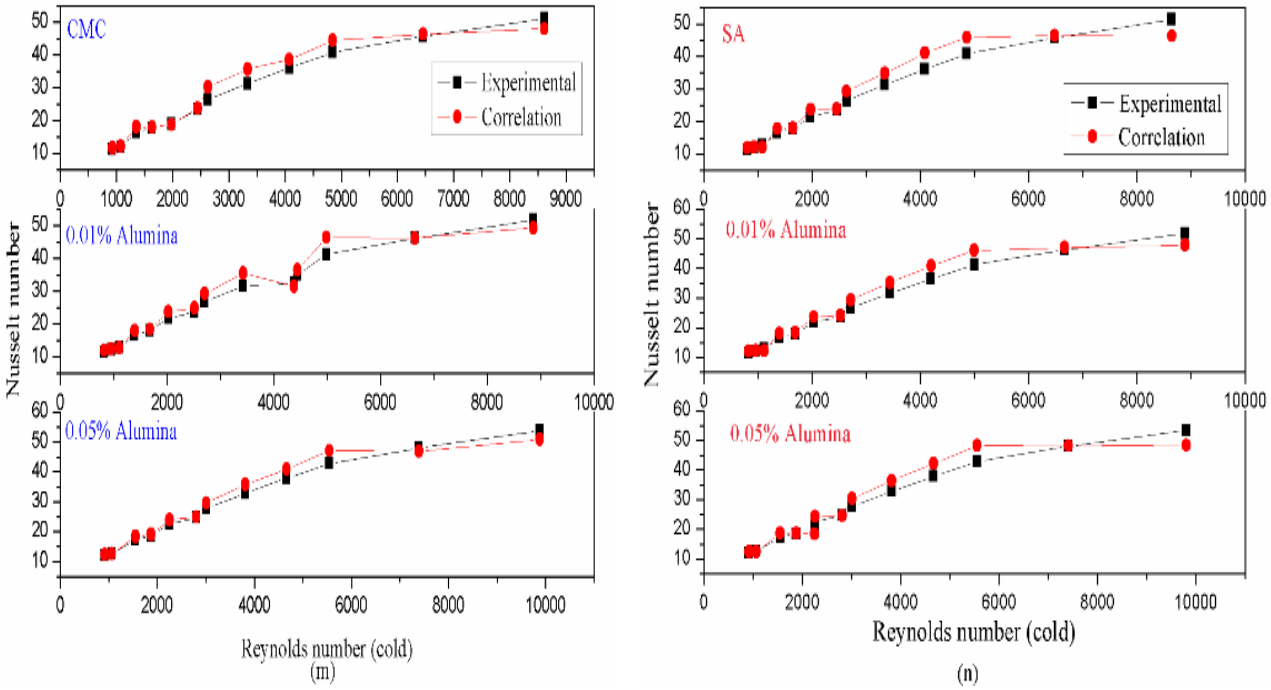


Figure.4. Comparison of experimental and correlation Nusselt number in a counter current flow for various concentrations of base fluid (CMC/SA) and nanoparticle.

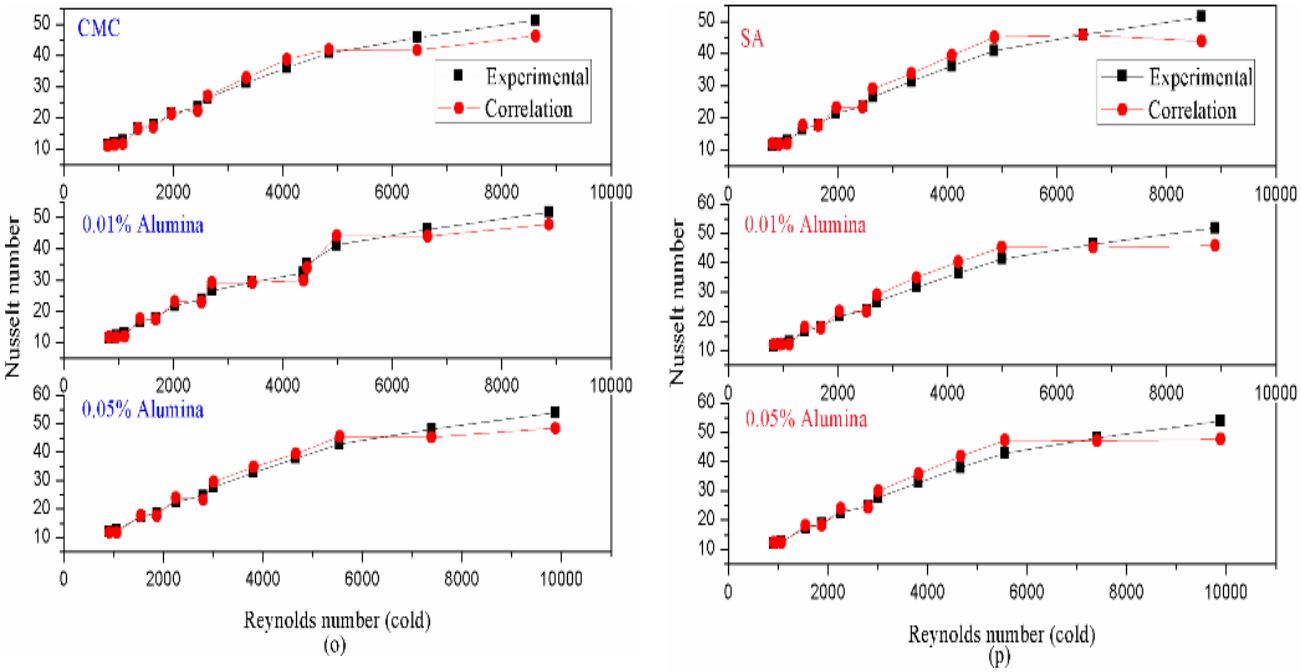


Figure.5. Comparison of experimental and correlation Nusselt number in a parallel flow for various concentrations of base fluid (CMC/SA) and nanoparticle

IV. Conclusion

The present work investigated the performance of plate heat exchanger (PHE) using two different non-newtonian base fluids (CMC and SA) with Al_2O_3 as nanoparticle. For experimental studies were carried based on three different concentration of base fluid such as 0.01%, 0.05% and 0.1% and two different nanoparticle concentrations 0.01% and 0.05% are used. The hot fluid and cold fluid inlet temperatures are maintained constants as 60ºC and 31ºC respectively, the volumetric flow rate of cold fluid was varied from 2 to 8 lpm while keep up the volumetric flow rate of hot fluid as constant 4 lpm. The result shows that increase in the concentration of nanoparticle and cold fluid flow rate increases the effectiveness of PHE. But the same time increase in the concentration of non-newtonian base fluid decreases the heat transfer co-efficient of PHE. Compare to CMC based nanofluid the SA based nanofluid gives the outstanding performance on heat transfer enhancement with lower concentration of base fluid. The Nusselt number correlations were developed for both CMC and SA based system with RMS deviation of 2.032 and 2.58 respectively. Then the developed correlation compared with the correlation found in the literature for non-newtonian fluid in PHE system. The developed correlation shows well fit to the system for the analysis of convective heat transfer coefficient.

Abbreviations

PHE	Plate heat exchanger
CMC	Carboxymethyl cellulose
SA	Sodium alginate
RMS	Root means square
lpm	Liter per minute
HTC	Heat transfer coefficients
LMTD	Logarithmic mean temperature difference (K)

List of symbols

A	Effective plate heat transfer area (m^2)
Cp	Specific heat of fluid at constant pressure (J/kg K)
D_h	Hydraulic diameter (m)
L	Plate length (m)
h	Convective heat transfer coefficient (W/m^2 K)

K	Fluid thermal conductivity (W/m K)
NRe	Reynolds number, dimensionless
Nnu	Nusselt number, dimensionless
NPr	Prandtl number, dimensionless
T	Temperature (K)
ΔT	Temperature difference (K)
U	Overall heat transfer coefficient (W/m^2 K)
v	Velocity (m^2/s)
w	Width of the plate (m)
k	Consistency index (Pa s^n)
n	Flow behavior index
K	Plate thermal conductivity (W/m K)
y	Dependent variable
a	Coefficient of x
x	Independent variable
b	Constant
z, a, b and c	Model parameters, dimensionless

Greek symbols

βg	Thermal expansion with acceleration due to gravity (*m/s^2K)
ρ	Density of the fluid (kg/m^3)
ϕ	Nano particle concentration
μ	Viscosity (Pa s)

Subscripts

b_1	Temperature inlet
b_2	Temperature outlet
nf	Nano fluid
bf	Base fluid

REFERENCES

[1]. Galeazzo FCC, Miura RY, Gut JAW, Tadini CC. Experimental and numerical heat transfer in a plate heat exchanger. Chem Eng Sci 2006;61:7133e8.
[2]. Lin JH, Huang CY, Su CC. Dimensional analysis for the heat transfer characteristics in the corrugated channels of plate heat exchangers. Int Commun Heat Mass 2007;34:304e12.
[3]. Zhang J, Diao Y, Zhao Y, Zhang Y. Experimental study of TiO2ewater nanofluid flow and heat transfer characteristics in a multiport minichannel flat tube. Int J Heat Mass Transf (2014);79: 628e38.
[4]. Nguyen CT, Roy G, Gauthier C, Galanis N. Heat transfer enhancement using Al_2O_3ewater nanofluid for an electronic liquid cooling system. Appl Therm Eng (2007);27:1501e6.
[5]. Putra N, Roetzel W, Das SK. Natural convection of nanofluids. Heat Mass Transf (2003);39:775e84.
[6]. Wu Z, Wang L, Sunden B. Pressure drop and convective heat transfer of waterand nanofluids in a double-pipe helical heat exchanger. Appl Therm Eng(2013);60:266e74.
[7]. Heris SZ, Esfahany MN, Etemad SG. Experimental investigation of convective heat transfer of Al_2O_3/water nanofluid in circular tube. Int J Heat Fluid Flow (2007); 28:203e10 .
[8]. Choi, S.U.S., Enhancing thermal conductivity of fluid with nanoparticles, Developments and Applications of Non-Newtonian Flows, FED-V. 231/ MD-V, vol. 66, ASME, New York, pp. 99–105 (1995).
[9]. Abinaya K., Thirumarimurugan M., Kannadasan T; Experimental Investigation Of Heat Transfer Enhancement Of Non-Newtonian Nanofluids(CMC) ; Journal of chemical, biological and physical sciences (JCBPS) 6 (1), 43 (2015).
[10]. Mohammed H.A., BhaskaranGShuaib., N.H., Abu-MulawehH.I. Influence of nanofluids on parallel flow square microchannel heat exchanger performance. IntCommu Heat Mass Transfer. 38 (2011) 1-9.
[11]. R. Lotfi, A.M. Rashidi, A. Amrollahi. Experimental study on the heat transfer enhancement of MWNT-water nanofluid in a shell and tube heat exchanger. Int Commu Heat Mass Transfer.39 (2012) 108-11.
[12]. Pandey S.D., NemaV.K..Experimental analysis of heat transfer and friction factor of nanofluid as a coolant in a corrugated plate heat exchanger. Exp Therm Fluid Sci. 38 (2012) 248-56.
[13]. Kwon Y.H., Kim D., Li C., Lee J., Hong D. Heat transfer and pressure drop characteristics of nanofluids in a plate heat exchanger. J Nanosci and Nanotech. 11 (2011) 5769–74.
[14]. Pantzali M.N., Mouza A.A., Paras S.V. Investigating the efficacy of nanofluids as coolants in plate heat exchangers (PHE). Chem Eng Sci 64 (2009) 3290–300.
[15]. Vajjha R.S., Das D.K. Specific heat measurement of three nanofluids and development of new correlations. ASME, J Heat Transfer. 131 (2009) 071601–7.
[16]. Karimi A., Amin Abdolahi Sadatlu M., Saberi B., Shariatmadar H., Ashjaee M. Experimental investigation on thermal conductivity of water based nickel ferrite nanofluids. Adv Powder Technol. (2015).
[17]. Huminic A., Huminic G., Fleaca C., Dumitrache F., Morjan I. Thermal conductivity, viscosity and surface tension of nanofluids based on FeC nanoparticles. Powder Technology. 284 (2015) 78-84.

[18]. HemmatEsfe M., Karimipour A., YanW.M., Akbari M., Safaei M.R., Dahari M. Experimental study on thermal conductivity of ethylene glycol based nanofluids containing Al_2O_3 nanoparticles. Int J Heat Mass Transfer.88 (2015) 728-34.
[19]. Reilly IG., Tien C., Adelman M., Experimental study of natural convective heat transfer from a vertical plate in a non-Newtonian fluid. Can J Chem Eng 43(4):157–160(1965).
[20]. Afonso IM., Maia L., Melo LF., Heat transfer and rheology of stirred yoghurt during cooling in plate heat exchangers. J Food Eng 57:179–187(2003).
[21]. Fernandes CS, Dias RP, Noberga JM, Maia JM., Effect of corrugation angle on the hydrodynamic behaviour of power-law fluids during a flow in plate heat exchanger. In: Shah RK, Ishizuka M, Rudy TM, Wadekar VV (eds) Proceedings of the fifth international conference on enhanced, compact and ultra compact heat exchangers: science engineering and technology, Engineering Conferences International, USA (2005).
[22]. Fernandes CS, Dias RP, Nobrega JM, Afonso IM, Melo LF, Maia JM., Simulation of stirred yoghurt processing in plate heat exchangers. J Food Eng 69:281 290(2005).
[23]. Fernandes CS, Dias RP, Nobrega JM, Afonso IM, Melo LF, Maia JM., Thermal behavior of stirred yoghurt during cooling in plat heat exchangers. J Food Eng 76:433 439(2006).
[24]. Fernandes CS, Dias RP, Nobrega JM., Maia friction factors of power-law fluids in chevron-type plate heat exchangers. J Food Eng 89:441–447(2008).
[25]. Gut JAW, Pinto JM, Gabas AL, Romer JT., Pasteurization of egg yolk in plate heat exchangers thermo-physical properties and process simulation, Annual meeting, CA 123:16–21(2003).
[26]. Muthamizhi K, Kalaichelvi P., Development of Nusselt number correlation using dimensional analysis for plate heat exchanger with a carboxymethyl cellulose solution, Heat mass transfer 51:815-823(2015).
[27**]**. Rajasekharan S., Kubair V.G., Kuloor N.R., Heat transfer to non-Newtonian fluids in coiled pipes in laminar flow International Journal of Heat Mass Transfer; 1970, 13, 1583–1594.
[28]. Eastman JA, PhillpotSR, Choi SUSand Keblinski P "Thermal Transport in Nanofluids." Annual Review of Materials Research, 34(1), 219-246(2004).
[29]. Moradi M, Etemad SG, Moheb A., Laminar flow heat transfer of a pseudoplastic fluid through a double pipe heat exchanger. Iran J Chem Eng 3(2):13–19(2006).

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Simulation Studies in an Anaerobic Sequential Batch Reactor for the Treatment of Textile Dyeing Industry Effluent

T.Senthilkumar[1], S.Rajamohan, B.Gopalakrishnan[2], M.Rajasimman[2]

[1]Tamilnadu Pollution Control Board, Tirunelveli

[2]Department of Chemical Engineering, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India.

senthilkumarpcb@gmail.com

Treatment of textile dyeing industry wastewater was performed in sequential batch reactor and the data obtained from the reactor is used for the simulation in this work. The effect of reaction time on the quality of wastewater from SBR in terms of COD was investigated. Finding the apt reaction time is very much important for the design and operation of the reactor. The simulation studies were performed for the effect of various parameters like effluent concentration, flow rate, rate constant and different volume of reactor on effluent concentration with reaction time has been studied. This simulation results can be used to describe the Sequencing Batch Reactor (SBR) kinetics with the optimum operational characteristics.

***Keywords**: SBR, Modeling, Simulation, Wastewater, Dye industry*

I. Introduction

The textile dyeing industry consumes large quantities of water and produces large volume of wastewater from different steps during dyeing processes. Wastewater from various sections is often rich in color, containing residues of dyes and chemicals, etc. Depending on the type of textile manufactured and the chemicals used, the characteristics of textile dye industry effluent varies. Organic and inorganic substances in the textile effluents make the water unsuitable for use. These substances even in a lower quantity are found to be toxic to aquatic life [1]. The seriousness of water pollution depends on the quantity of water and chemicals used in the wet process [2]. The dye concentration in a dye house range from 0.01g/L to 0.25 g/L depending on the type of the dye and the process carried out [3, 4].

Treatment of textile dye effluent has been carried out by physical, chemical and biological methods. Physiochemical methods such as filtration, coagulation, adsorption, chemical flocculation etc. are employed. Some of the methods *viz.*, reverse osmosis, nanofiltration, multiple effect evaporator (MEE) are found to be effective but quite expensive [5]. Biological treatment offers a cheaper and environmentally friendlier alternative for color removal in textile effluents. A number of microorganisms have been found to be able to decolorize textile dyes including bacteria, fungi and yeasts [6, 7].

SBR is a variation of the activated-sludge process. In a SBR, all necessary processes are taking place in a single basin as time sequenced rather than space sequenced simultaneously in separate tanks as such in conventional activated sludge (CAS) system. In addition, the SBR system can be designed with the ability to treat a wide range of influent volumes whereas the continuous system is based upon a fixed influent flow rate. Thus, there is a degree of flexibility associated with working in a time rather than in a space sequence. ASBR is similar to SBR but during the reaction step, air is not supplied into the reactor. In its most basic form, the SBR system is a set of tanks that operate on a fill-and draw basis. Each tank in the SBR system is filled during a discrete period of time and then operated as a batch reactor. After desired treatment, the mixed liquor is allowed to settle and the clarified supernatant is then drawn from the tank.

The cycle for each tank in a typical SBR is divided into five discrete time periods: Fill, React, Settle, Draw and Idle. There are several types of fill and react periods, which vary according to aeration and mixing procedures. Sludge wasting may take place near the end of React, or during Settle, Draw, or Idle. Central to SBR design is the use of a single tank for multiple aspects of wastewater treatment [8].

ASBR is an excellent reactor to treat a variety of wastewaters; they could be applied to treat domestic wastewater, landfill leachate, industrial wastewater, biological phosphorus and nitrogen removal, etc. The

ASBR has the following advantages in a small-scale system: flexibility in operation, low construction and maintenance cost and simultaneous removal of nitrogen and phosphorus. There are several literatures mentioning the applicability of this promising process in wastewater treatment [9 – 20].

The monitoring and supervision of SBR processes are crucial to the detection and timely correction of faults. Prompt fault identification and correction is particularly necessary in biological processes because such processes may take days, weeks, or even months to recover from an abnormal state [21-22]. Mathematical modeling has proven very useful to study complex processes, such as the anaerobic granular sludge systems. The computational model used in this study is based on previously developed models. The objective is to simulate the ASBR using the results obtained from the treatment of textile industry wastewater.

II. Theory

The substrate concentration remaining at the end of the period is a variable function of the volume and the kinetic expression for substrate removal that applies during the fill period. The mass balance during the fill period is [23].

In – Out + Generation = Accumulation

$$QC_{so} - 0 + r_{cf}V = \frac{d}{dt}(VC_s) \quad (1)$$

$$r_{cf} = -kC_s \quad (2)$$

A mathematical model is developed by Yasemin Damar *et al*., (2012) [24] to find the substrate concentration at the end of each cycle in a sequential batch reactor. It is give as:

$$C_{sf} = \left[\frac{C_{so}Q}{kV_b} + \left(\frac{V_a}{V_b}C_{sf} - \frac{Q}{V_b}\frac{C_{so}}{k}\right)e^{k\left(\frac{-V_{ab}}{Q}\right)}\right].e^{-kt_r} \quad (3)$$

III. Materials and Methods

ASBR used in this work was constructed using 12 mm thick Plexiglas and are cylindrical in shape. The total volume of the SBR is 1.5 L of which 1 L is used as working volume and the remaining 0.5 L for head space. The filling and withdrawal of the effluent was done using peristaltic pumps. A mechanical stirrer is used for mixing the effluent at the speed of 10 rpm at frequent interval during the reaction time.

ASBR is filled with the textile dye industry effluent and it is inoculated with the mixed culture obtained from textile dye wastewater treatment pond. The pH is maintained at 7.0±1 and temperature is maintained at 32±1°C. The setup is left for 75 days for the acclimatization of microorganisms. The textile dye industry wastewater is pumped into the reactor regularly and the COD reduction, were monitored regularly.

ASBR is operated in 24 h cycle. The operating procedure of ASBR is as follows: Fill – 1h; react – 20 h; settle – 2 h and with drawl – 1h. During the fill period, textile dye wastewater is fed into the reactor and then it is allowed to react. Then sludge is allowed to settle after the react period. After the settling of bio-sludge, the supernatant was removed during the withdrawal time. After that, fresh textile dye wastewater is introduced into the reactor and the above operation is repeated. COD is analyzed for each condition as per standard methods of analysis, APHA [25]. Experiments were carried out in ASBR at various average substrate concentrations (780, 1560 and 2580 mg/L) and flow rate by varying the HRT (8, 6 and 4 d).

IV. Results and Discussion

The characteristics of textile dyeing wastewater was studied and it was given in Table 1. For the average initial substrate concentration of 780 mg COD/L, 1560 mgCOD/L and 2580 mgCOD/L, the results obtained from SBR (data not given) was used for simulation study. At the end of one cycle period, a maximum of 96% removal efficiency occurs for the initial substrate concentration of 780 mg COD/L.

The simulation studies were carried out based on the effect of average initial substrate concentration, HRT in terms of flow rate, rate constant, volume of a batch reactor when is initially (Va, m^3), volume of reactor when is empty (Vb, m^3) and available volume at the beginning of the fill period (Vab, m^3) on the effluent concentration. First simulation study was carried out by varying the initial substrate concentration to 780 mg COD/L, 1560 mg COD/L and 150 mg COD/L. The other parameters were fixed as flow rate – 0.0002083 m^3/h, rate constant – 0.39 h^{-1}, and available volume as Va-0.0002 m^3, Vb- 0.000268 m^3 and Vab- 0.00001 m^3. Calculations have been carried out using excel. The results obtained from a 24 hr cycle, ASBR was employed for the simulation studies.

Table 1. Characteristics of textile dyeing industry effluent

Parameters*	Values
pH	7.1 - 7.9
Color	Brown
TSS	615 - 750
TDS	4000 - 4325
BOD	1195 - 1306
COD	2500 – 2650

* All values except pH and color are in mg/L

For various influent substrate concentration, the other parameter were kept constant. The effect of influent concentration (C_{so}) on effluent concentration (C_{se}) for different reaction time is simulated and it is depicted in Fig. 1. From the figure, it is inferred that the, effluent concentration decreased with respect to time irrespective of concentration. It was also observed that increase in concentration decreases the removal rate.

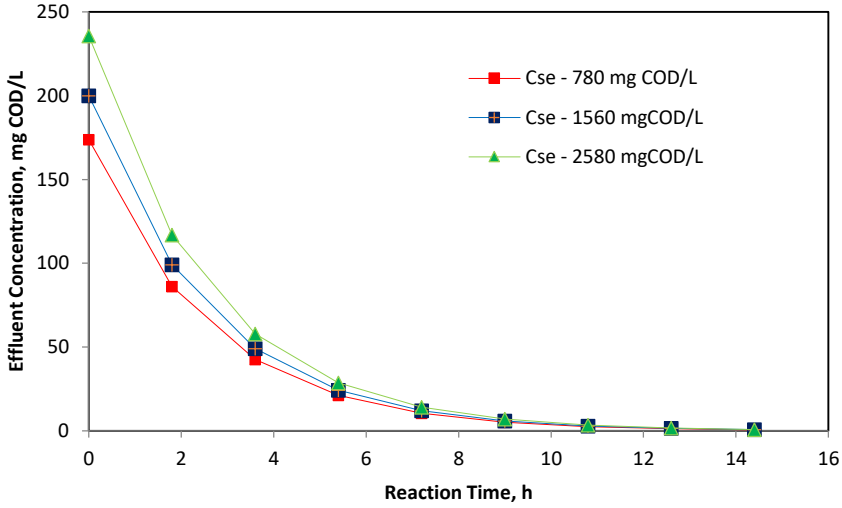


Figure 1. Effect of initial substrate concentration

In this work the flow rate values are varied by varying the HRT. For the various HRT, 4, 6, 8 days which corresponds to a flow rate denoted by Q1, Q2, Q3 respectively. The effect of flow rate on effluent concentration is shown in Fig. 2. From the figure it is observed that, the effluent concentration decreases with respect to time and also increase found that increase in flow rate decreases the removal efficiency of COD.

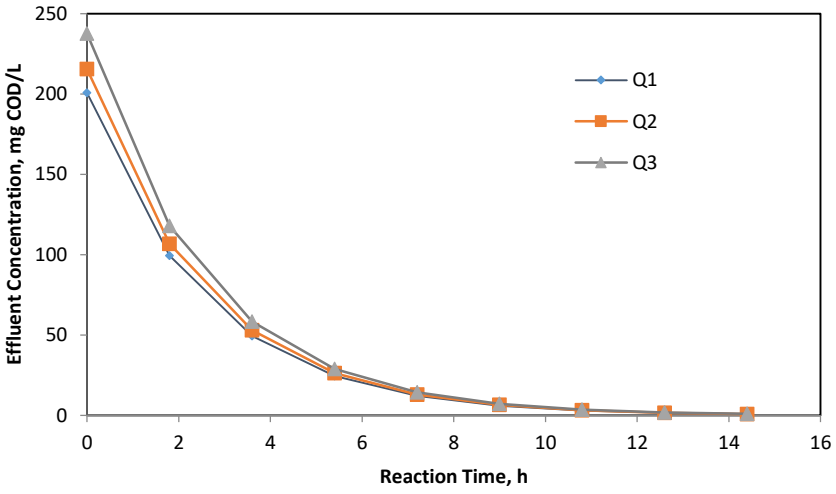


Figure 2. Effect of flow rate on effluent concentration

The effect of reaction rate constant is studied by varying the rate constant as 0.05, 0.39, 0.6 h-1. The effect of reaction rate constant (k) on effluent concentration was shown in Fig. 3. The figure shows that, the reaction rate constant range considered in this study has no effect on effluent concentration. The substrate concentration decreases with respect to time irrespective of rate constant.

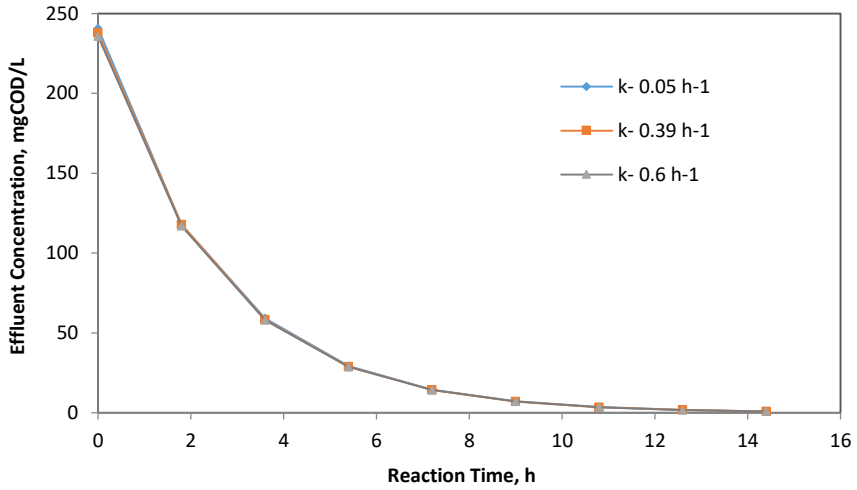


Figure 3. Effect of rate constant on effluent concentration

The effect of Va values was studied by varying the values as 0.00019, 0.0002 and 0.00021m^3. The Effect of volume of a batch reactor when is initially (Va) on variation of react time with effluent concentration is depicted in Fig. 4. When volume of a batch reactor when is initially is found to have no effect on removal rate. The substrate concentration decreases with respect time.

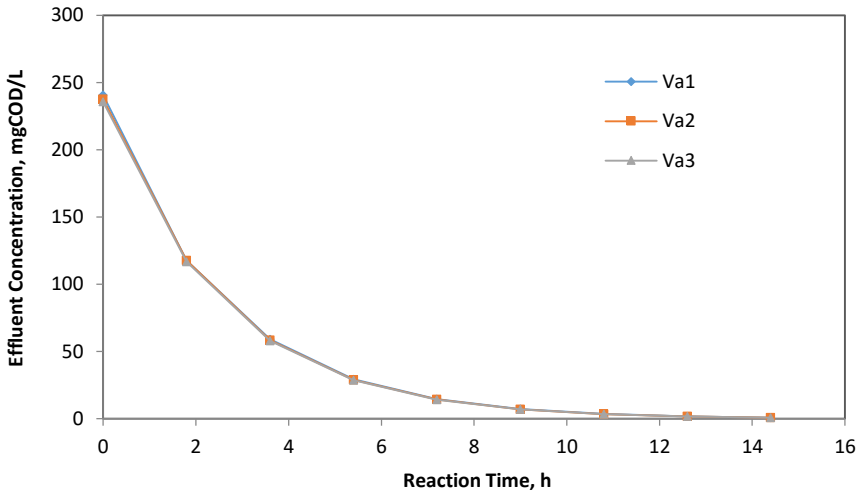


Figure 4. Effect of Va on effluent concentration

The effect of Vb values such as 0.000242, 0.000268 and 0.000268 m^3 were studied. Effect of volume of a batch reactor when is empty on variation of react time with effluent concentration is depicted in Fig. 5. The substrate concentration decreases with time and the volume of a batch reactor when is empty (Vb) has no effect on rate of removal.

The effect of Vab values such as 0.000009, 0.00001, 0.000011 m^3 were also studied. The Effect of available volume at the beginning of the fill period (Vab) on variation of react time with effluent concentration is shown in Fig. 6. Also the available volume at the beginning of the fill period (Vab) has no effect on rate of substrate removal.

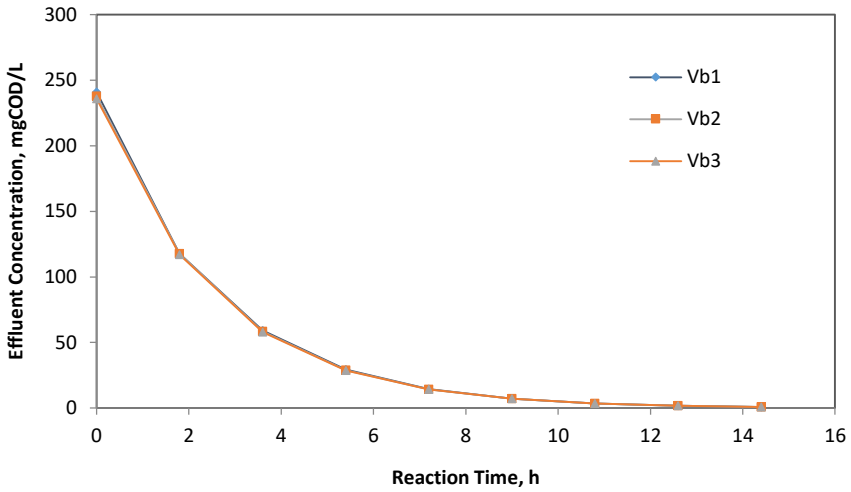


Figure 5. Effect of Vb on effluent concentration

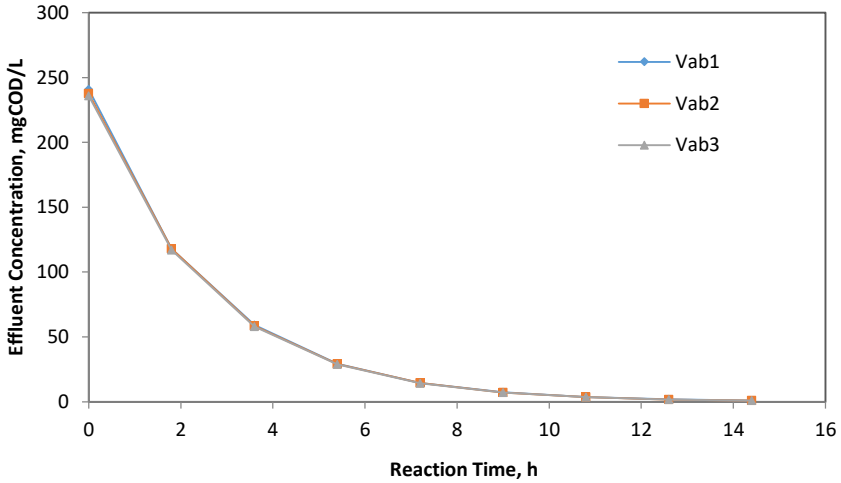


Figure 6. Effect of Vab on effluent concentration

V. Conclusions

In this work, simulation of anaerobic sequential batch reactor was carried out based on a kinetic model developed already. The effect of various parameters like initial average substrate concentration, flow rate, rate constant, and various volume of reactor conditions were studied for substrate removal. Based on the results obtained from this study, it has been found that the flow rate and substrate concentration has effect on substrate removal rate and other parameters does not have effect on substrate removal rate. From the results it has been observed that the theory applied in this study can be used to study the kinetic behavior of Sequencing Batch Reactor (SBR) for optimum operational conditions.

References

[1] M.Tholoana, Water Management at a Textile Industry A Case Study in Lesotho, University of Pretoria, 2007.
[2] M. Laxman, Pollution and its Control in Textile Industry, India, 2009.
[3] A.Reife, and H.Freeman, Environmental Chemistry of Dyes and Pigments, John- Wiley & Sons, Inc., New York, 2009.
[4] D.Wamburguh, and R.Chianelli, "Indigo Dye Waste Recovery From Blue Denim Textile Effluent: A By Product Synergy approach". New Journal of Chemist, Vol.32, 2008, pp.2189-2194.
[5] T.Do, J. Shen, G. Cawood, and R. Jeckins. "Biotreatment of textile effluent using *Pseudomonas* spp. Immobilized on polymer supports. In: Advances in biotreatment for textile processing. Hardin IR; Akin DE & Wilson JS (Eds). University of Georgia Press. 2002.
[6] N.Kirby, R. Marchan and G.McMullan. "Decolourization of synthetic textile dyes by *Phlebia tremellosa*", FEMS Microbiology Letters, Vol. 188, 2000, pp.;93–96.
[7] D.Wesenberg, I. Kyriakides and S.N. Agathos. "White-rot fungi and their enzymes for the treatment of industrial dye effluents". Biotechnology Advances, Vol. 22, 2003, 161–187.

[8] R.L. Irvine and L.H. Ketchum. The sequencing batch reactor and batch operation for the optimal treatment of wastewater. SBR Technology Inc., 2004.

[9] N. Hudson, J. Doyle, P. Lant, N. Roach, B. Bruyn, C. Staib. "Sequencing batch reactor technology: the key to a BP refinery [Bulwer Island] upgraded environmental protection system – a low cost lagoon based retro-fit". Water Science and Technology, Vol.43, 2001, pp. 339-346.

[10] S.V. Mohan, N.R. Chandrashekara, K.P. Krishna, B.T.V. Madhavi and P.N. Sharma. "Treatment of complex chemical wastewater in a sequencing batch reactor (SBR) with an aerobic suspended growth configuration". Process Biochemistry, Vol.40, 2005, pp.1501-1508.

[11] R. Boopathy, C. Bonvillain, Q. Fontenot, and M. Kilgen. "Biological treatment of low salinity shrimp aquaculture wastewater using sequencing batch reactor". International Biodeterioration & Biodegradation, Vol.59, 2007, pp. 16-19.

[12] Y.F.Tsang, F.L. Hua, H. Chua, S.N. Sin and Y.J. Wang. "Optimization of biological treatment of paper mill effluent in a sequencing batch reactor. Journal of Biochemical Engineering", Vol.34, 2007, pp.193-199.

[13] RP.Oliveira, JA. Ghilardi, SM. Ratusznei, JAD. Rodrigues, M. Zaiat and E. Foresti. "Anaerobic sequencing batch biofilm reactor applied to automobile industry wastewater treatment: Volumetric loading rate and feed strategy effects". Chemical Engineering and Processing, Vol.47, 2008, pp.1374-1383.

[14] E. Neczaj, M. Kacprzak, T. Kamizela, J. Lach, and E. Okoniewska. "Sequencing batch reactor system for the co-treatment of landfill leachate and dairy wastewater". Desalination, Vol. 222, 2008, pp.404-409.

[15] A. Rezaee and M. A. Khavanin. "Treatment of work camp wastewater using a sequencing batch reactor followed by a sand filter. American Journal of Environmental Sciences", Vol.4, 2008, pp. 342-346.

[16] F. El-Gohary, and A. Tawfik. "Decolorization and COD reduction of disperse and reactive dyes wastewater using chemical-coagulation followed by sequential batch reactor (SBR) process". Desalination, Vol. 249, 2009, pp. 1159–1164.

[17] A. Chiavola, R. Baciocchib, and R. Gavascib. "Biological treatment of PAH contaminated sediments in a sequencing batch reactor". Journal of Hazardous Materials, Vol.184, 2010, pp. 97-104.

[18] G.Durai, M. Rajasimman, N. Rajamohan. "Aerobic digestion of tannery wastewater in a sequential batch reactor by salt-tolerant bacterial strains". Applied Water Science, Vol.1, 2011, pp. 35–40.

[19] S.Sathian, M. Rajasimman, C. Karthikeyan. **"**Biodegradation and decolourization of textile dye wastewater using *Ganoderma lucidum".* 3Biotechnology, Vol. 1, 2013, pp. 71–79.

[20] M. Rajasimman, S. Venkatesh Babu and N. Rajamohan. "Biodegradation of textile dyeing industry wastewater using modified anaerobic sequential batch reactor –Start-up, parameter optimization and performance analysis". Journal of the Taiwan Institute of Chemical Engineers, Vol. 72, 2017, pp. 171–181.

[21] B. Lennox, GA. Montague, HG. Hiden, G. Kornfeld and PR. Goulding. "Process monitoring of an industrial fed-batch fermentation". Biotechnology & Bioengineering. Vol, 74, 2001, pp.125–135.

[22] P. Nomikos and J.F. MacGregor. "Monitoring batch processes using multiway principal component analysis". AIChE J Vol.40, 1994, pp. 1361–1375.

[23] R.L. Droste. Theory and Practice of Water and Wastewater Treatment, Wiley&Sons, New York, 1997.

[24] Yasemin Damar Asude Ates, Recep Ileri, "Treatment of Textile Industry Wastewater by Sequencing Batch Reactor (SBR), Modelling and Simulation of Biokinetic Parameters" International Journal of Applied Science and Technology, Vol. 2, 2012, pp. 302-318.

[25] APHA. Standard methods for the examination of water and wastewater, 16th edition. American Public Health Association, New York, 1992

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Preliminary analysis of Toxic Metal Contaminants in Common Food Products By Laboratory Chemical Reagents

S.Dinesh kumar[1], K.Manimaran[1] , A.Swedha[1] , V.Kavishree[1] , K.Krishnaveni[*2]

[1]*Under graduate student, Department of Chemistry, Dr.N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India.*

[2]*Associate Professor, Department of Chemistry, Dr.N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India.*

*Corresponding Author E-mail: Primary – ravikrishna2004@gmail.com

Secondary – dineshchemist22@gmail.com

Abstract: A normal human diet contains many chemical elements which are essential for human life but some heavy metals also present in the food items due to environmental pollution. Toxic metal analysis of food products is of major importance due to the raised consumer awareness and it is a need of an hour to evaluate and eradicate the presence of toxic trace elements in the food products. Toxic elements such as mercury, arsenic, cadmium, and lead, also termed as heavy metals, can destroy mental and central nervous system functioning and also severe damages to human kind. A qualitative assessment of heavy metal contamination (Cd, As, Pb and Hg) in some of the selected food samples collected from nearby local shops was done using simple laboratory chemical reagents. In the present work, the food products like Chocolates, Chilli powder, Turmeric powder, Rice, Wheat bread. Health Drink Powders and Sugar were taken to analyze presence of toxic metal. The purpose of the present work is to analyze the toxic metals present in the food samples using simple technique and to bring awareness to the public on the study of toxic metals.

Keywords: Metals, Harmful Diseases, Chemical Analysis, Lab reagents, Consumer Awareness.

I. Introduction

Food takes place a vital role in our day today life. Food article contain a large amount of various metallic elements especially sodium, potassium, iron, calcium, boron, magnesium, copper and zinc, etc. and heavy metals like Lead, Cadmium, Mercury, Arsenic, etc. Heavy metals enter into our body via food, water and air, at higher concentrations they can lead to health risks. These heavy metals were also indirectly adulterated with food items. Heavy metals are not only contaminated soils. These plants that in turn would be harmful for the user by taking in the plant material in the traditional manner(1). The heavy metals may present in water in a form of organometallic compound which will affect the thirteen different types of species in aquaculture food products(2). Many of these sea food products will be directly given to us in the cooked form. The concentration of heavy metals may increase if there is the change in their physical property, in some case, heavy metal concentration increases on heating(3).

Llobet et al, studied the dietary intake of heavy metals in the general population of Catalonia, Spain. They reported that the highest dietary intakes of As (223.6 µg/day), Cd (15.7 µg/day), Hg (21.2 µg/day), and Pb (28.4 µg/day) corresponded to male adults(4). The contamination of heavy metals in unavoidable in the present polluted environment. The detection of toxic contaminants in the food article is important since it is highly a need of an our research.

In many reports, the identification of toxic metals were done using atomic adsorption spectroscopy (AAS) (5). The present work is a preliminary effort to analyze the presence of heavy metals in the food samples by using simple chemical procedure.

II. Materials and Methods

Metals Analyzed: Arsenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg).

Reagents used: Dil. HCl, Conc. HNO_3, Conc. H_2SO_4, Ammonium hydroxide, H_2S (Gas), Ammonium molybdate.

Food Samples:

The food samples (Health drink powders, Chocolates, Turmeric powder, Sugar) used for the detection of heavy metals was collected from the local grocery shops in and around Coimbatore.

Method of Analysis:

The preliminary analysis of heavy metals present in the selected food samples was carried out by using following procedure:

Test for Arsenic:

A small amount of sample is taken in a dry test tube, acidified with few drops of dil. HCl and then Conc. HNO_3 is added. H_2S gas is passed through the above solution which is heated strongly and then 2-3 drops of Ammonium molybdate is added.

Test for Cadmium:

A small amount of sample is taken in a dry test tube and acidified with few drops of dil. HCl. Then a small amount of H_2S gas is passed through it. 2 drops of nitrating mixture (Conc. HNO_3 + Conc. H_2SO_4) is added and heated strongly. Few drops of ammonium hydroxide is added to the above solution and then H_2S gas is passed through it.

Test for Lead:

A small amount of sample is taken in a dry test tube. Then 2-3 drops of Conc. HCl is added.

Test for Mercury:

A small amount of sample is taken in a dry test tube. Then 2-3 drops of Conc. HCl is added.

III. Result and Discussion

The results obtained from the preliminary analysis were tabulated and the pictorial evidence of the work is depicted in the Fig.

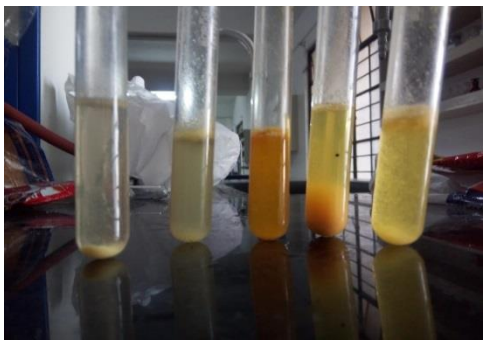


Fig 1: Yellow colour precipitate on Arsenic test.

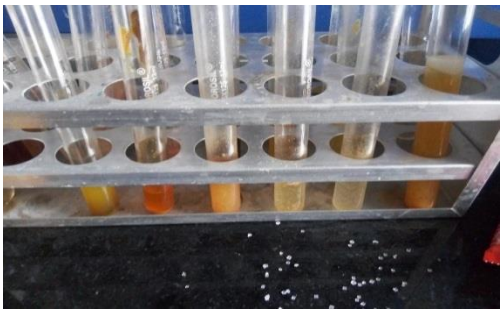


Fig 3: Yellow colour precipitate On Cadmium test.



Fig 2: Dark yellow colour Precipitate on Arsenic test

Arsenic:
Five samples of health drink powders were selected for the analysis and the tests were carried. Three out of five samples of health drink powders exhibit the yellow color (Fig 1) which shows the presence of Arsenic in the sample. For the other two samples, the result was found to be slightly yellow in color. Health drink samples are basically made up of wheat which is produced in greater number of arsenic content fields. The dark yellow color precipitate (Fig 2) which indicates the more contaminated by arsenic content and slight yellow color depicts the less contamination of arsenic.

Cadmium:
Six samples of Chocolates were tested for cadmium content. Four out of six samples produce the yellow precipitate (Fig 3). This indicates the presence of cadmium in the tested chocolate samples. Other two samples does not give the positive test for the presence of cadmium. Generally the chocolates are prepared from common cacao chocolate which consist of minimum amount of cadmium content that may be the reason for the positive response in the analyzed sample.

Lead:
Four turmeric powder samples were selected for analyzing the presence of lead and the corresponding chemical test were carried. Two out of four samples have shown the magenta coloration which is the positive test for the presence of the adulterant yellow lead salt such as lead chromate. Samples of common turmeric powder are generally adulterated with yellow lead salt.

Mercury:
Three samples of sugar were collected and the test was carried. One out of three samples of sugar has shown the presence of mercury content. The test solution gives slightly white precipitate which indicates that the content of mercury in present sugar. Other two sugar solution does not answer the chemical test.

Table 1: Preliminary Analysis of Toxic Metals in Selected Food Samples

Metal	Experiment	Samples	Observation	Result
Arsenic(As)	A small amount of sample is taken in a dry test tube and acidified with few drops dil. HCl. Then Conc. HNO_3 is added. After that a small amount of H_2S gas is added to it. Then this solution is heated strongly. After that adding 2-3 drops Ammonium molybdite.	Health Drink Powder(HDP)		
		HDP-1	Presence of yellow coloured precipitate.	Positive
		HDP-2	Presence of yellow coloured precipitate.	Positive
		HDP-3	Absence of yellow coloured precipitate.	Negative
		HDP-4	Presence of yellow coloured precipitate.	Positive
		HDP-5	Absence of yellow coloured precipitate.	Negative

Cadmium(Cd)	A small amount of sample is taken in a dry test tube and acidified with few drops of dil. HCl. Then a small amount of H_2S gas is added. Then 1 drop of nitrating mixture (Conc.HNO_3 +Conc.H_2SO_4) is added. Then heated strongly. After t that 2 drops of ammonium hydroxide is added. Then H_2S gas is added.	Chocolates(Ch) Ch-1 Ch-2 Ch-3 Ch-4 Ch-5 Ch-6	Presence of yellow coloured precipitate. Presence of yellow coloured precipitate. Presence of yellow coloured precipitate. Absence of yellow coloured precipitate. Absence of yellow coloured precipitate. Presence of yellow coloured precipitate.	Positive Positive Positive Negative Negative Positive
Lead(Pb)	A small amount of sample is taken in a dry test tube. Then 2-3 drops of Conc. HCl is added.	Turmeric Powder(TP) TP-1 TP-2 TP-3 TP-4	Magenta coloured solution obtained. Magenta coloured solution obtained. Absence of Magenta coloured solution Absence of Magenta coloured solution.	Positive Positive Negative Negative
Mercury(Hg)	A small amount of sample is taken in a dry test tube. Then 2-3 drops of Conc. HCl is added.	Sugar solution(Ss) Ss-1 Ss-2 Ss-3	Absence of White precipitate. Absence of White precipitate. Presence of White precipitate.	Negative Negative Positive

IV. Conclusion

Heavy metals are one of the common contaminant in our day to day life because of the polluted environment. Due to which many food products are being contaminated and causes harmful effects to the mankind. Direct or indirect consumption of heavy metal contaminated food products will increase the level of toxic metal percentage in our body. This should be educated to the peoples in the rural areas, so as the preliminary step the work being undertaken. The work will be continued by bringing awareness to the people by training/awareness program in future.

References

[1] Ababacar Maiga, Drissa Diallo, Ragnar Bye, and Berit Smestad Paulsen, Determination of Some Toxic and Essential Metal Ions in Medicinal and Edible Plants from Mali, *Journal Agricultural and Food Chemistry*, 2005, 53 (6), pp 2316–2321.

[2] Wen JyeMok, ShigeharuSenoo, TomohiroItoh, YasuyukiTsukamasa, Ken-ichiKawasaki, MasashiAndo, Assessment of a concentrations of toxic elements in aquaculture food products in Malaysia, Elsevier, Volume 133, Issue 4, 15 August 2012, Pages 1326-1332.

[3] Gemma Perelló, Roser Martí-Cid, Juan M. Llobet and José L. Domingo, Effects of Various Cooking Processes on the Concentrations of Arsenic, Cadmium, Mercury, and Lead in Foods, , *Journal Agricultural and Food Chemistry*, 2008, 56 (23), pp 11262–11269.

[4] J. M. Llobet, G. Falcó, C. Casas, A. Teixidó, and J. L. Domingo, Concentrations of Arsenic, Cadmium, Mercury, and Lead in Common Foods and Estimated Daily Intake by Children, Adolescents, Adults, and Seniors of Catalonia, Spain, , *Journal Agricultural and Food Chemistry*,2003, 51 (3), pp 838–842.

[5] Marcelo EnriqueConti, The content of heavy metals in food packaging paper boards: an atomic absorptionspectroscopy investigation, Elsevier, Volume 30, Issue 5, 21 November 1997, Pages 343-348.

Acknowledgement

We would like to thank our Management, College, Department, Faculty, Friends for supporting us throughout the work.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

EVALUATION OF POLLUTION PARAMETERS IN POULTRY WASTEWATER FROM LOCAL CHICKEN SHOPS

M.Udhaya Ganga[1] *, Dr.Bakyaraj [2], Dr.G.Nagamaniammai[1] and J.Suresh Kumar[1]

[1]Academy of Maritime Education and Training, Deemed to be University,
Kanathur, Chennai - 603 112.

[2]College of Poultry Production and management, Tamil Nadu Veterinary and Animal Sciences University,
Near District Livestock farm, Mathigiri, Hosur – 635 110, Krishnagiri (D.t.,)

ganga.happy7@gmail.com

Abstract*: Poultry waste water from the local chicken shop was collected and the quantitative analysis for the determination of pollution parameter was attempted. Analysis of Poultry processing waste water from local chicken shops was resulted in a way that the effluent was highly contaminated with the pollutants than the permissible level. Quantitative analysis was done to determine the pH, Total suspended solids, Total dissolved solids, Biological Oxygen Demand, Chemical Oxygen Demand, Oil and Grease, Nitrate and Phosphate levels in the Water taken for processing and the wastewater released into the drain. The pH 6.8 & 9.2, TSS 08 & 328 (mg/L), TDS 1296 & 1544 (mg/L), BOD < 2.0 & 602 (mg/L) , COD 08 & 815 (mg/L), Oil & Grease <1.0 & 14.0 (mg/L), Nitrate 0.14 & 12.69 (ppm) and Phosphate 0.04 & 19.81(ppm) are the levels for water sample and effluent sample respectively., The results of the pollution parameter analysis on the water taken for processing showed an increased range when compared with Indian Standard specification for drinking water. The study with an objective of comparative studies on quality parameter evaluation of water used for processing and effluent collected after processing and to bring out suggestion over the effluent processing. The pollution parameter study was done in-order to maintain the soil free from pollution parameters and to bring out some suggestions which can be a solution on effluent processing like Bioremediation, Electrocoagulation etc., Therefore, The best suggestion is the effluent water should not be allowed to mix with the municipal drain system without pre-treatment meeting sewage standards as per the Indian Standards.*

Keywords: Pollution parameters; BOD; COD; effluent treatment; waste management

I. Introduction

Poultry industry is one of the fastest growing segments of the agricultural sector in India today which has undergone a paradigm shift in structure and operation. A significant feature of India's poultry industry has been its transformation from a mere backyard activity into a major commercial activity in aspects of production which ultimately leads to the issues relating to environmental pollution in terms of high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), large amount of total suspended solid (TSS), and various other pollutants [1]. As is typical of many food processing industries, poultry processing is characterized by relatively high usage of water, most of it for non-consumptive purposes [2]. The quantity of wastewater discharged from processing operations in a poultry plant may range from 5 to 10 gallons per bird with 7 gallons being a typical value. Poultry processing wastewater is typically organic in character, higher than domestic sewage in BOD, and high with suspended solids and floating material such as scum and grease. Due to the increasing usage of water, the wastewater generation is tremendous and also having high concentration of pollutants fluctuating in wide range. Depending on the degree of treatment required poultry processors have the option of utilizing physical, chemical and biological treatment systems. Each system type possesses unique treatment advantages and operational difficulties.

The conversion of a live chicken into safe and wholesome meat products suitable for human consumption takes place in a series of processing steps [1]. Each step of the process utilizes potable water and generates by-products that combine to form the facility's wastewater stream. Poultry processing activities require large amounts of high quality water for process cleaning and cooling. In addition, process wastewater may contain high levels of nitrogen, phosphorus, residues of chemicals such as chlorine used for washing and disinfection, as well as various pathogens including salmonella and campylobacter

Waste Characteristics

The meat industry has the potential for generating large quantities of solid waste and waste waters with a biochemical oxygen demand (BOD_5) level of 600 milligrams per liter (mg/L) (this can also be as high as 8,000 mg/L) or 10 to 20 kilograms per metric ton (kg/t) of slaughtered animal and suspended solids level of 800 mg/l and higher, as well as, in some cases, offensive odors. The amounts of wastewater generated and pollutant load depend on the kind of meat being manufactured [3]. For example, the processing of gut has a major impact on the quantity and quality (BOD and chemical oxygen demand (COD) levels) of wastewater generated. The wastewater from a slaughterhouse can contain blood, manure, hair, fat, feathers, and bones. The wastewater may have a high temperature, and may contain organic material and nitrogen content. It may contain pathogens, including Salmonella and Shigella bacteria, parasite eggs, and amoebic cysts. Pesticide residues may be present from treatment of animals or their feed. Chloride levels may be very high (up to 77,000 mg/L) from curing and pickling processes. Smoking operations can release toxic organics into air. Rendering is an evaporative process that produces a condensate stream with a foul odor. All slaughtering wastes (generally 35% of the animal weight) can be used as by-products or for rendering [4–5]. The only significant solid waste going for disposal is the manure from animal transport and handling areas.

EFFLUENT LEVELS FOR POULTRY PROCESSING [6-7]

PARAMATERS	UNIT	GUIDELINE VALUE
pH	pH	6-9
BOD	mg/l	50
COD	mg/l	250
Total Nitrogen	mg/l	10
Total Phosphorous	mg/l	2
Oil and grease	mg/l	10
Total suspended solids	mg/l	50
Temperature increase	˚C	<3˚
Total coliform bacteria	MPN/	400

Note:
MPN – Most Probable Number
As the edge of a scientifically established mixing zone which takes into account ambient water quality, receiving water use, potential receptors and assimilative.

II. Materials and methods

In this experimental study, apparent instruments and equipment are utilized for the experiments of determination and estimation of several parameters [8]. Several pollutant parameters analyzed on this experiment study and the experiments done were as below:

1. Determination of pH
2. Estimation of total suspended solids
3. Estimation of total dissolved solids
4. Biological oxygen demand
5. Chemical oxygen demand
6. Determination of oil and grease
7. Determination of Nitrate
8. Determination of Phosphate

III. Result and conclusion

Table : 2 INDIAN STANDARD SPECIFICATIONS FOR DRINKING WATER IS: 10500

S.No.	**Parameter Requirement**	**Desirable Limit**
1	Colour	5
2	Turbiditry	10
3	PH	6.5 to 8.5
4	Total Hardness mg/L	300
5	Nitrate mg/L	45
6	Chloride mg/L	250
7	Mineral oil mg/L	0.01
8	Dissolved solids, Max mg/L	500

Table : 3 Analysis of pollution parameters on water and effluent sample

Parameter	Water Sample	Effluent Sample
pH	6.8	9.2
TSS	08	328
TDS	1296	1544
BOD	<2.0 *	602
COD	08	815
Oil& Grease	<1.0	14.0
Nitrate	0.14	12.69
Phosphate	0.04	19.81

*indicates detectable limit

The results for the analysis of water used for Primary and secondary poultry processing in the local chicken shop are presented in Table 2. There were no differences in the water quality parameters except Total dissolved substances (TDS) when compared with Indian standards.

The standard in drinking water for TDS is 500 milligrams per liter (mg/l)and our sample it was1296 (mg/L).The TDS test only provides a qualitative measure of the amount of dissolved ions, but it does not provide information about specific dissolved ions. Other indicators of high TDS are hardness, scale formation, bitter taste in drinking water caused by calcium carbonate (CaCO3) and magnesium carbonate ($MgCO_3$), and salty or brackish taste resulting from sodium chloride (NaCl) and potassium chloride (KCl). The latter can increase the corrosive ability of water.

However, an elevated TDS may mean that the water source contains elevated levels of nitrate and other contaminants If an indicator test shows elevated TDS, specific analysis is then required for each contaminant to determine potential health effects.

Poultry waste water from the local chicken shops was analyzed and presented in Table no. Almost all the pollution parameters (pollutants) were higher in level than the permissible level by the environment protection rules,1986. It indicates the waste water is highly enriched with nutrients like nitrogen, ammonia (a gas containing nitrogen and hydrogen), phosphorus and sulphur which lead to eutrophication in the water bodies

Total Suspended solids (TSS) are mineral and organic particles that remain suspended in water. TSS was found higher (1544 mg/L) than the normal level of 500mg/L. They sink only very slowly or are easily re-suspended by water turbulence. Suspended solids cause water to be turbid and this cloudiness reduces light levels. Turbidity can also be a sign of other pollution since nutrients, pesticides and metals can be attached to the suspended particles. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) tests are analytical methods for measuring the amount of oxygen consumed during the microbial or chemical breakdown of oxygen-depleting substances in water [9] such as sewage and farm slurry. High levels of BOD (602mg/L) and COD (815mg/L) indicate a heavily polluted water body making it less suitable for aquatic life. These levels

were much above than permitted levels, BOD (50mg/L)and COD(250mg/L) Poultry waste water showed that oil and grease content was higher (14mg/L) than the permissible level(10mg/L).Pollutants like oil, can have a tremendous impact on the ecosystem, especially if the water gets polluted. In a lake, it can wreak havoc on the ecological balance by stimulating plant growth and causing the death of fish due to suffocation resulting from lack of oxygen. The oxygen cycle will stop, and the polluted water will also affect the animal's dependant on the lake water.

IV. Conclusion

Analysis of Poultry processing waste water from local chicken shops was highly contaminated with the pollutants than the permissible level. Therefore, it should not be allowed to mix with the municipal drain system without pre-treatment meeting sewage standards as per the Indian Standards. Poultry waste should be washed away by safe potable and constant supply of fresh water at adequate pressure throughout the premises of slaughtering. The waste water from slaughter house is heavy in pollution and, the waste water treatment system should essentially comprise of: (i) self-cleaning type screening or two stage screening (Bar type); (ii) anaerobic treatment; (iii) aerobic treatment; and (iv) filter press for dewatering of the sludge [10]. Also we can suggest other solutions like Bioremediation, Electrocoagulation etc., and Effluent treatment plants at small scale level affordable to the local chicken shop keeper [11] It is the time to create awareness among the poultry meat shop owners about the pollutants from the poultry processing waste water and its impact on the ecosystem. The consequences of pollution may be direct, through the toxic effect of a substance. Or the consequences of pollution may be indirect [12.] Nitrogen and phosphorus are essential for plant growth, but excessive plant growth can be harmful to water. For example, enhanced phosphorus levels in surface waters (that contain adequate nitrogen) can stimulate excessive algal growth. In time, the process of algal decay reduces the oxygen available in the water, harming fish and other aquatic life [13]. Our use of water can cause pollution, not only because of substances we put into water either deliberately or accidentally, but also by abstracting water from rivers and lakes we reduce the ability of water bodies to tolerate the presence of potentially-polluting substances. Hence, the reduction of water pollution relies not only on preventing potentially-polluting substances entering water bodies, but also on reducing the amount of water we use.

References

1. M. J. Baker, D. W. Blowes, and C. J. Ptacek, Laboratory Development of Permeable Reactive Mixtures for the Removal of Phosphorus from Onsite Wastewater Disposal Systems. Environmental Science and Technology 32(15):2308-2316. 1998.
2. G. T. Kroyer, Impact of Food Processing on the Environment: An Overview. Lebensm. Wiss. Technology 28:547-552. 1995.
3. W.J. Campand E. Willoughby, Extended Aeration Purifies Effluent. Food Engineering 40:72-74. 1968.
4. R. E. Carawan 1989. Environmental Issues Facing Food Processors in the 1990's. In: 1989 Food Industry Environmental Conference Proceedings. Georgia Institute of Technology, Atlanta, Georgia. pp. 217-237.
5. R. E. Carawan, W. M. Crosswhite, J. A. Macon, and B. K. Hawkins, 1974. Waste and Waste Management in Poultry Processing. EPA-660/2-74-031. Environmental Protection Technology Service. U.S. Environmental Protection Agency, Washington, D.C.
6. International finance corporation, 2007.
7. S. C. Cates, D. W. Anderson, S. A. Karns, and P. A. Brown, Traditional Versus Hazard Analysis Critical Control Point-Based Inspection: Results from a Poultry Slaughter Project. Journal of Food Protection 64(6):826-832. 2001.
8. T. C. Chen, J. E. Hioll, and R. L. Hayes, Quality Characteristics of Raw and Treated Effluents from Mississippi Poultry Processing Plants. Poultry Science 55:2390-2395. 1976.
9. G. H. Teletzke,. Chicken for BBQ, Wastes for Aerobic Digestion. Wastes Engineering 32:135-139. 1961
10. R. Smith Wastewater Treatment System Upgrade at a Processing Plant. In: Proceedings of the 1996 National Poultry Waste Management Symposium. P. H. Patterson and J. P. Blake (Editors). Auburn University Printing Service, Auburn, Alabama. pp. 282-284. 1996.
11. S. P. Singh, R. L. Wesley, and E. A. Budd, 1973. Characteristics of Poultry Processing Effluents. Poultry Science 52:1478-1481.
12. D. T. McGrail Poultry Processing Wastewater – Advanced Treatment and Reuse. Technical Report EPA-600/2-76-304. U.S. Environmental Protection Agency, Washington, D.C. Sincero, A. P. and G. A. Sincero, 2003. Physical-Chemical Treatment of Waste and Wastewater. CRC Press, New York, New York. 1976.
13. APHA, Standard Methods for the Examination of Water and Wastewater. 18th Edition. A.E. Greenberg, L. S. Clesceri, and A. D. Eaton (Editors). American Public Health Association,Washington, D.C. 1992.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

ANTI BACTERIAL, SEM, DAMAGE PROBABILITY AND BEAM DISPLACEMENT OF GLYCINIUM MALEATE NANO PARTICLES

S JEYAKUMAR[1], K SENTHILKANNAN[2*], PRASANTH K[3], S JAIPRIYA[4],
ARVIND AKASH[3], AKILESH[3], PRAKASH A[3]
[1]MSAJ group of colleges Chennai, TN India.
[2]R and D, Edayathangudy G S Pillay Arts & Science College, Nagapattinam 611002 TN India.
[3]Sri Ramakrishna Mission Vidyalaya College of Arts and Science Coimbatore 641020
[4] Govt. Arts College, Coimbatore 18.
Email: mscgoldmedalist@yahoo.in

Abstract*: Glycinium Maleate (GM) synthesized as nanoparticles by ball milling method is subjected to SEM study for morphological survey, anti bacterial activity for bio classification of sample in pharmaceutical use, Damage probability by LASERs and beam displacement for filter applications.*

Keywords*: GM, nano, SEM, Anti bacterial.*

I. Introduction

Nano particles have many applications based on their size and shape and approach [6-7], here GM nano particles are mainly used for anti bacterial for bio pharma applications based on their size and change in behavioral property when the material is changed from macro granules to nano sized**.**

II. SEM study

SEM images show the agglomeration cum cluster f particles. It is due to ultrafine particles agglomerate in the open atmosphere and it portrays the surface morphological behavior of GM nano particles and here it is full of cluster of particles and some void gaps due to material atomic packing in the molecular system and no flaws observed as shown in Fig.1.

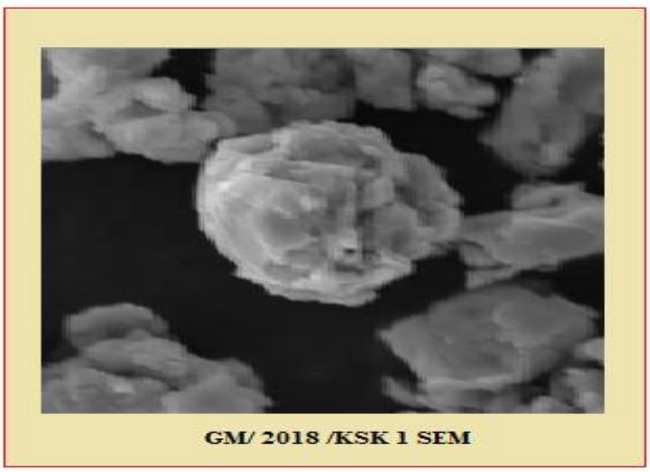


Fig.1 SEM of GM np

Fig.2 shows the XRD patterns of GM as a function of milling time. With increasing the milling time the peaks of element decrease in intensity and become broader, suggesting reduction in crystallite size. From initial to final crystal sizes are shown in the below mentioned pictorial representation.

Mill type	Retsch planetary ball
Milling time	40 hours
Wet milling medium	Toluene
Milling speed	250 rpm

Sample Crystallite Size (nm)

Sample	*Crystallite size*
GM initial powder	*205 nm*
GM after 15 hours	*54 nm*
GM after 24 hours	*37 nm*
GM after 30 hours	*28 nm*
GM after 40 hours	*21nm*

III. X-Ray Diffraction (XRD) Analysis

As a non-destructive testing practice, X-ray diffraction is a very very powerful tool for the analysis of crystalline structure. X-ray has wavelengths comparable to the crystalline lattice constants, thus it can be used for the accurate measurement of lattice parameter, crystallite size, lattice strain etc. Crystallite size is obtained from the X-ray diffractometer by using scherrer formula as given wavelength corresponding to Cu- Kα(1.5406 A°) , 2θ is the diffraction angle for the peak, k is a constant having value 0.9 and β indicates the full width at half maximum(FWHM) obtained by reducing instrument broadening from the FWHM of the sample as shown below.

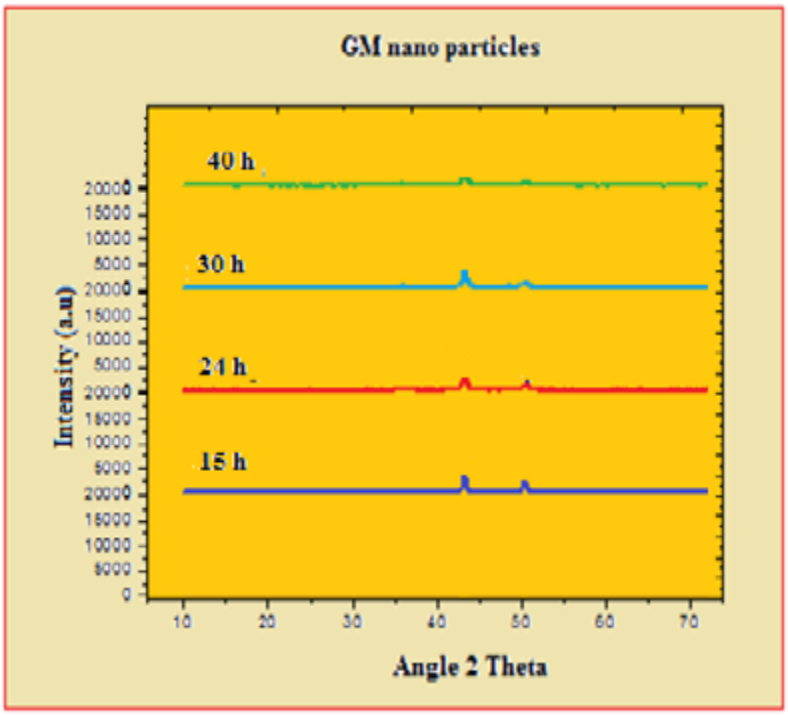


Fig.2 XRD of GM np

IV. Antibacterial Activity

Antibacterial activity of GM nano powders is investigated against Escherichia coli (E. coli), Bacillus subtilis (B.subtills) bacteria and the variation in the diameter of the inhibition zone as a function of the precursor solution is shown in Fig.3. The results reveal that the antibacterial efficiency of the GM is remarkably high in the case of 21nm. The antibacterial activity is enhanced with the size of particles. According to the mechanism related to the antibacterial activity of GM material, it is reported that the production of reactive oxygen species such as hydroxyl radicals. The emancipation of GM from the nano powder which plays a major role in the above said mechanism of antibacterial activity may be another reason for the enhancement in the antibacterial activity of the material.



Fig.3.Antibacterial Activity of GM np

V. Laser Damage and Filter and Angle settings

Bright line CW 10640 nm of power 2kW and diameter 2 mm similarity with 1J/cm2 Laser fluence is 63.661 kW/cm2 and % LDT is 31.83% and for and Bright line CW 1064 nm of power 2mW and diameter 2 mm similarity with 1J/cm2 Laser fluence is 0.06kW/cm2, Filter LDT is 20kW/cm2 and both cases probability is damage not probable.

For filters LWP 501.67 is the angle and beam displacement is 1.9764 mm and SWP -2393.77 is the angle and beam displacement is 2.3968 Compensation plate (2.0mm) -266.75 is the angle and beam displacement is -4.3733.

VI. Conclusion

The GM nano particles are synthesized by ball milling method and the SEM, XRD represents the morphological data and crystalline data and damage probability is by LDT approach and displacement by filter settings and antibacterial activity of GM also discussed and found GM np may be utilized for the opto electronic and bio pharma and by other applications based on sample size.

Reference

1. Jorge E. Munoz , Janeth Cervantes , Rodrigo Esparza , Gerardo Rosas; Iron nanoparticles produced by high-energy ball milling, J Nanopart Res (2007) 9:945–950, DOI 10.1007/s11051-007-9226-6.

2. Mohammad Baghar Rahaei, R.Yazdani rad, A.kazemzadeh,T. Ebadzadeh; Mechnochemical Synthesis of nano Tic powder; Powder Technology 217(2012)369-376 5. O.K.Tan, W.Cao,Y.Hu,W.Zhu; nanostructured oxides by high energy ball milling techniques:application as gas sensing materials; Solid State Ionics 172(2004)309-316.

3. Yadav, O.N.Srivastava; Synthesis of nanocrystalline Cerium oxide by high energy ball milling; Ceramics International 38(2012)5783-5789.

4. Musa Mutlu Can, Sadan Ozcan,Abdullah,Tezer Firat; Effect of milling time on synthesis of magnetic naoparticles; Material Science & Engineering 172(2010)72-75.
5. G.R. Khayati, k. Jangharban; The nanostructure Evolution of Ag powder synthesis by high energy ball milling; Advanced Powder Technology 23(2012)393-397.
6. Satya V. Ravikumar, Jay M. Jha, Krishnayan Haldar, Surjya K. Pal and Sudipto Chakraborty; Surfactant-Based Cu–Water Nanofluid Spray for Heat Transfer Enhancement of High Temperature Steel Surface; J. Heat Transfer 137(5), 051504 (May 01, 2015).
7. Ahmad Monshi, Mohammad Reza Foroughi,Mohammad Reza Monshi,Modified Scherrer
Equation to Estimate More Accurately Nano-Crystallite Size Using XRD, World Journal of Nano Science and Engineering, 2012, 2, 154-160.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Membrane Bioreactors for Treatment of Textile Effluent

Abarna Varshini. A, Madhumitha. M, Oviya Soundari. N, Sudhamathivadhani. E, Sagarika, Sivarajasekar. N
Department of Biotechnology, Kumaraguru Collage Of Technology
Coimbatore, Tamil Nadu,641049, India
abarna.16bt@kct.ac.in

Abstract: *Waste water from textile industry is the most prevailing problem that have been faced recently. It is estimated that nearly 10000 different dyes and* $7x\ 10^5$ *tonnes are used annually worldwide. Nearly two lakhs tonnes effluent are released annually. The dyes should be treated before discharging into the environment because it is highly toxic and contains carcinogenic substance like aromatic compounds/hydrocarbons. There are no distinct way in which the effluent can be 100% recovered without any toxic and carcinogenic substance which cause high danger to environment. They show severe effects at low concentration i.e. high toxicity. Membrane Bioreactor(MBR) is the only solution to this problem because it is highly effective and low cost process. There are many recent innovation and membrane cost reduction will enabled to use this technique more nowadays.Membrane bioreactor is the integrated form of biological degradation of organic and inorganic contaminants using membrane filtration. Membrane bioreactor is quite efficient than conventional bioreactor due to its distinct advantages. Though it has various advantages, there is a major drawback in MBR that is membrane fouling which decreases the lifespan of MBR. In this review paper we discussed about the various treatment of textile effluent, their working principle, and the application of membrane bioreactor and also the drawback of membrane bioreactor along with the way of controlling the membrane fouling. Further research aspects are considered for feasible process to treat the textile effluent.*

Keywords: *Effluent, toxic, carcinogenic, membrane bioreactor (MBR), fouling.*

I. Introduction

Textile industries are one of the major water consuming sector .In textile industry, the production of textile involves various process like fiber preparation, yarn spinning, sizing, and knitting, desizing, bleaching, dyeing, printing and finishing. This all process requires water and the liberation of waste water contains trace amount of organic, inorganic, biological and carcinogenic contaminants like COD, BOD, suspended solids, salts, surfactants, oxidizing and reducing agents, strong colours that are harmful to the environment and humans.

In order to treat the waste water various technology was evolved, in that the advanced technology of treating waste water is membrane bioreactor (MBR).[11]

In the contemporary world, one of the major issues concerned is the scarcity of water resources and inability to manage waste water. Despite several conventional methods coming into spot light, none are good enough so as to manage the need and the available resources. Those that are available are prone to contamination by the effluents such as salts, enzymes, surfactants, oxidising and reducing agents. They are suspended into the environment in several forms possibly as solids, COD, BOD, change in pH or even colour. [1]

The main environmental concern today and for the years from now on will be development of eco-friendly methods intending the purification of chemically contaminated water. This is also focussed on preventing further damage to the environment. One such domain is the textile industry effluents. The quality of the textile industrial water outlet into the surrounding bodies depends upon the employed colours, dyestuffs and accompanying chemicals as well as the process itself. [2]

Lately in the century, encompassing a vast number of options for water recycling, membrane bioreactors have been trending and are known to have the greatest advantage of energy efficient treatment of domestic and other wastewaters. In such bioreactors, membranes are generally placed external to the bioreactors with an energy usage of 3-7.3 Kw h/m3. [3]

The reactor is said to be a simple conventional system with a combination of an activated sludge which is in need of tertiary steps like filtration. [4] The choice of the membranes also results from the classification and characterization with contradictory constraints. [1]

So the at most need of the year is the advancement of a membrane bioreactor that incorporates biological treatments, chemical precipitation, membrane technology, activated carbon adsorption and several other energy saving technologies. This will possibly make the membrane technology a highly promising one.

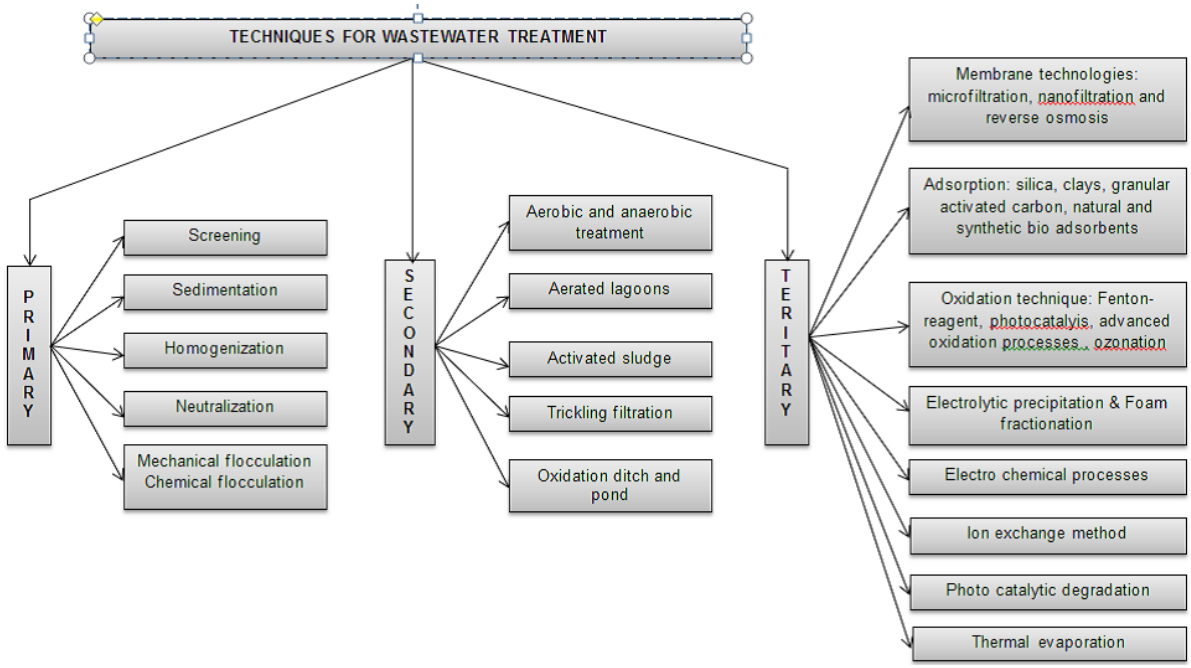
I. Various Method For The Treatment Of Textile Effluent:

Waste water is a major environmental crisis which results in the decrease of fresh water availability. Hence various methods are emerged to treat the textile effluents, that are oxidation method, physical method and biological method.

The treatment of effluent is catagorised into

- Primary Treatment
- Secondary Treatment
- Teritary Treatment [13]

The figure(1) represents the various techniques available for the treatment of textile effluemt

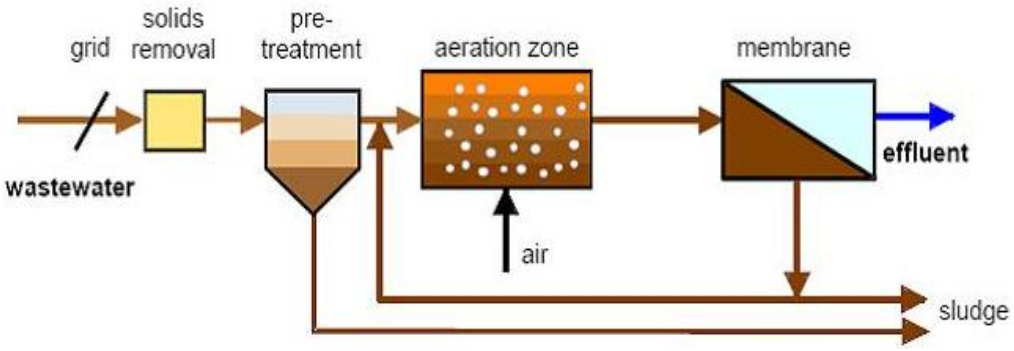


Various Method For Treating The Textile Effluent [13]

II. Membrane Bio-Reactor :

Membrane bioreactor is evolved during 1960's by Dorr-Oliver. But in recent years the application of membrane bioreactor has started to attract the serious attention, because of its significant advantage . In membrane bioreactor initially the waste water is pre -treated and then followed to aeration and membrane filtration .[10]
The membrane bioreactor process can be described as an recycling of the waste water using biological treatment , where microbes are used to degrade the organic pollutants that are then filtered by the submerged membranes.
Five types of membrane configurations are currently in operation :

- Hollow fibre
- Spiral –wound
- Plate and frame
- Pleated filter cartridge
- Tubular



Schematic diagram of external process of membrane bioreactor [13]

Pre-treatment:
To avoid the unwanted solid particles in the waste water, which enters the membrane treatment screening is essential. Pre-treatment process minimizes the accumulation of solid particle and prevents the membrane from damaging the debris and particles **.**It reduces the operating cost.

Secondary treatment:
Secondary treatment is the heart of waste water treatment process whereas the previous steps of treatment consisted in removing the suspended solids by settlement this step of treatment consists in removing the major part of dissolved pollution mainly Bod5 and ammonia during the aeration phase specific bacteria eat the

Membrane Bioreactor-Effluent Treatment [15]

dissolved organic matter and transform it to suspended solids other suspended solids other specific bacteria oxidize the ammonia into nitrates then during the non-aeration phase also called anoxic phase ,other type of bacteria convert the nitrates into nitrogen gas which is released into the atmosphere to ensure a good balance between food and bacteria . The bacteria settled in the clarifiers are continuously returned at the inlet of the tank they are called returned activated sludge the upstream part of the activated sludge tank is covered so that odorous air is trapped and extracted towards other treatment units.

III. Principle

Membrane reactor combines the activated sludge and membrane filtration process. It includes the micro filtration and ultrafiltration process with the pore size of 10 nm to 0.5 microns. Membrane reactor benefits for the physical barrier separation using biological treatment [16]. It eliminates the sedimentation and filtration process which is used for waste water treatment. Activated sludge process is the type of suspended growth system i.e. the biomass includes the bacteria, protozoa, cell tissues is in suspension in the sewage. The organisms are the aerobic type. When these organisms contact with the organic substance of the sewage it oxidized or stabilized the organic compounds in the aeration tank. Then the oxidized effluent goes to the secondary sedimentation tank and sludge formed. The sludge includes organic matters and active microbes some of these sludge recycled back to the aeration tank ,this process is known as activated sludge .This will increases the rate of treatment of the aeration tank. Using membrane filtration process these microorganism, organic pollutants are filtered through semipermeable membrane. The membrane process includes micro filtration, ultrafiltration and nano filtration.

IV. Drawback Of Membrane Bioreactor

Fouling is the major drawback in MBR. Fouling occurs due to organic substances (polymers and polysaccharides)/ biofouling and inorganic fouling. Fouling is the process which causes low efficiency due to the deposition of suspended particles on its membrane surface or pores.it serves as a barrier. Fouling is of different forms: cake formation, pore clogging, pore narrowing.

Fouling Formation [7]

These forms lead to the increased resistance towards membrane. Thus, the permeability of the membrane gets deteriorated

The main causes of fouling are

- Precipitation of organic matter
- Biofilm formation on its membrane surface
- Aging of membrane

V. The Way Of Controlling The Membrane Fouling

The systematic cleaning can help to prevent foulants from building on the membrane. There should be cleaning cycles scheduled monthly or at regular basis to provide the greatest benefit. The maintenance strategies can vary depending upon each membrane filtration design and can employ one or more cleaning methods, such as:

Physical Cleaning or Mechanical Cleaning: Gas scour, sponges, water jets or backflushing using permeate or pressurized air**.**

Biological Cleaning: Biocides are used to remove all viable microorganisms

Chemical Cleaning: The usage of acids and bases to remove foulants and impurities.

- Chemical coagulants like ferric chloride and alum to control fouling in submerged MBR.
- Zinc oxide can also be used

VI. Applications of MBR

- The main advantage of MBR is it reduces importance of biomass sedimentation.
- The treated water from MBR will be of higher quality due to the remove of particulants on its surface.(high effluent quality).
- Lower sludge production.
- Compact
- High effluent quality [6]
- High volumetric load possible[7]

VII. Conclusion

Membrane bioreactors now have grown in the field of effluent treatment and have shown tremendous changes and stopped the various kinds of pollutions. Through MBR we can purify the textile effluent and it can be discharged into the river or pond. Through this method there is no destruction of organisms and they are ecofriendly. In this paper we have seen the working principle and the importance of membrane bioreactor in textile industry. This method is expensive but it is highly effective,reliable and robust. The use of this membrane based separation or filtration process not only enable us to achieve high degree dye removal/efficiency. It minimizes the harmful effects of textile effluents. Mbr was found to be more efficient method than anyother conventional treatments.

VIII. References

[1] Kim, J., Kim, K., Ye, H., & Lee, E. (2011). Anaerobic Fluidized Bed Membrane Bioreactor for Wastewater Treatment, *45*(2), 576–581.

[2] Melin, T., Jefferson, B., Bixio, D., Thoeye, C., Wilde, W. De, & Koning, J. De. (2006). Membrane bioreactor technology for wastewater treatment and reuse, *187*(February 2005), 271–282. https://doi.org/10.1016/j.desal.2005.04.086

[3] Badani, Z., Ait-amar, H., Si-salah, A., Brik, M., & Fuchs, W. (2005). Treatment of textile waste water by membrane bioreactor and reuse, *185*, 411–417. https://doi.org/10.1016/j.desal.2005.03.088

[4] Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., & Kroiss, H. (2005). Removal of selected pharmaceuticals , fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants, *39*, 4797–4807. https://doi.org/10.1016/j.watres.2005.09.015

[5] Brik, M., Schoeberl, P., Chamam, B., Braun, R., & Fuchs, W. (2006). Advanced treatment of textile wastewater towards reuse using a membrane bioreactor, *41*, 1751–1757. https://doi.org/10.1016/j.procbio.2006.03.019

[6] Stephen Chapman, C., Law, I., & Solutions, I. B. L. Membrane Bioreactors (MBR) for Municipal Wastewater Treatment–An Australian Perspective.

[7] Larsson E., & Persson, J. (2004). Viability of membrane bioreactor technology as an advanced pre-treatment for onsite wastewater treatment.

[8] Hajibabania, S., Verliefde, A., Drewes, J. E., Nghiem, L. D., McDonald, J., Khan, S., & Le-Clech, P. (2011). Effect of fouling on removal of trace organic compounds by nanofiltration. *Drinking Water Engineering and Science*, *4*(1),71-82.

[9] Visvanathan, C., & Aim, R. B. (2002). Membrane Bioreactor Applications in Wastewater Treatment.

[10] Mutamim, N. S. A., Noor, Z. Z., Hassan, M. A. A., Yuniarto, A., & Olsson, G. (2013). Membrane bioreactor: applications and limitations in treating high strength industrial wastewater. *Chemical Engineering Journal*, *225*, 109-119.

[11] Jyoti, J., Alka, D., & Kumar, S. J. (2013). Application of membrane-bio-reactor in waste-water treatment: a review. *International Journal of Chemistry and Chemical Engineering*, *3*(2), 115-122.

[12] Holkar, C. R., Jadhav, A. J., Pinjari, D. V., Mahamuni, N. M., & Pandit, A. B. (2016). A critical review on textile wastewater treatments:possibleapproaches. *Journalofenvironmentalmanagement*, *182*,351-366.[13] Srebrenkoska, V., Zhezhova, S., Risteski, S., & Golomeova, S. (2014). Methods for waste waters treatment in textile industry.

[14] Suryawan, I. W. K., Helmy, Q., & Notodarmojo, S. (2018, January). Textile wastewater treatment: colour and COD removal of reactive black-5 by ozonation. In *IOP Conference Series: Earth and Environmental Science* (Vol. 106, No. 1, p. 012102). IOP Publishing.

[15] Scott, S. (2005). Application of Membrane Bioreactor Technology to Wastewater Treatment and Reuse

[16] Jyoti, J., Alka, D., & Kumar, S. J. (2013). Application of membrane-bio-reactor in waste-water treatment: a review. *International Journal of Chemistry and Chemical Engineering*, *3*(2), 115-122

[17] Cic¸ek, N., Franco, J. P., Suidan, M. T., Urbain, V. & Manem, J. (1999). Characterization and comparison of a membrane bioreactor and a conventional activated-sludge system in the treatment of wastewater containing high molecular weight compounds, Water Environment Research, 71, 64–70.

[18] Kennedy, M., Kim, S. M., Mutenyo, I., Broens, L., & Schippers, J. (1998).Intermittent crossflushing of hollow fiber ultrafiltration systems, Desalination, 118, 175–188.

[19] Le-Clech, P.; Fane, A.; Leslie, G. (2005) MBR Focus: The Operators Perspective. Filtration & Separation, June pp. 2023.

[20] Linden, S. (2006a). Industrial MBR Issues. Novel MBR Technologies for Industrial Wastewater Treatment and Reuse, WEF Webcast, May.

[21] Liu, R., Huang, X., Wang, C., Chen, L., & Qian, Y.(2000). Study on hydraulic characteristics in a submerged membrane bioreactor process, Process Biochemistry, 36, 249–254.

[22] Marcucci, M., Nosenzo, G., Capannelli, G., Ciabatti, I., Corrieri, D., & Ciardelli, G. (2001). Treatment and reuse of textile effluents based on new ultrafiltration and other membrane technologies, Desalination, 138, 75–82..

IX. Acknowledgments

The heading of the Acknowledgment section and the References section must not be numbered.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Industrial Wastewater Treatment Using Membrane Bioreactors

Mithra, Deepthi Suresh, Uthish, Keerthana, Poornima, Bharati Priyamvatha, Sivarajasekar. N

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore-641049, INDIA

keerthana1.16bt@kct.ac.in

Abstract*: Membrane bioreactor technology (MBR) an efficient advancement for the waste water treatment. The combination of activated sludge involving micro and ultra-filtration is a feasible way for the treatment. This review article gives an insight overview of the application of membrane bio reactor for the industrial waste water treatment. The waste water involves various residual organic, inorganic components and it may vary according to nature of the source. Several risk factors are involved in the operation of this technology of which fouling is the major one that can be reduced using the fouling reducers such as the activated carbon. The technology has been advanced from optimization to developments in architectural designing of the bioreactors. This article summarizes the techniques, recent trends and approaches towards the membrane bioreactor application in industries for waste water treatment.*

Keywords*: recent trends of MBR, application, techniques, risk factors.*

I.Introduction

The fresh water availability is becoming increasingly limited in many areas of the world but at the same time to maintain and support the growing population, world requires increased quantity and quality of water. Parts of Africa, Asia, India, and China are found to have great water scarcity. Recycling and reuse of water is the only way to meet the world's scarcity. The commonly used methods for industrial wastewater treatment are chemical and biological methods. Among these two methods, the most used method is biological method which is environmental and economic friendly [1]. Industrialization has increased in the worldwide which led to the generation of large quantity of industrial effluent with organic content which if treated will result in a significance amount of energy. Industrial effluents also contains suspended solids which can be separated by membranes which changes the turbidity of wastewater and the suspended solids come close to zero. Membrane technology is the recent development for treating the industrial wastewater.

Membrane bioreactor is a biochemical process which involves the use of both 1) Membrane separator for liquid and solid separation and 2) Suspended growth bioreactor for biochemical process [2]. Another post-treatment option is to use micro or ultra-filtration membranes combined with anaerobic reactors. Over the past decades, industrial wastewater treatment was achieved by submerged membrane bioreactor. MBR technology for industrial wastewater treatment has also gained attention because of the sturdiness of the process, very specific bio refractory, high organic loadings, difficult to treat compounds and inhibiting are the major characteristics of industrial waste streams that render alternative treatment techniques like MBR technology.

II.Existing Solutions

Table 1.Discharge level of toxic contaminants in the industrial effluents [3] (water pollution control act, 1974)

SNO	COMPONENTS	MARGINAL LEVEL
1	Mercury	0.01mg/l
2	pH	5.5 – 9
3	Suspended solid	250mg/l
4	Oil and grease	10 mg/l
5	Copper	3 mg/l
6	Iron	3mg/l
7	Zinc	5 mg/l
8	Nickel	3 mg/l
9	Cyanide	0.2 mg/l
10	Cadmium	2mg/l

Nowadays the industries are releasing a highly contaminated industrial effluent which has been disturbing the hydrological cycle as shown in table 1.To prevent this there are various techniques available to treat the waste

water in optimal cost and in an effective way. Depending on the amount of waste water released the various techniques are employed as shown in figure 1[3].

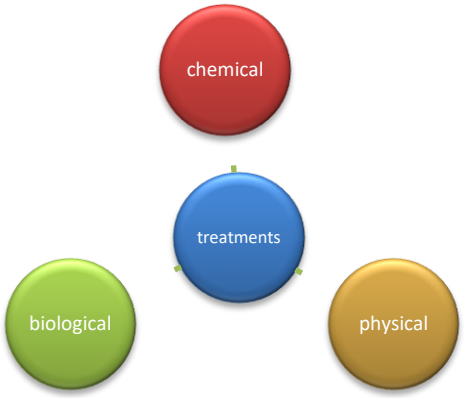


Figure 1.Different techniques employed to treat industrial wastewater

One of the conventional way of treating effluent water by the conventional activated sludge system (CAS). Ardern and Lockett in England developed this system. It is a biological method of waste water treatment which involves microorganisms and waste water is agitated and aerated. The biological solid are separated from the treated waste water and is recirculated for the aeration process. The Three types of organisms are responsible for the operations are bacteria, plants and animals. These organisms feed on organic substances and therefore treat the water. The advantage of CAS is producing the odor free treated water. But it is not suitable for reuse and recycling. And it is also not suitable for some industrial waste. To overcome these drawbacks membrane bio reactors are used [4].

III.Membrane Bioreactor

Membrane bioreactor technology is a modern waste water treatment method [2]. It is defined as integrating the biological degradation of wastewater with membrane filtration. For biomass separation, coupling the membrane with biological process are often used. It is quite effective in removing organic and inorganic contaminants. The bio reactor and membrane unit have specific functions :(i) degradation of organic matter by microorganisms; (ii) removal of microorganisms from treated waste water. The membranes are physical barrier for solids suspended in the water and production of water free solids, bacteria and viruses.

This method can be particularly used in water scarcity area and where water recycles and reuse is required. The industries where the technology can be implemented are petrochemical, pharmaceutical, cosmetics, dairy, automotive etc[4].

IV.Membrane Materials

The membranes are often made with polymers or ceramic materials. The advantage of polymers is low cost production but it may vary in pore size. Ceramic membranes have quality and longer lifetime but economically not feasible. Most commonly polymeric membranes are used. They may be either in the form of hollow fiber, plate, or frame [5].

Table 2.Characteristics of Different Polymer Membrane Material

Source: Scott et.al

MATERIALS	ADVANTAGE	DISADVANTAGE
Cellulose acetate	low-cost	Biologically active, Narrow pH range
Polyvinylidene fluoride	Tolerance to high chlorine content	Can only sustain pH <10
Polypropylene	Low cost, tolerance to high pH	No chlorine tolerance
Polyacrylonitrile	Low cost, Used for ultrafiltration membranes	Low chemical resistance
Polyethersulphone & Polysulphone	Chlorine resistant, Economically feasible	Extra support is required for brittle materials.

V.Types of Bioreactor

Membrane bioreactor system can be classified into four groups based on its application in wastewater treatment namely:

1. Bubble-less Aeration Membrane Bioreactors
2. Extractive Membrane Reactors
3. Membrane Separation Reactors
4. Recycle Membrane Reactors[6]

Bubble-less Aeration Membrane Bioreactors

Efficiency of the process is controlled by availability of air.80-90% of oxygen diffused in sludge process is vented to atmosphere due to inefficient mode of air supply. A synthetic polymer membrane is placed in between the gas and liquid phase to avoid bubbles. These membranes are either in the plate-and-frame form or hollow fiber module. Current research prefers hollow fiber module because it provides high surface area for oxygen transfer and occupies a small volume in the reactor [6].

Extractive Membrane Reactors

It enhances the performance by making the membrane to achieve high degree of separation while components transport from one phase to another. This causes biological degradation of wastewater pollutants. The extractive membrane reactor is operated in two ways:

1. Mode 1:
   Membrane is immersed in bio-medium tank. The toxic compounds are transported to the surrounding bio-medium due to concentration gradient and degraded.
2. Mode 2:
   External circuit is formed by the membrane with the bio-medium tank. The toxic pollutants in the wastewater are circulated on the shell side of the membrane modules. The toxic pollutant is degraded in the medium under optimum conditions [6].

Membrane Separation Reactors

Membrane separation bioreactor is used to separate bio solids in sludge process. This system overcomes the sedimentation steps in the conventional process. This biomass separation technology is a combination of membrane filter and suspended growth reactor for biodegradation. The membrane unit is either external or immersed in the bioreactor. In the conventional process, quality reusable water was achieved by carbon adsorption, multimedia filtration on the secondary effluents. The membrane separation reactor overcomes this by degrading bacteria and viruses in the first step [6].

Recycle Membrane Reactors

In industries, the recycle membrane reactors are utilized essentially in two basic configurations: Tubular and Beaker type. In the beaker type, the feed wastewater with together with biocatalyst is kept in a beaker and the membrane immersed in the vessel is used for filtration. In the tubular type, the biocatalyst is trapped either in the shell side or tube side and mainly used in large scale industries. This bioreactor has been tested in industries for the remove aromatic pollutants and pesticides [6].

VI. Advantages of Membrane Bioreactor in Industrial Wastewater Treatment

The specific advantages of membrane bioreactor in industrial wastewater treatment include the following:

- The membrane bioreactor system readily maintains the slow growing organisms like nitrifying bacteria and those capable of degrading the complex organics.
- Produce water for reuse through reverse osmosis.
- Eliminates particulate, colloidal and high molecular weight components.
- Short Sludge Retention Time (SRT) favors anaerobic process by increasing the biomass production while long SRT favors aerobic digestion of bio solids. Therefore control of SRT gives optimum control of microbial population as well as offers operation flexibility.
- At long SRT, high mixed liquor concentration in the reactor allows to treat the wastewater by minimizing the biomass yield.
- Applying the MBR system, there is a possibility for more compact biological reactor.
- The non-biodegradable compounds are discharged with the sludge instead of being discharged with the treated water.
- Due to retention of all microbial seed material, the initial startup process is rapid.
- It can readily eliminate biological nitrogen and phosphorous if configured. Certain metals can be eliminated by addition of chemicals externally and promotes the retention of resulting slats and hydroxides.
- The MBR system operates unattended except for occasional performance checks and the mechanical components maintenance.

- MBR system eliminates the concern for changing the biomass settling characteristics such as filamentous growth and related cost implications like polymer addition or chlorine addition to remove filamentous growth [1].

VII.Future Development

The membrane suppliers are increasingly focusing on the MBR applications which are likely to result in cost-effective embodiments as improvements are made in the membrane component efficiency.Infuture,cost-efficient anaerobic MBR systems and alternative MBR system are likely to emerge which not only involves in the biomass-effluent separation but also for other purposes[7].

VIII.Conclusion

This study concluded that MBR is the most modern technology for wastewater treatment. Membrane bioreactor for waste water treatment is rapidly accepted all over the world [2].The membrane bioreactors are effective in treating and producing the high quality effluent water. The effluent can be reused with the industries or it can be released to the surface water. Because of its ease in operation and compactness make it ideal for use in remote areas .MBR can be used in any industries or municipal wastewater treatment ,reducing pollution in water bodies[5].The research is undergoing in this area to develop a solution to treatment of recalcitrant industrial water[4].

References

[1] Jyoti, J., Alka, D., & Kumar, S. J. (2013). Application of membrane-bio-reactor in waste-water treatment: a review. *International Journal of Chemistry and Chemical Engineering*, *3*(2), 115-122.

[2] Fazal, S., Zhang, B., Zhong, Z., Gao, L., & Chen, X. (2015). Industrial wastewater treatment by using MBR (membrane bioreactor) review study. *Journal of Environmental Protection*, *6*(06), 584.

[3] Kulkarni, A. A., Deshpande, M., & Pandit, A. B. (2000). Techniques of Wastewater Treatment. 1. Introduction to Effluent Treatment and Industrial Methods. *India. Resonance*, *5*, 11-56.

[4] Attiogbe, F. (2013). Comparison of membrane bioreactor technology and conventional activated sludge system for treating bleached kraft mill effluent. *African Journal of Environmental Science and Technology*, *7*(5), 292-306.

[5] Scott, S. (2005). Application of Membrane Bioreactor Technology to Wastewater Treatment and Reuse.

[6] Visvanathan, C., & Aim, R. B. (2002). Membrane Bioreactor Applications in Wastewater Treatment.

[7] Sutton, P. M. (2006). Membrane bioreactors for industrial wastewater treatment: Applicability and selection of optimal systems configuration. *Proceedings of the Water Environment Federation*, *2006*(9), 3233-3248.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

QUANTUM DOTS- APPLICATIONS AND RECENT ADVANCEMENTS

Vasudha Priyadharshini[1], N. Sivarajasekar[1]

Department Of Biotechnology, Kumaraguru College of Technology

Coimbatore-641049, INDIA

vasukisubramaniyam098@gamil.com

Abstract: Quantum dots are Nano sized materials of size 1-3 nm which exhibit fluorescence when excited at certain wavelengths. QD's are classified based upon metal or material which is used as a precursor for the synthesis. Cadmium, Silicon, Carbon, & graphene quantum dots are the most prevalent nanodots that are by far reported in the literature. The versatility of these Nano dots such as fluorescence, highly tunable photoluminescence (PL) property, small size, biocompatibility, highly, exceptional multi-photon excitation (up-conversion) property, electromagnetic chemiluminesence , surface passivation with organic molecules for biocompatibility, and chemical inertness paved a pathway to research community by employing them in myriad of fields such as semiconductor materials, biosensors, cell imaging studies, bio labelling and in detection of ions. This review paper will discuss on the newer approaches used for the synthesis of these quantum dots and their applications in the aforementioned areas.

Keywords: Quantum dots, Carbon dots, Graphene dots, CdSe

I. Introduction

Quantum dots are a new class of semiconductor nanomaterials which are gaining importance in the current era due to their unique properties such as tunable photoluminescence, fluorescence, biocompatibility and unique physicochemical properties owing to their small size and zero dimension range of such materials.[1] Quantum dots range from 1-10 nm and have smaller radius due to the quantum confinement. Quantum dots are applied in various branches of science like biosensors, optoelectronic devices, bio labelling and cell imaging studies, photovoltaic solar cells due to the unique properties. Quantum dots are synthesized based on two approaches i.e , bottom up and top down approaches. Quantum dots possess semiconductor properties and are usually made up of heavy metals such as Cadmium, Silicon. These heavy metal doped carbon dots have tunable photoluminescence and fluorescence properties which provides to be alternative to organic dyes that are used for bio labelling and provide higher photoluminescence and prevents photo bleaching.[2] The main advantage of the quantum dots are they are easy to synthesize and possess low cytotoxicity, biocompatible towards cells during detection and drug delivery. Due to their nano size and tunable luminescence properties they are used as smart nano drug carriers for targeted drug delivery. This paper proposes to highlight on myriad of fields that are used and their mode of synthesis and their applications in diverse fields.

II. Synthesis- Approaches

Many chemical and physical methods were designed and developed by research groups worldwide since 2006 for the production of carbon dots and graphene quantum dots. Primarily carbon and graphene dots that were synthesized were suspended in solvents and were used in fluorescence measurements. Many popular methods were developed amongst which hydrothermal carbonization, laser ablation, pyrolysis, electrochemical oxidation, sonication were predominantly used. Carbon dots and Graphene Dots exhibit multicolour fluorescence emissions at different excitation conditions. But their quantum yields were low to very low. Hence surface functionalization or surface passivation of the quantum dots was necessary to obtain high quantum yields. Polymeric materials such as PEG (Poly ethylene Glycol). Polyethylenimine (PEI) were used for the surface passivation to obtain fluorescence. Presence of amino groups in these polymers led to multiphoton excitation. Also many articles reported on heavy metal surface passivated nano dots that were cytotoxic to in vitro and in vivo biological assays. Hence development of green synthesis or plant based carbon or graphene derivatives was the need of the hour for biological studies. Research was thus focused on synthesizing green alternatives by

switching on to the use of waste biomass such as vegetable and fruit waste, plants for the synthesis of carbon and graphene dots were focused due to their sustainable and low cost production of CD's and GD's that provided high quantum yield with low cost substrates. Table. Depicts the use of green precursors for the synthesis of various CD's and GD's and their applications in diverse fields.

Applications	*Example*	*References*
LED light	Neem(Azadirachta indica) & Fenugreek(Trigonella foenum-graecum) derived GQD's	[3]
Tumor & Bacterial cell detection	Mannose & Folic acid , Papaya powder derived Pomegranate	[4][5][6]
Bio imaging	Orange juice, Sugar cane baggase, PEG, Silk , Lantana camara berries, gelatin Rose heart radish	[7][8][9][10]
Targeted drug delivery	Trapa bispinosa peel	[11]
Ion detection	Bovine Serum Albumin, Prawn shells, Lantana camara berries, apple juice, rose heart radish	[12][10][13][14]

III. Drug delivery studies

In a study conducted by in the year for studying the effects of Nano carriers conjugated with Graphene Quantum dots, it was found by the research group that GQDs capped MMSN nanoparticles act as a multifunctional platform for synergistic therapy with controlled drug release, magnetic hyperthermia, and photo thermal therapy. Doxorubicin was used as the model drug in this study with Magnetic Mesoporous Silica Nanoparticles encapsulated with GQD to prevent the leakage of the model drug DOX. pH mediated controlled drug release was observed in the breast cancer 4T1 cell lines. Also the synergy between the MMSN/GQD capped DOX showed higher efficiency in the killing of cancer cells. [15] Huang and his colleagues conducted a study on paramagnetic graphene quantum dots as a dual modality. Bio imaging and tumour targeted drug delivery in in vivo and in vitro models. In In vitro study the HeLA & HepG2 cell lines were used for viability assay and Zebra fish model was in vivo evaluation of toxicity to the embryonic development of zebrafish. The GQD's used in this study were used as contrast agent in th MRI imaging for the non-invasive cancer detection. It was also established and has been proven to be successful in the targeted delivery of DOX via these Nano carriers. [16]. The use of green fluorescent labelled graphene quantum dots were used in the prognosis of pancreatic cancer. **Gemcitabine,** the drug used for the pancreatic cancer treatment was encapsulated in the albumin nanoparticles was incorporated in the green fluorescent GQD's. This Nano carrier showed excellent bioavailability and excellent bio imaging properties by enhancing the effects of the drug. [17]

IV. Quantum dots in Bio imaging studies

QD's are preferred in bio imaging studies over organic dyes due to their unique characteristics. One of the major practical applications of QD's in bio imaging is in the FRET studies. (Fluroscene resonace energy transfer.) FRET uses organic dyes for tagging the protiens in C-terminal and their N-terminal. When two organic dyes are used in as the donor and acceptor of photons in FRET technique there occurs an overlapping of the fluorophores and the detection of the dynamics in molecular level becomes difficult. Hence a new technique was then designed by exploiting the concept of quantum dots using two inorganic nanocrystals to understand the dynamics of molecular mechanism of proteins instead of organic dyes.

V. Quantum dots in food science

Food contamination by bacterial species is a major concern in food & packaging industries. Conventional dyes such as Rhodamine, Fuoresceinisothiocyanate (FITC) and tetramethylrhodamineisothiocyanate (TRITC) are used in the detection of pathogens or contaminants. The major disadvantage observed with the use of these organic dyes is their ability to photo bleach quickly under the introduction of the fluorescence light source. Another major disadvantage on the use of organic dyes is that they require specific wavelengths to excite the dye for detection. [18]Hence investigations have been carried out with QD's as they are stable than the organic dyes for the labelling and detection of food borne pathogens such as as E.coli , listeria, salmonella. [19] The use of CdSe quantum dots were used in the detection of food borne pathogens with immunoassays as fluorescence labels for immediate detection. On the other hand for a research group experimented on QDs are used as fluorophore probes for the first time developed a detailed protocol to understand the gluten molecular network in flatbread and zein matrix in corn extrudates. They also obtained a 3 dimensional image of the arrangement of gluten in the flatbread using Zen 2009 imaging software (Carl Zeiss Micro Imaging GmbH) that could be used

to tag and track molecules of interest in food microstructures by using carboxyl activated QDs conjugated to gluten and zein.[20] [21]

VI. Opportunities and Challenges in Quantum dots

Even though Quantum dots such as CdSe, CdTe, GQD, CQD'S with various surface passivation effects are prepared are used due to their versatility and due to their excellent non photo bleaching capacity, multiphoton luminesce, biocompatibility and low cost production, the QD's suffer from some trivial drawbacks. The cons of Quantum dots are listed below. [22]

- Quantum dots have lower quantum yields i.e the ratio between the absorbed and the emitted energy is low when synthesized from green precursors.
- Controlling the shape and size of the QD's during the synthesis is difficult
- Certain QD's that are used in biological applications are suspended in solvents which may render cytotoxic the cells
- Sometimes the QD's are unable to penetrate into the cells due to their large physical size compared to the cell size.
- CdSe based QD's need to be surface passivated with polymers in order to prevent leaching of CdSe in the bio imaging or drug delivery studies.

VII. Conclusion

Quantum dots for biological research will become a indispensable tool in the future. Quantum dots can be used increasingly use to elucidate the molecular mechanisms of protein binding, signalling, and regulation. They will be used as smart nanocarriers and drug delivery mechanisms. In the near future , applications of quantum dots as cell reporters and replacement of organic dyes in biochemical assays will increase. Incorporation of QD's in studying the protein structures in food molecules will gain momentum and will lead to the understanding of rheology of the food particles at microlevel. Green synthesis of Nanodots are becoming a versatile technology where in the heavy metal based nanodots will be replaced with plant based nanodots which will be comparatively less cytotoxic to the cells and will prove to be an excellent probe in drug delivery and signalling studies.

REFERENCES

[1] A. P. Alivisatos, "Published by : American Association for the Advancement of Science Semiconductor Clusters , Nanocrystals , and Quantum Dots," *Am. Assoc. Adv. Sci.*, vol. 271, pp. 933–937.

[2] F. Yang, S. Sonkar, and L. Yang, "Carbon-based quantum dots for fluorescence imaging of cells and tissues," no. February 2014, 2016.

[3] C. Phadke, A. Mewada, R. Dharmatti, and M. Thakur, "Biogenic Synthesis of Fluorescent Carbon Dots at Ambient Temperature Using Azadirachta indica (Neem) gum," 2015.

[4] X. Su *et al.*, "A graphene quantum dot@Fe3O4@SiO2 based nanoprobe for drug delivery sensing and dual-modal fluorescence and MRI imaging in cancer cells," *Biosens. Bioelectron.*, vol. 92, pp. 489–495, 2017.

[5] N. Wang, Y. Wang, T. Guo, T. Yang, M. Chen, and J. Wang, "Biosensors and Bioelectronics Green preparation of carbon dots with papaya as carbon source for effective fl uorescent sensing of Iron (III) and Escherichia coli," *Biosens. Bioelectron.*, vol. 85, pp. 68–75, 2016.

[6] M. Zhang *et al.*, "Bright quantum dots emitting at ~ 1 , 600 nm in the NIR-IIb window for deep tissue fluorescence imaging," no. June, 2018.

[7] B. Behera and S. Mohapatra, "ChemComm Simple one-step synthesis of highly luminescent carbon dots from orange juice : application as excellent bio-imaging agents w," no. July 2015, pp. 1–4, 2012.

[8] Z. L. Wu *et al.*, "One-pot hydrothermal synthesis of highly luminescent nitrogen-doped amphoteric carbon dots for bioimaging from Bombyx mori silk-natural proteins," *J. Mater. Chem. B*, vol. 1, no. 22, pp. 2868–2873, 2013.

[9] Q. Liang, W. Ma, Y. Shi, Z. Li, and X. Yang, "Easy synthesis of highly fluorescent carbon quantum dots from gelatin and their luminescent properties and applications," *Carbon N. Y.*, vol. 60, pp. 421–428, 2013.

[10] R. Bandi, R. Dadigala, B. R. Gangapuram, and V. Guttena, "Green synthesis of highly fluorescent nitrogen – Doped carbon dots from Lantana camara berries for effective detection of lead(II) and bioimaging," *J. Photochem. Photobiol. B Biol.*, vol. 178, no. November 2017, pp. 330–338, 2018.

[11] A. Mewada, S. M. Shinde, and N. Mishra, "Green synthesis of biocompatible carbon dots using aqueous extract of Trapa bispinosa peel," no. October 2017, 2013.

[12] S. S. Wee, Y. H. Ng, and S. M. Ng, "Synthesis of fluorescent carbon dots via simple acid hydrolysis of bovine serum albumin and its potential as sensitive sensing probe for lead (II) ions," *Talanta*, vol. 116, pp. 71–76, 2013.

[13] G. Gedda, C. Y. Lee, Y. C. Lin, and H. F. Wu, "Green synthesis of carbon dots from prawn shells for highly selective and sensitive detection of copper ions," *Sensors Actuators, B Chem.*, vol. 224, pp. 396–403, 2016.

[14] Y. Xu *et al.*, "Green synthesis of fluorescent carbon quantum dots for detection of Hg2+," *Chinese J. Anal. Chem.*, vol. 42, no. 9,

pp. 1252–1258, 2014.
[15] X. Yao *et al.*, "Graphene Quantum Dots-Capped Magnetic Mesoporous Silica Nanoparticles as a Multifunctional Platform for Controlled Drug Delivery, Magnetic Hyperthermia, and Photothermal Therapy," *Small*, vol. 13, no. 2, 2017.
[16] C. L. Huang *et al.*, "Application of paramagnetic graphene quantum dots as a platform for simultaneous dual-modality bioimaging and tumor-targeted drug delivery," *J. Mater. Chem. B*, vol. 3, no. 4, pp. 651–664, 2015.
[17] A. Manuscript, "Materials Chemistry B," 2017.
[18] Y. T. Mohamed and A. H. Ibrahim, "Extraction of Copper from Waste Solution Using Liquid Emulsion Membrane," vol. 2012, no. January, pp. 129–134, 2012.
[19] E. Mohamadi, M. Moghaddasi, A. Farahbakhsh, and A. Kazemi, "A quantum-dot-based fluoroassay for detection of food-borne pathogens.," *J. Photochem. Photobiol. B Biol.*, 2017.
[20] N. Sozer and J. L. Kokini, "Use of quantum nanodot crystals as imaging probes for cereal proteins," *FRIN*, vol. 57, pp. 142–151, 2014.
[21] J. C. Bonilla, F. Bozkurt, S. Ansari, N. Sozer, and J. L. Kokini, "Applications of Quantum Dots in Food Science and Biology," *Trends Food Sci. Technol.*, 2016.
[22] J. K. Jaiswal and S. M. Simon, "Potentials and pitfalls of fluorescent quantum dots for biological imaging," vol. 14, no. 9, 2004.

VII. Acknowledgments

We would like to thank Kumaraguru College of Technology for giving this opportunity under the guidance of Dr. N. Sivarajasekar of Department of Biotechnology .

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

A Concise review on removal of commercial textile dyes from water

Dhiranesh. N

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu 641049, India

tpradeepika15@gmail.com

Abstract: The reduction of pollution has become more important now a days. These cause mutagenic effects, skin problems, allergic diseases and other human problems in birth. The norms of the government has become very strict in textile industries. The degradation of the dyes in the wastewater is more important to avoid pollution the various methods which used in dye degradation are adsorption using various adsorbent, chemical decomposition, photodegradation and microbial decoloration. Here different types of dyes and the efficient degradation methods for each dyes are going to take place. The main aim is to provide a cost efficient method for degradation.

Keyword: *dye; Pollution; remediation; removal*

I. Introduction

Dyes are colouring substances is an aqueous solution used for dying fibre. Dyes are major pollutant in the textile dying industries. There many diseases caused by the dyes which are not treated and released after treating. Dyes are not only released by textile industries but also by dying dyestuff manufacturing. These dyes can be degraded by various method like photodegradation , chemical degradation , biological degradation. Some of the methods and effluents used for the degradation will be harmful for the mammals [1]. The colour present in the dye also make the water to be unpleasant. Many dyes are taken here and the efficient method of degrading the dyes will be seen in this paper[2].

II. Commercial dye sequestration by various methods

ALIZARIN

Alizarin is an organic compound of structure $C_{14}H_8O_4$. This the dye first dye to be discovered from root of the plant. It is used as Red dye in textile industries. This first dye to be synthetically produced. It is used as staining agent in biological researches. Here the dye is removed by photo-degradation technique[3]. The TiO_2 is added to 50 ml of dye and a dispersion is prepared. Nd:YAG laser is used as a radiation source at 532 nm. The capillary tube used in this method where the same is used through out to reduce the error of measurement. Using centrifuge the TiO2 is removed. At 50ml the process was efficient. The major adsorption of alizarin was found at 420 nm[4].

CRYSTAL VIOLET

Crystal violet is a triarymethane dye used in histological stain and bacterial stain. The method of developing this dye was produced by a German chemist named kern and caro. This crystal violet dye is only used in textile industries. This is degraded using bio-degradation using culture. The P.chrysosporium is used as the culture bacteria. 50 µl of crystal violet is taken and the culture is prepared. The cis allowed to grow for 6 days after that the crystal violet gets degraded by the bacteria added[5].

JANUS GREEN B

Janus green B is a dye used in histological staining and a stain for the mitochondria. This was introduced by Leonor michaelis. This colour is based on the oxygen present. The dye is degraded using intestinal anaerobes. The dye is taken and 2ml of cell suspension, 2ml of 0.4M of phosphate buffer (pH-7.4). The whole setup is carried out in 37 degree for one hour[6].

METANIL YELLOW

Metanil Yellow is a azo class dye. This also used as pH indicator. This also used as adulterant for turmeric powder. The dye is removed using absorbent. Bottom ash and de-oiled soya is as the absorbent in the process. The study is performed in different temperature like 30,40 and 50 degree. A volumetric flask is taken 50ml of dye and 50ml of the absorbent is taken in different concentrations. The flask is given continuous mechanical agitation. Finally the absorbent is kept in 495 nm to find the degradation of the dye[7].

MALACHITE GREEN

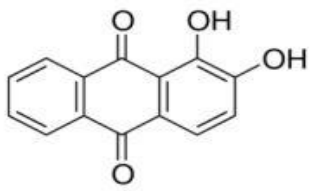
Malachite Green is an organic compound used as dyestuff. This used as dying for the silk leather and paper. This is triarylmethane dye. This is a chloride salt. Kocuria rosea is the bacteria used in the biodegradation of the dye. The microbial culture is inoculated in the 100ml of nutrient broth and kept for 24 hour incubation. Then the inoculation is added to the dye and kept for some time. After that the sample is centrifuged at 15000 rpm to remove the cell mass. Thus the dye is degraded using the bio-degradation method[8].

PURPURIN

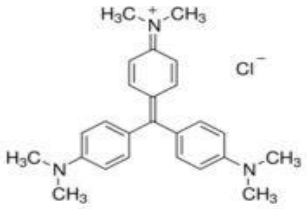
Purpurin is an anthraquinone dye. This dye is naturally red or yellow colour. This is the dye used in the textile industries. The dye is degraded using ageing process. Here the xenon lamp is used at a light range of 200-900 nm and 765 W/m2. It is kept for 720 min both in air and nitrogen atmosphere. This method is carried out in an oxygen free environment[9].

SUDAN II

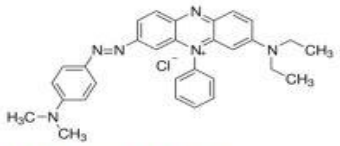
Sudan II is also azo dye. This is used to find lipid protein bound. This is red powder. This has melting point 156-158 degree. This is degraded using bio-degradation using culture. Here the bacteria is taken from human gastrointestinal track. The study is performed in different temperature like 30,40 and 50 degree. A volumetric flask is taken 50ml of dye and 50ml of the absorbent is taken in different concentrations[10].



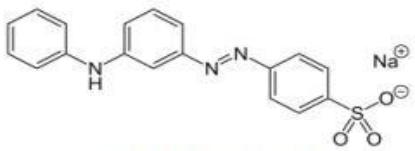
ALIZARIN



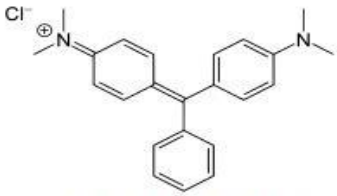
CRYSTAL VIOLET



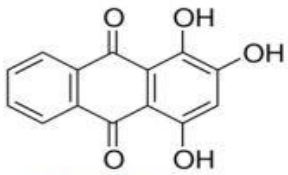
JANUS GREEN B



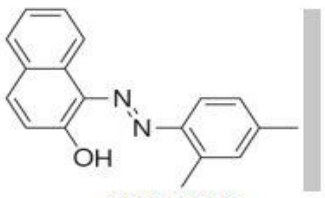
METANIL YELLOW



MALCHITE GREEN



PURPURIN



SUDAN II

III. Conclusion

Dyes are more hazardous to the lives surviving in the world. It causes many types of mutagenic diseases and also it is fatal to the organism in the world. So the dyes must treated to the extant where they don't cause any problem to the lives present in the surrounding. There by we have discussed about various dyes and the degradation methods .We have used photo-degradation, bio-degradation, chemical degradation. The effective methods each dyes are done in the above paper. The best degradation techniques are listed in the above[11].

References

[1] S. Muthusaravanan *et al.*, "Phytoremediation of heavy metals: mechanisms, methods and enhancements," *Environmental Chemistry Letters*, 2018.

[2] A. Rehorek, M. Tauber, and G. Gübitz, "Application of power ultrasound for azo dye degradation," *Ultrason. Sonochem.*, vol. 11, no. 3–4, pp. 177–182, 2004.

[3] N. Sivarajasekar, N. Mohanraj, S. Sivamani, I. Ganesh Moorthy, R. Kothandan, and S. Muthusaravanan, "Comparative modeling of fluoride biosorption onto waste Gossypium hirsutum seed microwave-bichar using response surface methodology and artificial neural networks," in *2017 International Conference on Intelligent Computing, Instrumentation and Control Technologies, ICICICT 2017*, 2018.

[4] G. Liu, T. Wu, J. Zhao, H. Hidaka, and N. Serpone, "Photoassisted degradation of dye pollutants. 8. Irreversible degradation of alizarin red under visible light radiation in air-equilibrated aqueous TiO2 dispersions," *Environ. Sci. Technol.*, vol. 33, no. 12, pp. 2081–2087, 1999.

[5] J. A. Bumpus and B. J. Brock, "Biodegradation of crystal violet by the white rot fungus Phanerochaete chrysosporium.," *Appl. Environ. Microbiol.*, vol. 54, no. 5, pp. 1143–1150, 1988.

[6] K.-T. Chung, G. E. Fulk, and M. Egan, "Reduction of azo dyes by intestinal anaerobes.," *Appl. Environ. Microbiol.*, vol. 35, no. 3, pp. 558–562, 1978.

[7] A. Mittal, V. K. Gupta, A. Malviya, and J. Mittal, "Process development for the batch and bulk removal and recovery of a hazardous, water-soluble azo dye (Metanil Yellow) by adsorption over waste materials (Bottom Ash and De-Oiled Soya)," *J. Hazard. Mater.*, vol. 151, no. 2–3, pp. 821–832, 2008.

[8] G. Parshetti, S. Kalme, G. Saratale, and S. Govindwar, "Biodegradation of Malachite Green by Kocuria rosea MTCC 1532.," *Acta Chim. Slov.*, vol. 53, no. 4, 2006.

[9] S. S. Phugare, D. C. Kalyani, A. V Patil, and J. P. Jadhav, "Textile dye degradation by bacterial consortium and subsequent toxicological analysis of dye and dye metabolites using cytotoxicity, genotoxicity and oxidative stress studies," *J. Hazard. Mater.*, vol. 186, no. 1, pp. 713–723, 2011.

[10] V. J. Sharavanan, A. Aapakudal, M. Sivaramakrishnan, and R. Kothandan, "Resolving Class Imbalance in Meteorological Datasets for Predicting Dengue Outbreak," vol. 10, no. 5, pp. 590–594, 2017.

[11] N. Sivarajasekar, T. Paramasivan, S. Muthusaravanan, P. Muthukumaran, and S. Sivamani, "Defluoridation of water using adsorbents - A concise review," *J. Environ. Biotechnol. Res.*, vol. 6, no. 1, pp. 186–198, 2017.

Acknowledgments

Author is sincerely thankful to Dr. N. Sivarajasekar, Assistant Professor-II, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore- 641 049, for their contribution and guidelines regarding our work.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

The b – chromatic coloring for Silicate and Benzenoid Networks

K. P. Thilagavathy*[1], K.R.Aranganayagam[2] and A. Santha[3]

[1,2] Assistant Professor, Kumaraguru College of Technology, Coimbatore.

[3]Associate Professor, Kumaraguru College of Technology, Coimbatore.

*E.mail: kp_thilagavathy_22@yahoo.com

Abstract: In this research investigation the b-chromatic coloring for silicate and various benzenoid network are discussed. Different benzenoid derivates have been studied, namely benzene (G_1) and its derivative networks (G_2), naphthalene derived network (G_3), polyhex (G_4) and alkyl substituted benzene derivatives (G_5).

Keywords: *b-chromatic, silicate network, benzenoid network*

I. Introduction

Graph theory is a branch of mathematics which deals with the inter relationships between objects. The impact of graph theory in science and technology is evident from its active use in various fields such as genomics in biochemistry, communication networks and coding theory in electrical engineering, algorithms and computation in computer science and scheduling in operations research etc. (Shariefuddin and Ashay, 2007). In recent years, mathematicians and chemists are interested in exploring the applications of graph theory towards molecular determination systems in chemistry. Chemical graph theory and colouring is one the branches under this broad area that shows wide scope to provide molecular information, especially on the molecular arrangements, chemical reactions, kinetic parameters and specific reactions. Several books and literature are available to support these kinds of applications (Balaban, 1976, Graovac et al., 1977, Trinajstic, 1992, Balasubramanian, 1985).Chemists have been using the structural formulae to represent the topological structure of chemical substances and also various types of flow diagrams to discuss the reaction network.

The concept of b-chromatic number was introduced by Irwing & Manlove in 1999. The b-chromatic number $\varphi(G)$ of a graph G is the largest integer k such that G admits a proper k - colouring and every colour class has a representative vertex that is adjacent at least to one vertex in each other class. This type of colouring is called b-colouring. In order to connect graph theory to chemical systems, b-chromatic colouring for silicate and various benzenoid networks is discussed in this paper.

II. The B-Chromatic Number of Silicate Networks

The largest, most complicated and fascinating mineral in the earth is SiO_4.Silicatesare obtained by fusing metal oxides or metal carbonates with sand. In the language of chemistry, the corner vertices of SiO_4tetrahedron represent oxygen ions and the centre vertex represents silicon ions. A Silicate network can be constructed in different ways. The efficient approximation algorithm to determine the achromatic number of silicate network was reported by Sharmila Mary Arul (2013). We now discuss the b-chromatic coloring of Silicate network.

A Cycle graph of length n is the graph C_n on n vertices $\{v_0, v_1, ...v_{n-1}\}$ with n - edges $(v_0, v_1), (v_1, v_2), ..., (v_{n-1}, v_0)$. A block of the graph G is a maximal connected sub graph of G that has no cut vertex. If G itself is connected and has no cut vertex, then G is a block. The block cut point graph of G is a path. A K_4-chain is a connected graph in which all blocks are complete graphs on 4 vertices and the block cut point is a path P .The results are shown below,

$$\text{The b-chromatic number of } tK_4 = \begin{cases} 4, & t \le 5 \\ 5, & t = 6 \\ 6, & t = 7 \\ 7, & t \ge 8 \end{cases}$$

Proof:

Case (i): When *t=1*

The graph is a complete graph K_4, it represents a complete graph with 4 vertices. Its b-chromtic number is 4.

Case (ii): When *t=2,3,4,5*

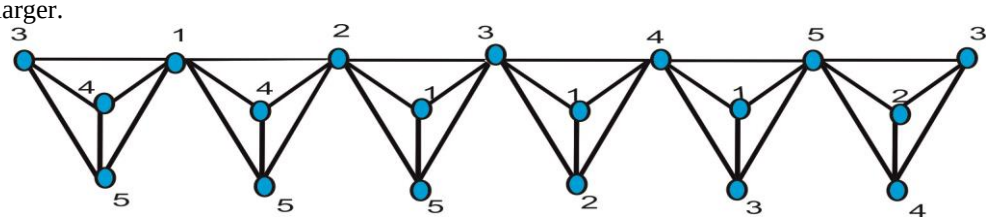
The maximum degree in the graph is 6when t=2, the number of vertices having this maximum degree is 1. Similarly for t=3,4,5 no other vertices have degree greater than or equal to 4. Hence the b-chromatic number is 4.

Case (iii): When *t=6,7*

When t=6,7 the number of vertices having the maximum degree are 5 and 6 respectively. Hence we can accommodate 5, 6 colours for a b-chromatic colouring.

Case (iv): when $t \geq 8$

The maximum degree in the vertices is six. Hence we can accommodate only seven colours even when t becomes larger.



$\varphi[6K_4] = 5$

Fig-1

III. The B-Chromatic Number of Benzenoid Networks

Multiprocessor interconnection networks are often required to connect thousands of homogeneously replicated processor memory pairs, each of which is called a processing node. Honey comb networks are widely used in computer graphics, cellular phone base stations, image processing and in chemistry as the representation of benzenoid hydrocarbons. Honey comb networks are better in terms of degree, diameter, total number of links, cost and bisection width than mesh connected planar graphs.

The cyclic structure in organic compounds is made of carbon atoms in a hexagonal pattern. Hexagonal structures do appear a lot in nature. A regular hexagon has 6 lines of symmetry and all its internal angles are of measure 120 degrees. The degree of any such network is three. Honey comb meshes can be built from hexagons in various ways. The simplest way to define them is to consider the portion of the hexagonal tessellation which is inside a given convex polygon. The honey comb $HC(1)$ is a hexagon. The honey comb network $HC(2)$ is obtained by adding six hexagons to the boundary edges of $HC(1)$. Inductively $HC(n)$ is obtained from $HC(n-1)$ by adding a layer of hexagons around the boundary of $HC(n-1)$. In this section, different benzenoid networks, namely benzene (G_1) and its derivative networks (G_2), naphthalene derived network (G_3), polyhex (G_4) and alkyl substituted benzene derivatives (G_5)are discussed.

The b-chromatic number of simple Benzene (C_6) network is $\varphi[HC(1)] = 3$.

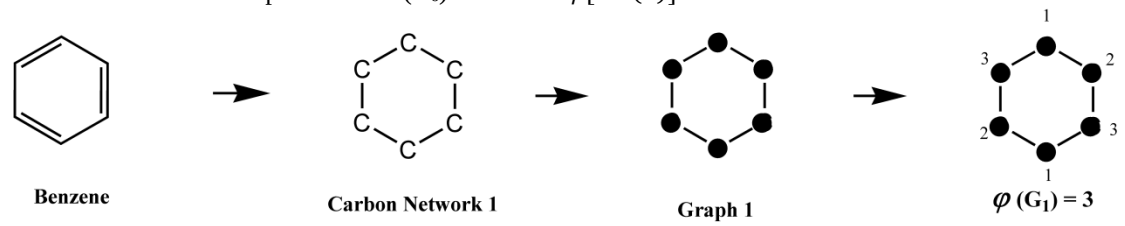


Fig-2

A *p*-Xylene (*substituted benzene derivative*, C_8) is a cycle of length 6 with 2-pendent edges at diametrically opposite vertices of the cycle. The b-chromatic number of *p*-Xylene is 3.

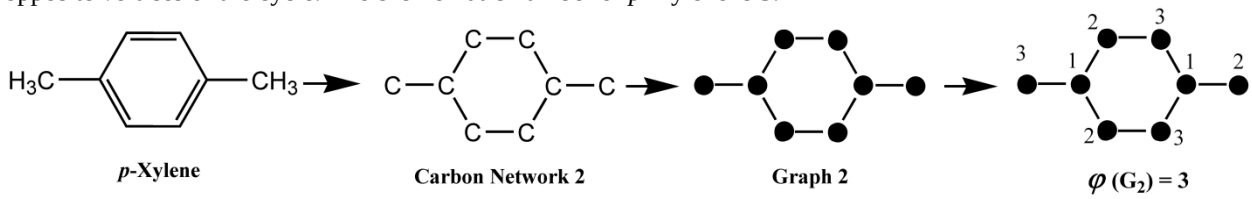


Fig-3

'A t-layer of1,4,5,8-Tetramethyl-naphthalene (*Naphthalene derivative*, C_{14}) is defined as the union of honey rolls H_i, $i = 1,2,3...,n$ where H_i is isomorphic to the above graph 2. For any honey roll graph H_i, the b-chromatic number is 4, $i = 1,2,3...,n$ (Fig.4)

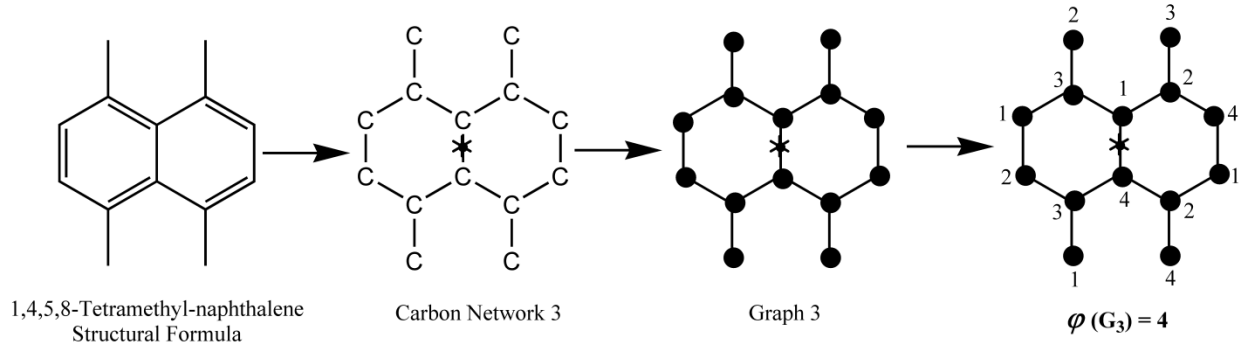


Fig-4

The b-chromatic number of coronene derivative, C_{24} (Honey Comb) network is $\varphi[HC(n)] = 4, n \geq 2$ (Fig. 5)

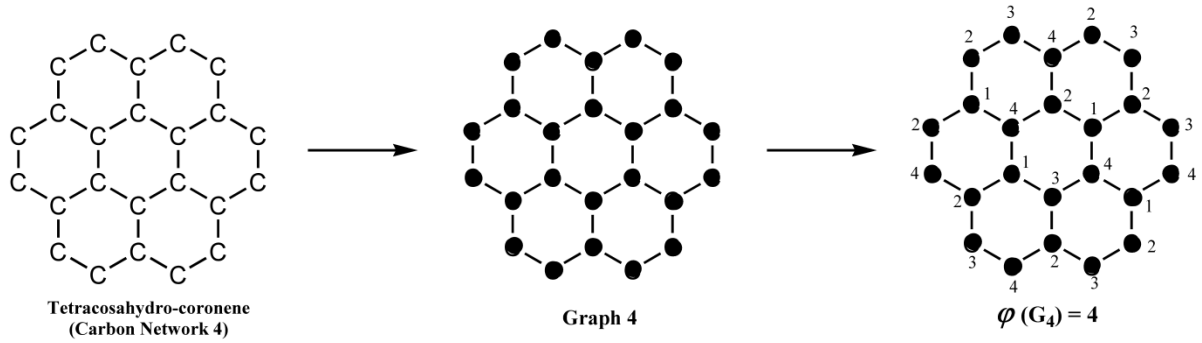
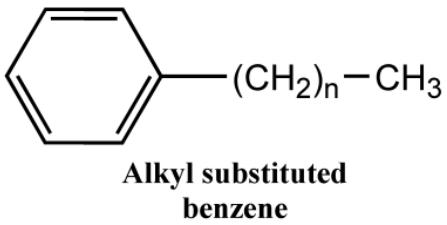


Fig-5

The b-chromatic number for alkyl substituted benzene derivatives are studied and it is observed that all the derivatives shows the similar and unique coloring index of $\varphi(G_5), n = 3$.



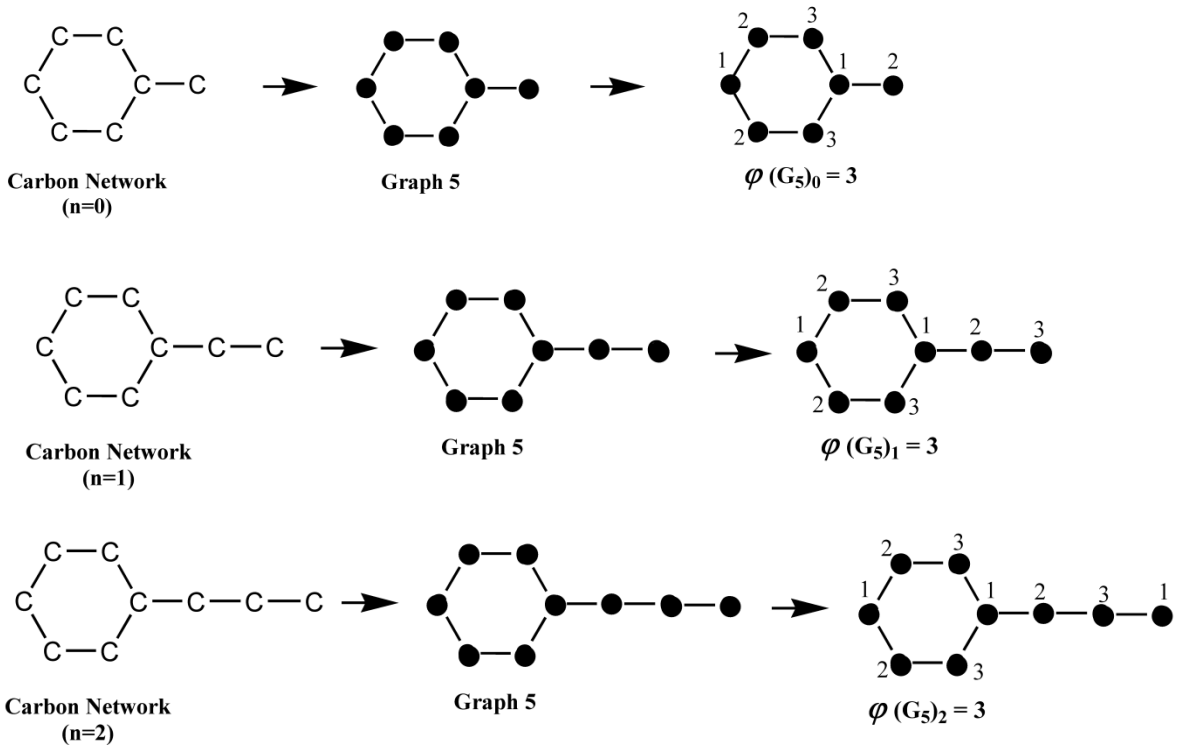


Fig-6

These can be explained as follows:
For $n = 0$ (methyl benzene):$\varphi(G_5)_0 = 3$, For $n = 1$ (ethyl benzene): $\varphi(G_5)_1 = 3$
For $n = 2$ (propylbenzene): $\varphi(G_5)_2 = 3$,
For any number n(Alkyl substituted benzene) of $\varphi(G_5)_n = 3$. This indicates that the compounds having a different chemical environment but they all have b-chromatic number as 3.

IV. Conclusion

An attempt has been made to correlate graph colouring and chemical compounds, by finding the b-chromatic numbers of silicate and Benzenoid networks. We hope that this may lead to finding a connection between the chemical properties of these networks and their b-chromatic numbers.

References

[1] T. Balaban(ed.),Chemical applications of graph theory, Academic Press, London1976.

[2] K.Balasubramanian, "Recent Chemical Applications of Computational Combinatorics and Graph Theory", in D.H. Rouvray (ed.), Computational Chemical Graph Theory, Nova Sci. Publ., Commack, New York, (1990) pp. 67-104.

[3] A.Graovac, I.Gutman, and N. Trinajstic, "Topological Approach to the Chemistry of Conjugated Molecules", Springer-Verlag, Berlin, 1977.

[4] Sharmila Mary Arul "On the achromatic Number of Silicate Networks"International Journal of Pure and Applied Mathematics,Volume 87 No. 6 (2013), 771-780.

[5] Shariefuddin Pirzada and Ashay Dharwadker "*Applications of Graph Theory*", Journal of the Korean society for Industrial and Applied Mathematics vol. 11 No. 4(2007).

[6] K.P.Thilagavathy, A.Santha"*A Note on Achromatic and b-chromatic number of graphs"*International Journal of Applied Mathematics and Statistics,53(1)(2016),104-110.

[7] K.P.Thilagavathy, A.Santha "*The achromatic and b-chromaticcolouring of central graph of Book graph and shadow graph of path graph"*International Journal of pure and Applied Mathematics vol. 113(2017) No.8,1-8.

[8] N.Trinajstic," Chemical Graph Theory", 2nd Ed., CRC Press, Boca Raton, FL, 1992.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Optimization of Endoglucanase production using mixed substrate by *Trichoderma viride*

S.Kavitha[1]*, Janani.B[2], J.Praveen Kumar[1], K.Balasubramani[1], G.Surendran[1]
[1]JCT College of Engineering and Technology, Pichanur, Coimbatore, Tamil Nadu,India.
[2] Karpagam Academy of Higher Education, Coimbatore
*e-mail: kavitha212418@gmail.com

Abstract: The production of Endoglucanase by Trichoderma viride using mixed substrate (Wheat Bran, Rice Bran).In this work, the application of Response Surface Methodology (RSM) is presented for optimization of production of endoglucanase using agrowaste.The goal was to improve production conditions in batch process, i.e., to minimize the substrate concentration and to increase the product concentration of endoglucanase. A full factorial Central Composite Design (CCD) was employed. Analysis of variance (ANOVA) showed a high coefficient of determination value (R^2=0.97) and satisfactory prediction second-order regression model was derived. Maximum endoglucanase activity was predicted and experimentally validated. The endoglucanase production was significantly affected by fermentation time, inoculum size, pH and temperature. The optimum values of the parameters X_1, X_2, X_3, X4 were found to be 29 °C, pH 5.47, 71.83 hrs and 2.16 mL respectively.

Keywords : *Trichoderma viride, Kinetics, Endoglucanase, Fermentation, Response Surface Methodology*

I. Introduction

Cellulose is the most abundant polymer in the biosphere .Cellulose is the major part of plant biomass. Therefore the wastes generated from forests, agricultural fields and agro industries contain large amount of utilized or underutilized cellulose [1,2]. Despite a worldwide and enormous utilization of natural cellulosic sources, there are still abundant quantities of cellulose-containing raw materials and waste products that are not exploited or which could be used more efficiently [2,5]. It is possible to transform this polymer into glucose an excellent substrate for industrial fermentation by means of several chemical and bioconversion methods [3,4]. Although a large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell free enzymes capable of completely hydrolyzing crystalline cellulose *invitro.* Fungi are the main cellulase-producing microorganisms, though a few bacteria and actinomycetes have also been reported to yield cellulase activity [6]

The medium composition of each medium component is crucial parameters that regulate enzyme activity. The conventional one facto at a time measure techniques are time consuming, labor incentive and often erroneous. Statistical optimization are very effective for process optimization of commercial industrial enzyme .when many factors and their interactions regulate the desired esponse.RSM is an effective tool for optimizing the process by solving the multivariable equation. To enhance the production of endoglucanase combinatorial interactions of medium components are good strategy [8].

II. Materials and Methods

2.1 Organisms and Culture Maintenance

Trichoderma Viride MTCC 167 strain was obtained from the stock culture of Microbial Type Culture Collection, Chandigarh and maintained on 4% potato- dextrose -agar medium. The cultures maintained by regular transfer onto new slants and were stored at 4˚C.

2.2 Substrate

RB and WB dried at 65°C overnight used in the fermentation medium [12]. Both those substrates collected from the respective industries.

2.3 Production Medium

The production medium contained (g/l) KH_2PO_4 – 1.0, $CaCl_2$-0.1, $MgSO_4.7H_2O$–0.2, $(N\ H_4)_2\ SO_4$ – 0.5, KCL-0.5,L-asperginie,yeast extract and 5g of RB and WB (different proportions) were mixed with the liquid medium in 1:3 ratio in 250 ml Erlenmeyer flasks and autoclaved at 121 ^{O}C for 20 min. The mixed substrate inoculated with 1 ml spores containing 10^7 spores /ml [1].

2.4 Analytical Techniques

Batch fermentation carried out on mixture of substrate hydrolyzed with trace elements. The endoglucanase activity, substrate concentration, pH, temperature and biomass were determined every 24 hours for 6 days. Culture supernatant was determined according to Mandels et al [12].

A Reactive mixture of 0.5 ml of 1% (W/V) carboxymethyl cellulose solution in 0.05 M citrate buffer (pH 4.8) and 0.5 ml of culture supernatant was incubated at 50 ^{O}C after incubation for 30 min the reducing sugars released were assayed by adding 3 ml of DNS reagent. Controls were prepared with 10 min boiled enzyme [12].

One unit of endoglucanase activity expressed as the amount of enzyme required to release 1µ mol reducing sugars per ml under the above assay conditions by using glucose as standard curve [10].

For cellulose evaluation, anthrone method was implemented 1ml of sample, 8ml of 0.1% anthrone solution and 4 ml of distilled water, being this solution incubated in boiling water for 15 min. The spectrophotometer measuring made against crude cellulose to 620 nm [1].

For biomass estimation, 5ml portion of culture broth centrifuged for 20 minutes and the supernatant discharged. The resulting pellets were dried and dry weight was estimated [12].

III. Optimization of the process parameters involved in endoglucanase production

The effects of temperature, pH, fermentation time and inoculum size were studied using central composite design (CCD) for the fungal strain of *Trichoderma viride*. The effects of four major key factors responsible for enzyme production were studied at five different levels (-α, -1, 0, +1, +α) by CCD. The recommended number of tests at the center is seven. Hence, the total number of tests (N) required for the four independent variables is:

$$N = 2^n + 2n + n_c = 2^4 + (2 \times 4) + 6 = 30$$

Once the desired ranges of values of the variables are defined, they are coded to lie at±1 for the factorial points, 0 for the center points and ±α for the axial points [12].

Table .1 The coded and actual values of the variables in CCD for optimizing the parameters in endoglucanase production by *Trichoderma viride*

	Independent variables	**Coded Values**				
		-2	-1	0	1	2
T. viride	Temperature	25	28	30	35	40
	pH	4	4.5	5	5.5	6
	Fermentation time	24	48	72	96	120
	Inoculum size	0.5	1	2	3	4

3.1 Response Surface Methodology (RSM) for endoglucanase production

The second-order model used to fit the response to the independent variable is shown in equation .The data obtained from RSM on endoglucanase production are subjected to analysis of variance (ANOVA) [1].

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \ldots\ldots\ldots\ldots 3.1$$

Where, Y is the predicted response *k* the number of factor variables, X_i and X_j are the input variables that influence the response variable Y, β_0 is the intercept, β_i represents the linear effect of X_i, βij represents the

interaction between X_i ,X_j and βii represents the quadratic effects of X_i. However, in this study, the independent variables are coded as X_1, X_2, X_3 and X_4.

$$x_i = \frac{X_i - X_o}{\Delta x_i}, \; i=1,2,3.....k, \qquad \ldots\ldots\ldots\ldots 3.2$$

Where x_i is the dimensionless value of an independent variable. X_i is the real value of an independent variable, X_o is the real value of the independent variable at the center point, and ΔX_i is the step change value. Thus, the second order polynomial model equations for the four factors take the following form[22].

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 \qquad \ldots\ldots\ldots 3.3$$

IV. Optimization of endoglucanase production using response surface methodology (RSM) for *Trichoderma viride*

RSM was performed to examine the combined effect of four different process parameters (independent variables), on the production of endoglucanase for which 30 experiments were carried out. The regression coefficients were calculated and the data was fitted to a second order polynomial equation. The response, Y (Endoglucanase activity, U/ml) by *Trichoderma viride*, can be expressed in terms of the following regression equation.

$$Y = 98.7500 - 0.292917X_1 - 0.156250X_2 - 0.305417X_3 - 0.189583X_4 - 1.75615X_1^2 - 1.35990X_2^2 - 1.25490X_3^2 - 1.21865X_4^2 - 0.664375X_1X_2 + 0.0293750X_1X_3 + 0.256875X_1X_4 + 0.0756250X_2X_3 + 0.634375X_2X_4 + 0.228125X_3X_4 \qquad \ldots 4.1$$

Where Y is the response value and X_1, X_2, X_3 and X_4 are the coded levels of temperature, pH, fermentation time and inoculum size respectively. The adequacy of the model was checked using analysis of variance and the results are represented in Table 1 and 3**.** The independent variables were fitted to the second order model equation and examined for the goodness of fit. Several indicators were used to evaluate the accuracy of the fitted model and the results are shown in Table .3.

4.1 Statistical analysis

In Statistical testing of the model was performed with the Fisher's statistical test for analysis of variance. ANOVA of the regression model for Y demonstrated that the model was significant due to an *F*-value and a very low probability value ($P < 0.001$). The *P*-values are used as a tool to check the significance of each of the coefficients, which in turn indicate the pattern of the interactions between the variables. Smaller value of *P* indicated that it was more significant to the corresponding coefficient. This case X_1, X_4, X_1X_2, X_1X_4, X_1^2, X_2^2, X_3^2 and X_4^2 were significant model terms. These results indicate that the incubation temperature, pH, inoculum size, fermentation time bores a direct relationship to endoglucanase production. The interaction between incubation temperature and pH were significant

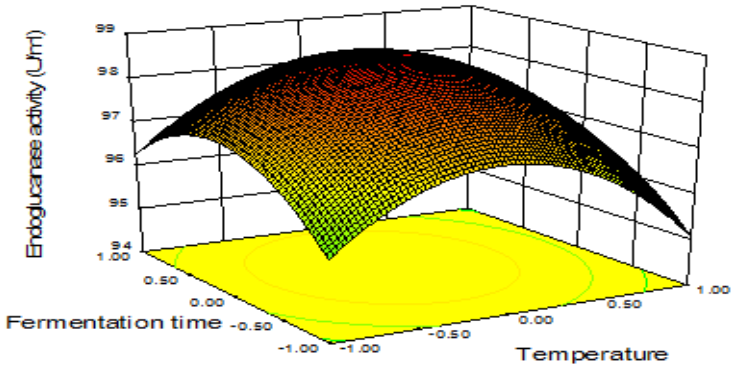


Figure 1 The combined effect of fermentation time and temperature of endoglucanase activity at constant pH and inoculum size.

4.2 Response of endoglucanase activity

In order to determine the optimal levels of each variable for maximum endoglucanase production, three dimensional response surface plots and contour plots were constructed by plotting the response (Endoglucanase activity) on the Z axis against any two independent variables, while maintaining other variables at their optimal levels which is helpful for understanding both the main and the interaction effects of these two factors. The response surfaces can be used to predict the optimum range for different values of the test variables and the major interactions between the test variables can be identified from the circular or elliptical nature of the contours.. The orientation of the principal axes of the contour plots between the variables fermentation time and temperature, pH and temperature, temperature and inoculums size indicated that the mutual interactions between these set of variables had a significant effect on the production of endoglucanase. Under certain conditions, a maximal contour (endoglucanase production 98.75 U/ml) could be determined, meaning that further change in temperature and pH would not increase the endoglucanase yield any further.

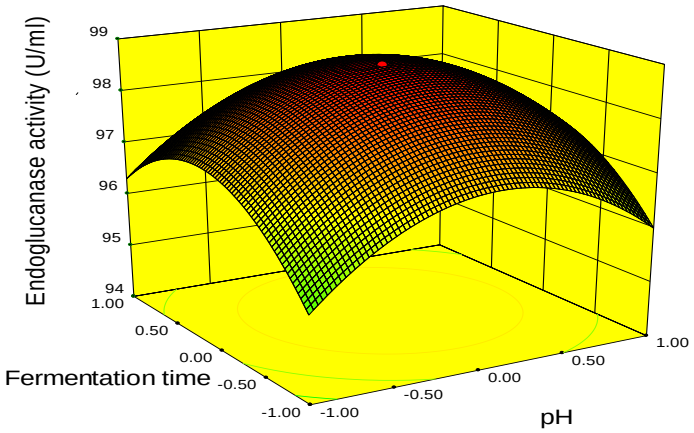


Figure 2 The combined effect of fermentation time and pH of endoglucanase activity at constant temperature and inoculum size.

4.3 Optimization by response surface modeling

One of the main aims of this study was to find the optimum process parameters to maximize the adsorption of chromium, from the mathematical model equations developed in this study. The quadratic model equation was optimized using quadratic programming (QP) to maximize of adsorption of chromium within the experimental range studied. The optimum values of the parameters X_1, X_2, X_3, X4 were found to be 29 °C, pH 5.47, 71.83 hrs and 2.16 ml respectively. Furthermore, this study revealed that owing to the high concentration of endoglucanase activity

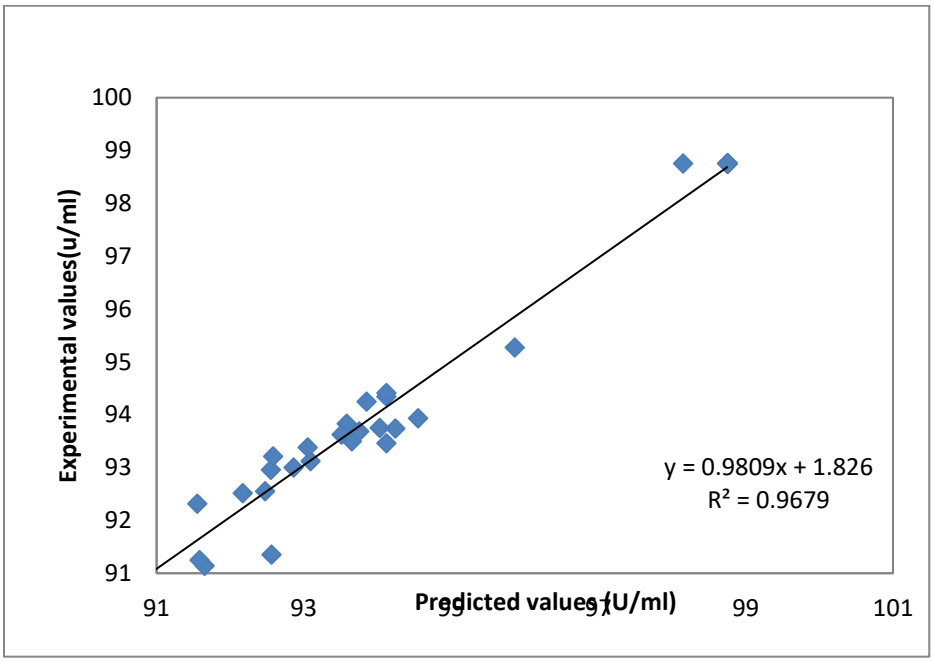


Figure 3 Comparison of predicted and experimental values for the endoglucanase production

V. Conclusions

Based on the design of experiment approach over conventional strategy, it was found that interaction between the process parameters significantly affect the endoglucanase production using agro waste. However, in this study, only selected critical process parameters were studied. Parameters for the model are estimated by statistical regression equations based on endoglucanase activity data for various substrates. A second-order polynomial equation was adequate to predict the permeability as the response. An R2 value of 0.97 ensures a sufficient adjustment of the model with the experimental data. The results showed that as the values of process variables increased, the yield also increased but only up to the midpoint of range of variables and thereafter the yield decreased even though the values of variables increased. The endoglucanase production was significantly affected by fermentation time, inoculum size, pH and temperature. The optimum values of the parameters X_1, X_2, X_3, X4 were found to be 29 °C, pH 5.47, 71.83 hrs and 2.16 ml respectively. Furthermore, this study revealed that owing to the high concentration of endoglucanase activity, the optimum concentration is achieved at different process parameters. However, more investigations are needed on different types of agro waste and different operating conditions before such conclusions can be generalized.

Table2 Regression coefficients and their significances from the results of CCD for endoglucanase production from *Trichoderma viride*

Term constant	Regression Coefficient	Estimation Coefficient	t-Statistics	P-Value
Constant	98.7500	0.2322	425.353	0.000
X_1	-0.2929	0.1254	-2.336	0.033
X_2	0.1563	0.1254	1.246	0.231
X_3	0.3054	0.1254	2.436	0.027
X_4	0.1896	0.1254	1.512	0.150
X_1*X_1	-1.7561	0.1149	-15.289	0.000
X_2*X_2	-1.3599	0.1149	-11.839	0.000
X_3*X_3	-1.2549	0.1149	-10.925	0.000
X_4*X_4	-1.2186	0.1149	-10.609	0.000
X_1*X_2	-0.6644	0.1536	-4.327	0.001
X_1*X_3	0.0294	0.1536	0.191	0.851
X_1*X_4	0.2569	0.1536	1.673	0.114
X_2*X_3	-0.0756	0.1536	-0.492	0.629
X_2*X_4	0.6344	0.1536	4.131	0.001
X_3*X_4	0.2281	0.1536	1.486	0.157

Table 3 Analysis of variance (ANOVA) for the quadratic polynomial model of endoglucanase production from *Trichoderma viride*

Source of variation	Degrees of freedom(DF)	Seq sum of squares (SS)	Adj Mean square(MS)	F-value	P-value
Regression	14	196.396	14.0283	37.18	<0.000
Linear	4	5.746	1.4366	3.81	0.023
Square	4	175.155	43.7887	116.06	<0.000
Interaction	6	15.495	2.5825	6.84	<0.001
Residual Error	16	6.037	0.3773	-	-
Lack-of-Fit	10	6.037	0.6037	-	-
Pure Error	6	0.000	0.0000	-	-
Total	30	202.433	-	-	-

REFERENCES

[1] Rajasekhar Reddy B, et al (2005), Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*, African J. Biotechnology, 5(5), pp.472-476.

[2] M. Karmarkar, R.R Ray (2010). Extracellular endoglucanase production by *Rhizopus oryzae* in solid and liquid state fermentation of agro wastes, Asian J. Biotechnology, pp.27-36.

[3] G. Immanuel, et al(2006), Effect of different growth parameters on endoglucanase activity by bacteria isolated from coir retting effluents of estuarine environment, Int. J. Environ. Sci. Tech., 3(1), pp. 25-34.

[4] A.C.Ene,et al (2005),Studies on the use of agricultural wastes for cellulase enzyme production by *Aspergillus niger*, Research J. Agri. Bio. Sci., 1(4): pp.325-328.

[5] Omojasola, et al (2008). Cellulase production by some fungi cultured on pineapple waste, Nature and science, 6(2), pp. 1545-1547.

[6] G. Immanuel (2010), Production and partial purification of cellulase Mycologia, 91(2), pp.359-364.

[7] Tamas Juhasz, et al (2003), Production of β – Glucosidase in mixed culture of Aspergillus Niger and Trichoderma ressei, Food Technol. Biotechnol.,41(1), pp. 49-53.

[8] N.K. Abu Bakar, et al (2010), Isolation and selection of appropriate cellulolytic mixed microbial cultures for cellulases production from oil palm empty fruit bunches, Biotechnol., pp.73-78.

[9] Chellapandi.P, Himanshu M. Jani (2008), Production of endoglucanase by the native strains of Streptomyces isolates in submerged fermentation, Brazilian Job Microbiology, 39, pp. 122-127.

[10]Mohammad Muhsin Javed, et al (2005), Cotton Saccharifying activity of cellulases produced by co-culture of *Aspergillus niger* and *Trichoderma viride*, Research. J. of Agri. and Bio.Sci, 1(3), pp. 241-245.

[11]Sudhakar.P and Nagarajan P (2010), Production of chitinase by solid state fermentation from rice bran, Int.Jou.Envi.Science, 1:5.

[12]Kavitha.S and Nagarajan.P (2011).Production of endoglucanase by *Aspergillus niger* using agro residue, .J.Che.Eng.Research Vol (3), pp.89-96.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Fluorescent Biomarkers in Environmental Monitoring

Lekha K[1], Kerensa Miriam Sheen[2], and Kumaravel K.[*]
Laboratory of Molecular Biology and Genetic Engineering,
Department of Biotechnology, Kumaraguru College of Technology,
Chinnavedampatti, Coimbatore – 641 028, Tamilnadu, India.
[*]kumaravel.k.bt@kct.ac.in

Abstract*: The world is being inflicted by various toxic substances that affect its life forms. Anthropogenic causes are the main reasons for the harm caused to the natural environment, home to a million species. Diverse flora and fauna are becoming extinct due to the disturbances in their ecosystem. Pollution abatement is possible with the help of biomarkers. Biomarkers help us to know the pollution levels, the type of pollutant and can be used to assess the extent of toxicity in that environment. This review focuses on the various fluorescent biomarkers used in different ecosystems and compares the methods of detection. Other biomarkers can also be used but it depends on the species and the level of sensitivity required for the specific site. Periodic monitoring after cleaning up of pollutants can be estimated using biomarkers and this helps us understand how much more abatement is required until the area is safe and will thrive again and contribute as an aesthetic beauty.*

Keywords*: biomarkers, fluorescent biomarkers, GFP, FISH*

I. Introduction

Today the world is facing immense stress due to anthropogenic activities. Pesticides, sewage, detergents, medical waste, toxic metals, chemicals, pathogens, musk fragrances, preservatives, UV filters, insect repellents, organophosphate flame retardants, phthalate esters, per fluorinated compounds, and industrial phenols and other xenobiotics have accumulated in the natural environment as well as inside living organisms. Contamination of aquatic ecosystems by herbicides and pesticides are other major issues that we are facing. In addition to this, petroleum products in the marine environment may undergo photo-oxidation, evaporation, dissolution, microbial degradation, emulsification and sedimentation which need to be detected [1]. Toxicological studies need an improved way in which the causes of pollution and the response to the stress caused can be evaluated both accurately and economically.

To understand the effects of pollution caused by man-made activities, and the accumulation of different pollutants in different living organisms, researchers are looking more towards biomarkers as effective analytical tools. A biomarker is generally defined as a change in a biological response which can be related to exposure or to toxic effects of environmental chemicals. Biomarkers can be classified under three main categories, i) biomarkers of exposure that are used to quantify the xenobiotic with the target, ii) biomarkers of effect are used to study the harm caused to health and iii) biomarkers of susceptibility indicates how the affected organism responds to the toxic substance [2].

The advantages of biomarkers are that low levels of substance can be detected using advanced analytical techniques. Therefore, the levels of pollution in a place can be monitored by using biomarkers. Biomarkers can also play a major role in monitoring the improvements caused by remediation until the complete removal of pollutants in a locality. Generally, studies have been performed on human samples to estimate the response in the presence of major chemical and metallic pollutants of air, water and soil. But various ethical reasons have to be considered while acquiring samples from human beings. Researchers are now considering using other organisms such as fish, as an ideal biomarker in response to biomonitoring marine and freshwater ecosystems.

Fluorescence is a phenomenon used in labelling method to better enable visualisation. Red colour that has a longer wavelength can penetrate longer distances in cell. But issues with orange and red coloured probes have been reported. There are issues of mis-foldings and mis-targeting. The points to consider are the size of the probe, the toxic effects it may cause, brightness, the localisation at the specific tissue or organelle, sensitivity to pH – (some organelles have lower pH and some isoforms of GFP can get quenched there), their behaviour at the cell and molecular level, background fluorescence, colour variant and linkers. In this review we specifically see how fluorescence helps in monitoring pollution. Fluorescent biomarkers as well as dyes are being used to understand the various polluted ecosystems.

II. Fluorescent Biomarkers

Fluorescent biomarker genes were shown to be essential for monitoring the levels of remediation in different temperatures. A study conducted by [3] uses the GFP gene to tag *Arthrobacter chlorophenolicus* A6 bacteria which degrades chlorophenol in the soil and the *luc* gene was used to monitor the metabolic activity of the cell population in the soil. It was seen that the cells were viable at lower temperatures of 5℃ rather than 28 ℃. Hence this form of bioremediation is better suitable for colder habitats. The fluorescence can be monitored using a fluorescent microscope and flow cytometer. GFP is capable of emitting fluorescence in blue-light and does not say anything about the metabolic state of the cell. Luc on the other hand tells us about the metabolic status of the cell as the amount of light emitted is dependent on the amount of enzyme acting on the substrate luciferin.

GFP is used in bioremediation due to its gamut of advantages. No substrate is required for it to exhibit fluorescence. It does not alter the functionality of the proteins with which it is fused. It is being used in biosensors for measuring the concentrations of biologically relevant pollutants. This is possible by transcriptional fusions and the measure of expression of reporter gene gives the amount of pollutant [4].

There are some limitations to GFP's use in environmental monitoring. Studies were conducted by [5] on GFP expressed from *Bacillus subtilis* (an ideal model organism), where the stability of GFP was tested under different conditions as a biomarker of exposure, and whether it can be used in field studies to determine environmental contaminants of soil and marine sediments. GFP fluorescence was estimated by luminescence spectroscopy, and it was observed that at near neutral conditions, GFP fluorescent intensity is better, i.e. at pH 7, GFP fluorescence intensity is better. Therefore, GFP can be considered as a good biomarker for environments near neutral conditions.

Bioremediation of cold climatic regions contaminated with polychlorinated biphenyls by using strains obtained from that region which can withstand those harsh environmental conditions. They can be tagged with GFP to monitor the level of abatement. Pseudomonas strains are known to degrade biphenyls. The activity of wild strains and tagged strains were found to be the same by [6] which showed that the introduction of the gfp gene did not in any way affect the normal functioning of the organism. Even the PCB-degrading enzymes of Pseudomonas sp. Cam-1 and Sag-50G were found to be cold adapted at 4°C. Epifluorescence microscopy was used to see the green fluorescence.

Webb, S. R [5] also proposed a new method to decontaminate soil which has been polluted due to chemicals such as trinitrotoluene (2,4,6-TNT). This compound present in soil, and when leached out of the soil, leaves the soil in excess of alkaline conditions. This decontamination technique would use a fluorophore that that can be genetically engineered via a plasmid into a microorganism or polymer, and both approaches could be tested for intensity of GFP fluorescence via suitable imaging applications. The result is a genetically engineered plasmid which is able to with stand fluxes in pH, temperature, and other environmental conditions. Experimental work to prove this theory was conducted by [7] wherein *lux* genes from *Vibrio fischeri* were analysed as potential bioreporters in the contamination of soil samples by the PAHs naphthalene and toluene.

Rhodococcus species is able to degrade chlorimuron ethyl in soil. It has been tagged with GFP to enable monitoring of the soil pollution levels and to keep track of its viability in the soil. Studies were conducted by [8] to analyze its effect on the microbial community and the results showed that it did not influence the community to a great extent. Fluorescent microscopy was used to count the cells in addition to CFUs. The advantage of using this method was that the cell count obtained was higher in fluorescent microscopy than in CFU. The difference was attributed to viable but non-culturable population of cells that still exhibited fluorescence. Therefore, this is another example which shows that fluorescent markers are useful in pollution abatement.

Special fluorescent proteins of scleractinian coral tissue have been discovered to be responsible for red pigmentation corresponding to an immune response to trematode infections, as studied by Palmer and colleagues [9]. Here red fluorescent proteins provide a systemic defence mechanism against trematode infections, but its scope in terms of localized infections in *Pories compressa* tissue is yet to be studied in great detail.

III. Fluorescent Techniques for Environmental Monitoring

Fluorescent compounds can also indicate the effects of oil pollution on aquatic ecosystems. Studies have shown that fluorescent aromatic hydrocarbons (FACs) present in the bile of benthic fish can act as biomarkers of exposure to polynuclear aromatic hydrocarbons (PAHs) contamination of marine and freshwater ecosystems [10]. However, variability can occur in the degree of exposure, and this can be due to many factors such as age, gender, mobility and others. Tests were performed on the benthic fish species *Ameiurus nebulosus*—known commonly as the brown bullhead—to study the degree of increased levels of FACs present

in the bile of this fish when exposed to PAH-contaminated sediments deposited below the lake basin [11]. The results of this study indicate that the FAC levels in the biliary fluids of *Ameiurus nebulosus* increased 173-fold as PAHs in the sediments were metabolized by the fish, and then rapidly declined as the PAH levels in the lake basin decreased. By this data, it has been shown that bile FAC levels in most benthic fish species can be used as a biomarker of exposure to PAHs.

Some fluorescent dyes help in genotoxic analyses. For example, acridine orange, a fluorescent marker stains immature or polychromatic erythrocytes and has been used to assess fish cells. It can be visualized by using a fluorescence microscope. The micronuclei and main nuclei are stained a strong green-yellow fluorescence and immature erythrocytes can be seen as red fluorescence due to their RNA content in the cytoplasm. This technique is simple as it involves staining after slide preparation and identification of any aberration is done by using an epifluorescence microscope at 1000x as described by [12].

For bioremediation of a site, the amalgamated work of biochemical, genomic and phylogenetic analyses is required but there is a limitation in growing the various microbes from the environment even when resources are plentiful. This can be overcome by using techniques like FISH which is Fluorescence In-Situ Hybridization. The function of microorganisms in the environmental processes can be studied by using metagenomic strategies like micro-autography combined with FISH. Substrate uptake by microorganisms in extreme halophilic environment can be studied using this approach as done by [13]. These techniques help in wastewater treatment and also in studying about a new species or already available species in a given environment and how it contributes to the various environmental cycles. Halogenic compounds are produced by marine organisms by nature in addition to the toxic substances that we dump into the oceans. They can be monitored using these techniques [14].

An interesting application of note is the GENECARD-FISH technique. Bacterial *Dehalococcoidesmccartyi* strains have tceA and vcrA genes that produce the enzyme RDases, which can reduce harmful chlorinated compounds into harmless ethene. A new protocol was developed called geneCARD-FISH (catalyzed reporter deposition fluorescence in situ hybridization) which had been proved to be better than qPCR since it was able to give accurate quantitative results over a wide concentration range as shown by [15]. This method involves the following steps. First the DIG-labelled probe is synthesized followed by sample preparation, hybridization, probe conjugation with HRP (horse radish peroxidase), signal amplification and finally fluorescence microscopic analysis. Thus, this innovative technique can be used to monitor ground water pollution and aid in bioremediation.

Another area where fluorescence is of essence is in groundwater contamination monitoring. Trichloroethene (TCE) which are carcinogenic can be dechlorinated to VC(vinyl chloride) and then to ethene by using Dehalococcoidesmccartyi. The genes responsible for producing the enzymes were found to be tceA and vcrA. This organism is difficult to analyse since it has complex issues in downstream analyses. The fluorescence-activated cell sorting method makes it easy to monitor and abate pollution. It is combined along with whole genome amplification (WGA) after isolating the DNA. This combinatorial method was first done by [16] and now stands an example for future bioremediation. (DAPI, 358 nm 117 (excitation)/461 nm (emission); Alexa Fluor 488, 495 nm (excitation)/519 nm (emission)) DAPI and alexafluor 488 were used as fluorescent dyes. They were viewed using an epifluorescence microscope and sorted using a MoFlo high speed flow cytometer. The desired cells exhibiting the fluorescence were collected in this manner. This method when coupled with a broad specificity probe, 16S rRNA FISH probe it helped in separating multiple strains. Thus, this method serves as an ideal tool for isolating a species from a microbial community for genomic characterization and pollution monitoring.

Fluorescent-dye labelled assays provide a simple and rapid detection system to monitor organophosphorus pesticide metabolites such as trichloropyridinol (TCP), by using the principles of immunochromatographic assay techniques like ELISA. Here, rat plasma is taken and monitored for TCP as a biomarker of exposure to the bio-pesticide Chlorpyrifos [17]. The fluorescent dye labelled analog of TCP and the analyte (TCP) undergo a competitive binding with capturing antibody on the test strip. After a complete assay the test strip is subjected to fluorescence with the test strip reader. The fluorescence signal will be inversely proportional to the analyte concentration of TCP in the sample, therefore leading to the quantification of TCP in samples.

IV. Conclusion

The review shows that it is vital for us to control environmental pollution which leads to the destruction of ecosystems. Several bioremediation techniques are undertaken and followed throughout the world. Here we see how fluorescence plays a role in monitoring pollution. It is vital to identify the organisms which degrade toxic compounds and to identify suitable tags and biomarkers that can be used to track the levels of pollution. The effect and treatment must be monitored to ensure safe levels again. The advantages and merits of using GFP and

LUC genes have been mentioned in this review. The use of fluorescent biomarkers comes in handy when viable but non-cultivable cells are to be detected for environmental monitoring. The uses of FISH and GENECARD-FISH in efficiently monitoring contaminated sites are iterated. Fluorescent microscopy and flow cytometer have shown to be a quantitative tool for detecting the fluorescent dyes used for visualising the cells to monitor environmental pollution. Therefore, we come to understand that fluorescence comes in handy when it is difficult to culture the organisms and when the other methods of detection are not suitable due to complications and cost. Hence more understanding and modifications to existing methods of monitoring pollution is necessary for revival of ecosystems for the benefit of various life forms.

References

[1] Ines, Z., Amina, B., Mahmoud, R., & Dalila, S. M., "Aliphatic and aromatic biomarkers for petroleum hydrocarbon monitoring in Khniss Tunisian-Coast, (Mediterranean Sea)", Procedia Environmental Sciences, vol. 18, pp. 211-220, 2013.

[2] Franco, S. S., Nardocci, A. C., & Günther, W. M. R., "PAH biomarkers for human health risk assessment: a review of the state-of-the-art.", Cadernos de Saúde Pública, vol. 24, a569-s580, 2008.

[3] Backman, A., Maraha, N., & Jansson, J. K., "Impact of temperature on the physiological status of a potential bioremediation inoculant, Arthrobacter chlorophenolicus A6." Applied And Environmental Microbiology, vol. 70(5), pp. 2952-2958, 2004.

[4] Kumari, A., Kumar, R., Kapoor, K. K., & Sharma, P. K., "Applications Of Green Fluorescent Protein." Everyman's Science, pp. 369.

[5] Smith, C. B., Anderson, J. E., Fischer, R. L., & Webb, S. R., "Stability of green fluorescent protein using luminescence spectroscopy: is GFP applicable to field analysis of contaminants?", Environmental Pollution, vol 120(3), pp. 517-520, 2002.

[6] Ahn, Y. B., Beaudette, L. A., Lee, H., & Trevors, J. T., "Survival of a GFP-labeled polychlorinated biphenyl degrading psychrotolerant Pseudomonas spp. in 4 and 22˚ C soil microcosms." Microbial Ecology, vol. 42(4), pp. 614-623, 2001.

[7] Burlage, R. S., & Kuo, C. T., "Living biosensors for the management and manipulation of microbial consortia.", Annual Review Of Microbiology, vol. 48(1), pp. 291-309, 1994.

[8] Xiong, M., Hu, Z., Zhang, Y., Cheng, X., & Li, C., "Survival of GFP-tagged *Rhodococcu*s sp. D310-1 in chlorimuron-ethyl-contaminated soil and its effects on the indigenous microbial community.", Journal Of Hazardous Materials, vol. 252, pp. 347-354, 2013.

[9] Palmer, C. V., Roth, M. S., & Gates, R. D., "Red fluorescent protein responsible for pigmentation in trematode-infected Porites compressa tissues." The Biological Bulletin, vol 216(1), pp. 68-74, 2009.

[10] Willett, K. L., McDonald, S. J., Steinberg, M. A., Beatty, K. B., Kennicutt, M. C., & Safe, S. H., "Biomarker sensitivity for polynuclear aromatic hydrocarbon contamination in two marine fish species collected in Galveston Bay, Texas.", Environmental Toxicology and Chemistry: An International Journal, vol. 16(7), pp.1472-1479, 1997.

[11] Leadley, T. A., Balch, G., Metcalfe, C. D., Lazar, R., Mazak, E., Habowsky, J., & Haffner, G. D., "Chemical accumulation and toxicological stress in three brown bullhead (*Ameiurus nebulosus*) populations of the Detroit River, Michigan, USA.", Environmental Toxicology And Chemistry, vol. 17(9), pp. 1756-1766, 1998.

[12] Vieira, C. E. D., Costa, P. G., Cabrera, L. C., Primel, E. G., Fillmann, G., Bianchini, A., & dos Reis Martinez, C. B., "A comparative approach using biomarkers in feral and caged Neotropical fish: Implications for biomonitoring freshwater ecosystems in agricultural areas.", Science of the Total Enviornment, vol. 568, pp. 598-609, 2017.

[13] Rossello-Mora, R., Lee, N., Anton, J., & Wagner, M. "Substrate uptake in extremely halophilic microbial communities revealed by microautoradiography and fluorescence in situ hybridization." Extremophiles, vol. 7(5), pp. 409-413, 2003.

[14] Wagner, M., Nielsen, P. H., Loy, A., Nielsen, J. L., & Daims, H., "Linking microbial community structure with function: fluorescence in situ hybridization-microautoradiography and isotope arrays." Current opinion in biotechnology, vol. 17(1), pp. 83-91, 2006.

[15] Matturro, B., & Rossetti, S., "GeneCARD-FISH: detection of tceA and vcrA reductive dehalogenase genes in *Dehalococcoides mccartyi* by fluorescence in situ hybridization.", Journal of microbiological methods, vol. 110, pp. 27-32, 2015.

[16] Lee, P. K. H., Men, Y., Wang, S., He, J., & Alvarez-Cohen, L., "Development of a Fluorescence-Activated Cell Sorting Method Coupled with Whole Genome Amplification To Analyze Minority and Trace Dehalococcoides Genomes in Microbial Communities." , Environmental Science & Technology, vol. 49(3), pp. 1585–1593.,doi:10.1021/es503888y, 2015.

[17] Zou, Z., Du, D., Wang, J., Smith, J. N., Timchalk, C., Li, Y., & Lin, Y., "Quantum dot-based immunochromatographic fluorescent biosensor for biomonitoring trichloropyridinol, a biomarker of exposure to chlorpyrifos." Analytical Chemistry, vol. 82(12), pp. 5125-5133, 2010.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

OUTCOME AMBULATORY AND PRE AND POST WALK TEST VALUES OF TASK-ORIENTED TRAINING ON PHYSICAL APPROPRIATENESS USING LOCOMOTIVE VECTOR, TIME FACTOR OF STROKE SURVIVORS

R RANADEVAN[1*], S JEYAKUMAR[2], SHARMILA R[1]
SANTHI K[1], SUGANYA K[3], T RAJESHWARI[4], K SENTHILKANNAN[5*]
[1]Dept. of Biochemistry, Edayathangudy G S Pillay Arts & Science College, Nagapattinam
[2]MSAJ group of colleges Chennai, TN, India.
[3]Dept. of Chemistry, Edayathangudy G S Pillay Arts & Science College, Nagapattinam
[4]Dept. of Biotechnology, Edayathangudy G S Pillay Arts & Science College, Nagapattinam
[5]R and D centre, Edayathangudy G S Pillay Arts & Science College, Nagapattinam, TN.
E.Mail.ID- mscgoldmedalist@yahoo.in

Abstract*: The circuit training the study which enunciated the walking speed, walking endurance and functional ambulatory category scores of the stroke patients. The improvements in walking abilities of the subjects in the circuit training group can be due to improvements in the physical development. The subject who participates in task oriented circuit training showed highly significant improvement in ambulation than subject who received conventional therapy or any other types of heal.*

Keywords: *Stroke defaulters; Ambulation, Walk test, Locomotive Vector, Time Factor*

I. Introduction

The World Health Organization (WHO) defined stroke is the one of the developing clinical symbols of global disturbance of cerebral function of human beings, with symptoms lasting 24 hours or longer or leading to lethal effect, with no apparent cause other than of vascular origin. Stroke is the sudden loss of neurological function caused by an interruption of the blood flow to the brain [1-4]. Ambulatory restoration after stroke needs a more and more intensive and rigorous training that will holistically improve gait. Several studies have been conducted to examine the proper recovery of stroke victims or patients [5-8] and there is evidence to tell or suggest that timely initiation of treatment will improve the ambulation of them. Conventional therapy includes passive and active exercises, cryotherapy, stretching or various neuro-muscular facilitation techniques such as PNF, Brunnstrom approach, Roods approach, NDT etc. Circuit Training is one of the main exercises cum training techniques that can be employed to improve ambulation of stroke survivors [9-12]. Circuit training is a technique in which exercises are performed in successive stations with either a predetermined number of repetitions or for a set duration of time for healing or remedial purpose.

II. AIM OF THE STUDY

The aim of the present study is to find the effectiveness of multi task oriented circuit training for hemi paretic stroke patients with sanctioned ethical view.

2.1 OBJECTIVE

The prime objective of the study is

* To improve the ambulatory function

2.2 STUDY DESIGN

Pre and Post Experimental study

2.3 SAMPLE DESIGN

Non probability convenient sampling technique

2.4 SAMPLE SIZE

Control group=n(10)
Experimental(circuit) group=n(10)

STUDY SETUP

Clinical based setup

CRITERIAS

2.5 INCLUSION CRITERIA

Hemiparetic stroke patients.

First episode single stroke duration of <3 months.

Age 45-60 years of both sexes.

Ability to walk 10 meters independently with or without walking aid.

Functional ambulatory category score of 3 or more.

2.6 EXCLUSION CRITERIA

Aphasia

Cardiac arrhythmias

Uncontrollable Hypertension

2.6.1 STUDY DURATION

8 Weeks

2.7VARIABLES

2.7.1 Independent variable:

Task oriented circuit training

2.7.2 Dependent variable:

Ambulation

2.8 MATERIAL USED

i. Chair
ii. Couch
iii. Cycling
iv. Pillow
v. Sandbag
vi. Towel
vii. Treadmill

2.8.1 OUTCOME MEASUREMENT TOOLS

i. 6 minute walk test
ii. Functional ambulatory category score

III. PROCEDURE

Consent is obtained well by explaining the procedure to the individuals. A total of 20 patients of both genders were selected based on the inclusion criteria. All the patients are undergoing for pre test assessment for using outcome tool 6 minute walk test and functional ambulatory category scores. The subjects were conveniently assigned into two groups. Experimental group received circuit training. Control group received conventional therapy. Both groups received 3 times per week for 8 weeks. Then end of the week again all patients are underwent for post test assessment for the outcome measurement by using 6 minute walk test and functional ambulatory category score.

3.1 ASSESSEMENT PROCEDURE:

The tasks were explained to the patient and were instructed to perform the task. The score were evaluated to the performance of the patient. The tests were concluded before and after treatment procedure.

3.2 TREATMENT PROCEDURE

The subjects were chosen with accordance to the inclusion and exclusion criteria and assigned to experimental and control group. In each group 10 patients were allocated to the treatment procedure.

Experimental group(A) (n) = 10

Control group(B) (n) =10

3.2.1 EXPERIMENTAL GROUP (A)

The frequency of the circuit training was 3 times a week, for 8 weeks. The duration per training session was 35 minutes including:

i. warm - up (5 minutes)
ii. first circuit training (12 minutes)
iii. rest-interval(5 minutes)

iv. second /repeat circuit training (12 minutes)

The type and nature of exercise stations was to achieve a total lower limb as well as total body work-out.
WARM UP: 5mins

Sitting at a table and reaching in different direction for objects located beyond arms length to promote loading of the affected leg and activation of affected leg muscle

EXERCISES:

i. Treadmill walking
ii. Push-ups
iii. Squatting exercise
iv. Straight leg raise exercise
v. Stairs walking
vi. Cycling

STATION 1:
TREADMILL WALKING
Patient is made to stand on the treadmill with support.
The speed of the treadmill is 0.9 km/hour for 3 minutes.
STATION 2:
PUSHUPS
Patient position: prone lying
Supported by pillow at the abdomen
Ask the patient to perform 1 set of 10 repetitions in a minute
STATION 3:
SQUATTING EXERCISE
Ask the patient to bend their knees and sit on the chair with support
They perform 1 set of 15 repetitions in a minute.
STATION 4:
STRAIGHT LEG RAISE (SLR)
Patient position : supine lying
The patient lift their leg without bending the knee with the support of towel
They perform 1 set of 10 repetitions in a minute.
STATION 5:
STAIRS WALKING
The patient covers 2 steps climb up and down in 3 minute with the support of sandbag (to prevent buckling of knee)
STATION 6:
CYCLING
The patient sits on the cycle comfortably. They perform cycling for 3 minutes. The subject performed circuit training at light intensity at the beginning and hard intensity towards the end of session. The speed of the treadmill was between 0.9 km/hour and 3.0 km/hour. The speed of the cycling and resistance as well as squatting, straight leg raise and stair walking were adjusted to the training intensity.

3.2.2 CONTROL GROUP (B)

The control group received only the usual conventional therapy of passive and active exercises.

Upper limb strengthening exercises
Walking Re-education
Standing and balance retraining carried out between the parallel bars.

IV. DATA ANALYSIS

Pre and Post test mean difference of functional ambulatory category score

Group A	Pre test	Post test	Mean difference in min
Task oriented circuit training	3.0	4.5	1.5
Group B	Pre test	Post test	Mean difference
Conventional therapy	3.0	3.5	0.5

Pre and post test mean differences of 6 minute walk test

Group A	Pre test	Post test	Mean difference in m
Task oriented circuit training	17.75	27.75	10
Group B	Pre test	Post test	Mean difference
Conventional therapy	16.95	21.95	5

Comparison of the pre, post functional ambulatory category values within the groups

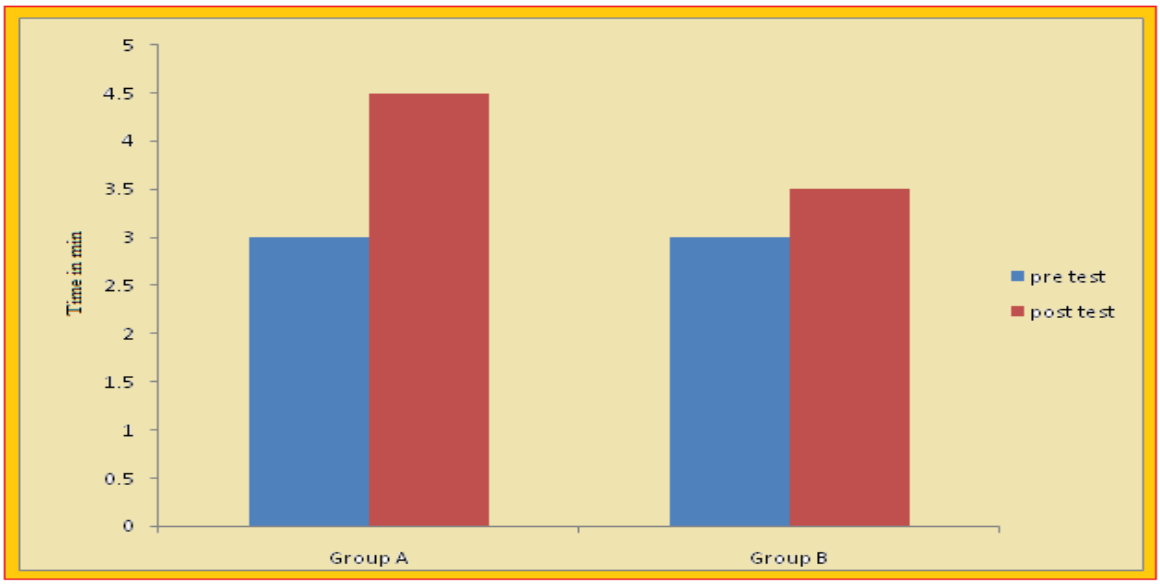


Fig.1 Time for activity with groups A and B in Bar chart

Comparison between the pre and post 6 minute walk test values within the groups

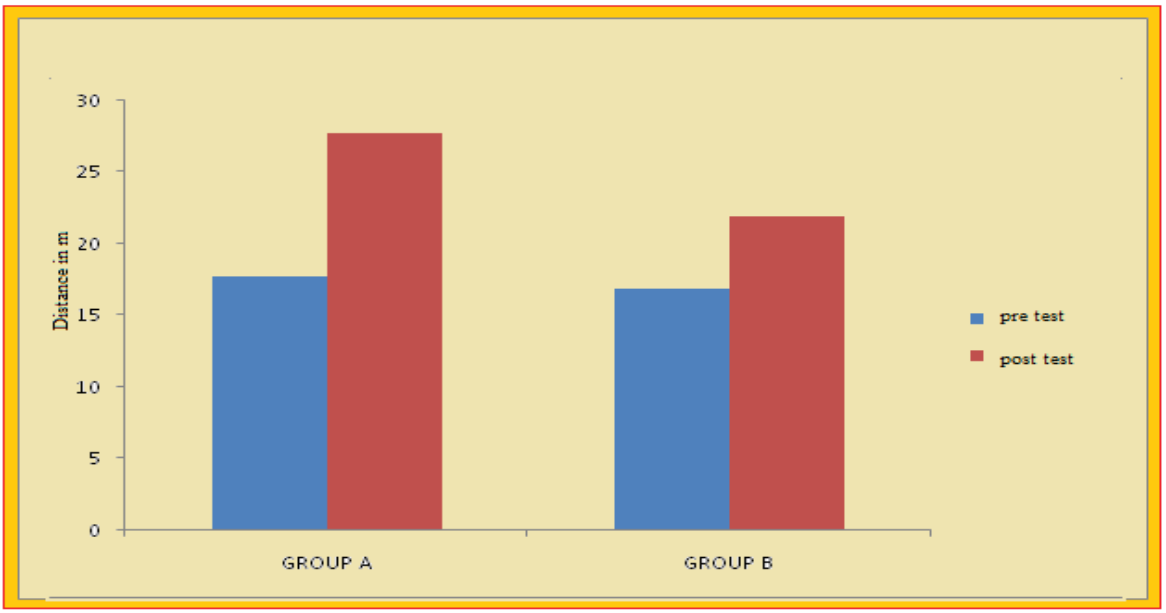


Fig.2 distance moved with groups A and B in Bar chart in the activities

V. RESULT

GROUP (A)

The experimental group who received task oriented circuit training has pretest mean value of functional ambulatory category score is 3 while posttest mean value of functional ambulatory category score is 4.5 thus, the significant difference between pretest and post test scores of functional ambulatory category score is 1.5 and the experimental group who received task oriented circuit training has pre test mean value of 6 minute walk test is 17.75 m while post

test mean value of 6minute walk test is 27.75 m thus, the significant difference between pretest and post test value of 6 minute walk test is 10m

GROUP (B)

The control group who received conventional therapy exercises has pretest mean value of functional ambulatory category score is 3 while posttest mean value of functional ambulatory category score is 3.5 thus, the significant difference between pretest and posttest scores of functional ambulatory category score is 0.5. The control group who received conventional therapy exercises has pretest mean value of 6 minute walk test is 16.95 m while post test mean value of 6 minute walk test is 21.95 thus, the significant difference between pretest and posttest value of 6 minute walk test is 5m

III.FUNCTIONAL AMBULATORY CATEGORY (FAC) SCORES

The functional ambulatory category scores were assessed while subjects were walking to evaluate the level of dependency of subjects in performing functional activities. FAC is a six point hierarchical rating scale that reflects the amount of assistance a person requires to walk.

SCORE 0- a subject is non-functionally ambulatory (cannot walk)
SCORE 1- a subject requires continuous manual contact
SCORE 2- a subject requires intermittent manual contact
SCORE 3- a subject requires verbal supervision/guarding
SCORE 4 - a subject is an independent ambulatory who can walk independent ambulatory who can walk freely on level surface only
SCORE 5- a subject is an independent ambulatory who can walk freely on any surface

IV.6 MINUTE WALK TEST (6MWT)

The 6MWT should be performed indoors, along a long, flat, straight, enclosed corridor with a hard surface that is seldom travelled. If the weather is comfortable, the test may be performed outdoors. The walking course must be 30 m in length. A 100-ft hallway is, therefore, required. The length of the corridor should be marked every 3 m. The turnaround points should be marked with a cone (such as an orange traffic cone). A starting line, which marks the beginning and end of each 60-m lap, should be marked on the floor using brightly colored tape.

Required Equipment

Countdown timer (or stopwatch)
Two small cones to mark the turnaround points
A chair that can be easily moved along the walking course
Sphygmomanometer

Patient Preparation

1. Comfortable clothing should be worn.
2. Appropriate shoes for walking should be worn.
3. Patients should use their usual walking aids during the test (cane, walker, etc.)
4. The patient's usual medical regimen should be continued.
5. A light meal is acceptable before early morning or early afternoon tests.
6. Patients should not have exercised vigorously within 2 hours of beginning the test.

V.MASTER CHART

FUNCTIONAL AMBULATORY CATEGORY SCORING

TABLE 1 GROUP A

S.NO	PRE-TEST	POST-TEST
1	3	4
2	3	4
3	3	5
4	3	4
5	3	5
6	3	5
7	3	4
8	3	5
9	3	4
10	3	5

MEAN VALUE:	3	4.5

TABLE 2 GROUP B

S.NO	PRE-TEST	POST-TEST
1	3	4
2	3	3
3	3	4
4	3	3
5	3	4
6	3	3
7	3	3
8	3	4
9	3	3
10	3	4
MEAN VALUE:	3	3.5

6 MINUTE WALK TEST SCORING

TABLE 3 GROUP A

S.NO	PRE TEST	POST TEST
1	17.5m	26.5m
2	19m	25m
3	16m	25m
4	18m	30m
5	20m	29m
6	16m	30m
7	19m	28m
8	20m	29m
9	17m	30m
10	15m	25m
MEAN VALUE	17.75 m	27.75 m

TABLE 4 GROUP B

S.NO	PRE TEST	POST TEST
1	16m	22m
2	15m	21m
3	20m	24m
4	17.5m	20.5m
5	19m	25m
6	15m	21m
7	16m	20m
8	19m	23m
9	15m	22m
10	17m	21m
MEAN VALUE	16.95 m	21.95 m

VI. CONCLUSION

The circuit training is utilized in this research cum diagnostic study resulted in increased walking speed, walking endurance and functional ambulatory category scores of the stroke defaulters and survivors. Therefore, the study shows that the circuit training can be used to improve ambulatory functional representation of stroke in acute stage. The results support earlier findings of studies on exercise training designed to improve walking functions of stroke survivors when compared with conventional therapy. The improvements in walking abilities of the subjects in the circuit training group can be due to improvements in the muscle strength and the endurance of lower limb muscles, improved cardio respiratory functions, speed, balance, neuromuscular control and coordination. This study is examined whether a 8-weeks task oriented circuit training program improved the ambulatory

function of stroke patients. The task oriented circuit training program (experimental group) was more effective in improving the ambulation of hemi paretic stroke patients than the general physical therapy program (control group) Comparison of the variation in the functional ambulatory category scores of the experimental and control groups before and after training revealed a significant difference. The variation in the experimental group was greater than in the control group. The subject who participates in task oriented circuit training showed highly significant improvement in ambulation than subject who received conventional therapy and the healing effect is taken care with proper ethical group permission for being human beings are experimented and involved and displacement vectors and time factor are used.

References

1. Duncan M, Sean K (2003). Circuit training. In: Porter S. Tidy's Physiotherapy (13th ed), London, Butterworth-Heinemann.), pp. 438-469.
2. English C, Hillier SL (2010). Circuit class therapy for improving mobility after stroke. Cochrane Database of Systematic Reviews. 7: 107120.
3. Enright PL, Sherrill DL (1998). Reference equations for the six-minute walk in healthy adults. American Journal of Respiratory and Critical Care Medicine. 158(5): 1384–1387.
4. Hartona S. experiences from a multicenter stroke register : a preliminary report. BULL WHO 1976;54:541-53
5. Hesse S, Bertelt C, Jahnke MT, Schaffrin A , Baake P, Malezic M, Mauritz KH (1995). Treadmill training with partial body weight support compared with physiotherapy in non-ambulatory hemiparetic patients. Stroke 26; 976-81.
6. Ivey FM, Hafer-Macko CE, Macko RF. Exercise rehabilitation after stroke. Neuro RX. 2006;3(4):439-450
7. Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS. Recovery of walking function in stroke patients: the Copenhagen Stroke Study. Arch Phys Med Rehabil.1995;76:2
8. Lawrence ES, Coshall C, Dundas R, Stewart J, Rudd AG, Howared R. Estiates of acute stroke impairments and disability in a multi-ethnic population. Stroke 2001; 32:1279-1284.
9. Mackay-Lyons MJ ; Makrides L. Exercise capacity early after stroke. Archives of physical medicine and rehabilitation. 2002 ; 83(12): 1697-1702
10. Macko RF, De Souza CA, Tretter LD, Silver KH, Smoth GV, Anderson PA (1997). Treadmill aerobic exercise training reduces the energy expenditure and cardiovascular demands of hemiparetic gait in chronic stroke patients. Stroke. 28(2): 326–30.
11. Macko RF, Katzel LI, Yatacho A, et al., Low velocity graded treadmill stress testing in hemiparetic stroke pts. Stroke 1997; 28(2) : 326-330
12. Mudge S, Barber PA, Stott NS (2009). Circuit based rehabilitation improves gait endurance but not usual walking activity in chronic stroke: a randomized controlled trial. Arch Phys Med Rehabil. 90(12): 1989-96
13. Olney SJ, Richards CL (1996). Hemiparetic gait following stroke. Part I: Characteristics. Gait & Posture. 4: 136–46.
14. Rose D, Paris T, Crews E, Wu SS, Sun A, Behrman AL, Duncan P (2011). Feasibility and effectiveness of circuit training in acute stroke rehabilitation. Neurorehabil Neural Repair. 25(2); 140-8.
15. Van de Port IGL, Wevers LEG, Lindeman E, Kwakkel G (2012). Effects of circuit training as alternative to usual physiotherapy after stroke: randomised controlled trial. British Medical Journal. 10:344.
16. Wevers L, van de Port I, Vermue M, Mead G, Kwakkel G (2009). Effects of task-oriented circuit class training on walking competency after stroke: a systematic review. Stroke. 40(7): 2450-9.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

ENHANCED PRODUCTION OF PECTINASE FROM A*SPERGILLUS AWAMORI* BY SOLID STATE FERMENTATION USING RICE BRAN

S.B.RISWANALI

Department of Chemical Engineering, Annamalai University

FEAT, Annamalai University,Annamalai Nagar, India

s.b.riswanali@gmail.com

Abstract: *present work deals with the production of pectic enzymes using Aspergillus awamori by solid state fermentation technology. Agro industrial wastes like rice bran is employed as fermentation media along with nutrient solutions. Analyses for the maximum production rate of pectinase are studied by varying the media composition, pH level and fermentation temperature to optimize these physio-chemical characters. The maximum Pectinase enzyme yield by Aspergillus awamori using rice bran ml of 67.59 U/l substrate was obtained. The optimum values for the independent variables were, Substrate concentration of 10 g., initial pH of fermentation media of 5, fermentation temperature of 40ºC.*

Keywords*: Pectinase, SSF, Aspergillus awamori, Rice bran*

I. Introduction

Pectinases is an enzyme that break down pectin .Pectin is a structural polysaccharide found in primary cell wall and middle lamella of fruits and vegetables. Pectinase splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. Pectinase softens the cell wall of the fruits and increase the yield of juice [1].

Pectic substances are mostly spread in fruits and vegetables, Pectinase enzymes are commonly used in the degradation of plant materials, like quickening the extraction of fruit juice from fruit, including apples and other fruits.In earlier1960 Pectinases have also been used in production of wine. They can be extracted from fungi such as *Aspergillus awamori* [2] *Penicillium atrovenetum, Aspergillus flavus ,Aspergillus oryzae* and *Aspergillus niger*[3]. The fungus produces pectinases enzymes to break the middle lamella in plants.

Pectinase are enzymes which is used to degrade pectic substances and are of importance to the food industry. These enzymes are used to assist filtration, extraction and clarification and to enhance the maximum yields in the production beverages and fruit juices. Pectinases (PG) have been employed in preparation of apple juice for , clarity ,higher juice yield [4], vegetables oil extractions, coffee and tea fermentations, [5], paper making and in textile industries [6].

Pectinase is widely used in food processing industry, waste water treatment, souring of cotton, in the alcoholic beverage , degumming of plant fibers bleaching of paper, [7] These enzymes classified. as pectin esterase (PE), depolymerizing enzymes and propectinase.

Solid-state fermentation has more than submerged fermentation for production of increasing enzyme yield and regulating the biosynthesis of particular composition of enzyme mixtures [8]. SSF has more advantages over submerged fermentation system, includes ,requirement of simple fermentation equipments, , higher concentration of the products ,High volumetric productivity,less effluent generation, easy product separation, no foam generation and elimination of the need for rigorous control of many Parameters during fermentation. [9].

II. Material and Methods

Aspergillus awamori (MTCC 548) is used in this study purchased from IMTECH, Chandigarh. They were subculture on PDA agar slants and maintained. The fermentation medium was made with rice bran is used. Various parameters were studied to optimize the conditions to get the maximum production of pectinase. The fermentation medium was sterilized in an autoclave (121º C for minutes). After cooling the medium was inoculate with 10^8 spores from the spore suspension of *Aspergillus awamori* MTCC 548 for rice bran . The fermentation was carried out for 144 hours in a batch reactor and samples were drawn at regular time interval and analyzed for total pectinase enzyme activity.

A. Enzyme extraction

The crude pectinase was extracted from fermented rice bran by mixing distilled water with 10 g of fermented ricebran and stirred for 20 min. In the shaker, The crude pectinase was filtered and then finally ,centrifuged for 20 min.The supernatant was used for enzymatic measurements by DNS method [11].

B. Total pectinase assay

A properly diluted sample of 0.5 ml was mixed to a solution containing 2 ml of 1% citrus pectin in acetate buffer (pH 4.8) in a test tube. Samples are kept at the temperature of 45°C for 30 min. in a water bath, then the content was cooled, added with 2.5 ml of DNS reagent. Ultimately 10 ml of distilled water was added to it .The content was measured at 540 nm using UV/Vis Biospectrophotometer (ELICO BL 198).
One unit of total pectinase enzymatic activity is defined as the amount of enzyme required to produce one micromole of glucose per hour.

III. Results and Discussion

A. *Effect of substrate concentration on Pectinase production*

The effect of substrate concentration on enzyme activity in solid-state fermentation using *Aspergillus awamori* was studied. The experiments with five different Substrate concentrations namely 5 g, 7.5 g, 10 g, 12.5 g and 15 g by keeping all other parameters kept constant for the fermentation period of 144 h. The pectinase enzyme activity was found to increase exponentially reached a maximum 50.08 U/ml of pectinase activity at 10 g substrate concentration. The results are given in Table I and Fig. 1.

B. *Effect of initial pH on production of pectinase*

The effect of initial pH on pectinase production was studied at different pH namely 3, 4, 5, 6 and 7 by using substrate concentration of 10 g of rice bran. The results are given in Table II and Fig. 2. As initial pH was increased from 3 to 5, the pectinase activity also increased. Further increase in initial pH after 5 the pectinase activity was reduced. It reflects the preference of fungi *Aspergillus awamori* to lower pH for its metabolism and growth. A maximum pectinase activity of 56.25 U/ml was obtained with mixed substrate for a fermentation period of 144 h and at pH value of 5. Hence optimum pH value was chosen as 5.

C. *Effect of temperature on production of pectinase*

The optimum temperature on the pectinase enzyme production was studied using 10g substrate concentration**.** The experiments were conducted at different temperatures namely 30ºC, 35ºC, 40ºC, 45ºC and 50ºC by keeping all other parameters constant for the fermentation period of 144 h. The results are given in Table III and Fig. 3. As temperature increases from 30ºC to 40ºC, the pectinase enzyme activity was increased and maximum pectinase activity of 67.59 U/ml was found at 40ºC. Further increase in temperature, the pectinase activity was decreased till the end of fermentation. Hence, optimum temperature was chosen as 40ºC and was used for further studies. The decrease in enzyme activity at higher temperature may be due to enzyme denaturation.

TABLE I. PECTINASE ACTIVITY ON SUBSTRATE CONCENTRATION

S.No	Substrate Concentration	Pectinase ActivityU/mL
1	5	12
2	7.5	34
3	10	50.08
4	12.5	38
5	15	32

TABLE II. PECTINASE ACTIVITY ON INITIAL PH

S.No	Initial pH	Pectinase Activity U/mL
1	3	31.01
2	4	41.28
3	5	56.25
4	6	47.65
5	7	42.63

TABLE III. PECTINASE ACTIVITY TEMPERATURE

S.No	Temperature	Pectinase Activity U/mL
1	30	27.58
2	35	46.28
3	40	67.59
4	45	58.48
5	50	41.12

FIGURE 1 EFFECT OF SUBSTRATE CONCENTRATION ON PECTINASE ACTIVITY FROM *ASPERGILLUS AWAMORI* USING RICE BRAN AS SUBSTRATE

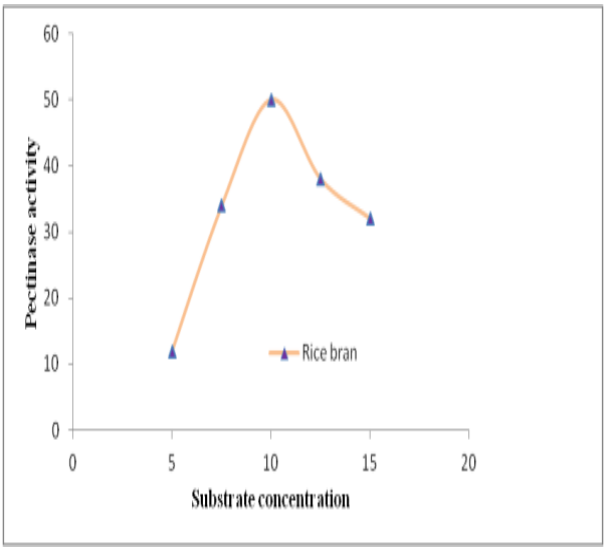


FIGURE 2 EFFECT OF INITIAL PH ON PECTINASE ACTIVITY FROM *ASPERGILLUS AWAMORI* USING RICE BRAN AS SUBSTRATE

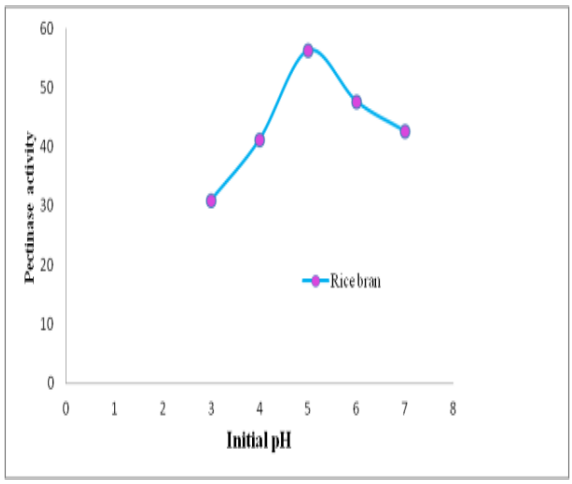
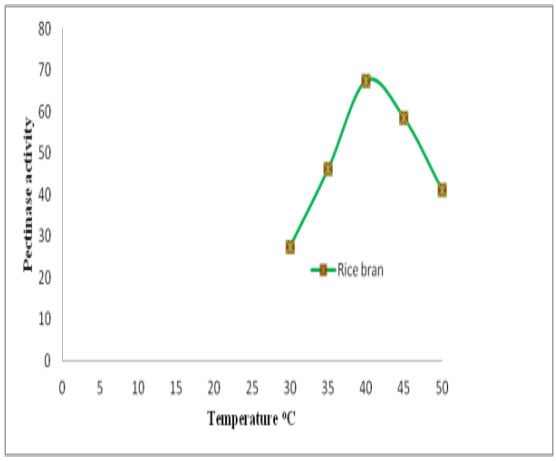


FIGURE 3 EFFECT OF TEMERATURE ON PECTINASE ACTIVITY FROM *ASPERGILLUS AWAMORI* USING RICE BRAN AS SUBSTRATE



IV Conclusion

Optimization of process parameters namely substrate concentration, temperature and pH were carried out using *Aspergillus awamori* microorganism in solid state fermentation. The maximum total pectinase activity of 67.59 U/ml was achieved using rice bran as substrate .The optimum values for the independent variables were, Substrate concentration of 10 g., initial pH of fermentation media of 5, fermentation temperature of 40°C. for maximum production of pectinase.

Acknowledgement

The authors express their sincere thanks to the Department of Chemical Engineering, Annamalai University for providing the necessary facilities for the successful completion of this research work

References

[1]. Ashfaq Khan, Sanjay Sahay, Neha Rai, ,Production and optimization of pectinase enzyme using aspergillus niger strains in solid state fermentation, Research in Biotechnology, 3(3), 19-25 , (2012).

[2]. A Blandino, T Iqbalsyah, SS Pandiella, D Cantero, C Webb. Polygalacturonase production by *Aspergillus awamori* on wheat in solid-state fermentation. Appl Microbiol Biotechnol. 58,164–169, 2002.

[3]. Adebare Johnson Adeleke, Sunday Ayodele Odunfa, Afolake Olanbiwonninu, Mojisola Christiana Owoseni, Production of cellulase and pectinase from orange peels by fungi, Nature and Science, ,10(5). (2012).

[4]. J Oszmiański, A Wojdylo, J Kolniak. Effect of enzymatic mash treatment and storage on phenolic composition, antioxidant activity and turbidity of cloudy apple juice. J Agric Food Chem. 57:7078–7085, 2009.

[5]. GS Hoondal, RP Tiwari, R Tiwari, N Dahiya, QK Beg. Microbial alkaline pectinases and their applications: a review. Appl Microbiol Biotechnol. , 59,409–418, 2000.

[6]. S Molina, F Pelissa, B Viteroilo ,Screening and improvement of pectinolytic fungi for degumming of texture fibers. Braz J Microbiol 32, 320-326, 2008.

[7]. Catarina Almeida,Tomáš Brányik,Pedro Moradas- Ferreira, José Teixeira, Continuous Production of Pectinase by Immobilized Yeast Cells on Spent Grains, Journal of Bioscience and Bioengineering, 96(6). 513–518, (2013).

[8]. SR Patil , A Dayanand ,Production of pectinases from deseeded sunflower head by Aspergillus niger in submerged and solid-state conditions. Bioresource Technology, 97,2054 -2058, 2006.

[9]. H. kh Q. Ai and M.M.D. Zulkali, " Design aspects of Bioreactors for solid state fermentation: A Review" chem. Biochem. Eng, Q, 25 (2) 255-266, 2011.

[10]. Miller, G.L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Journal of Analytical Chemistry, 31, 426-428,1959.

Acknowledgments

The authors express their sincere thanks to the Dept. of Chemical Engineering, Annamalai University,India for providing the necessary facilities for the successful completion of this research work

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Biosynthesis of Cholesterol Lowering Drug from *Aspergillus terreus* by Solid State Fermentation

Subhagar Seraman[1] & Aravindan Rajendran[2]
Department of Chemical Engineering, Annamalai University
Annamalai nagar, Chidambaram, Tamil Nadu, India
subhagaraja@gmail.com, http://www.annamalaiuniversity.ac.in

Abstract: Lovastatin ($C_{24}H_{36}O_5$), a potent drug for lowering blood cholesterol acts by competitively inhibiting the enzyme HMG-CoA reductase which catalyzes the rate limiting step of cholesterol biosynthesis. The strain *Aspergillus terreus* MTCC 279 was obtained from the Institute of Microbial Technology, Chandigarh, India, was found to produce higher concentration of lovastatin. In order to screen the best solid substrates for lovastatin production by *A. terreus*, the following substrates were experimented; including besan flour (*Cicar aritinum*), ragi (*Eleusine coracana*), baggase, millet (*Pennisetum Typhoides*), rice flour, wheat bran, black gram (*Vigna mungo*), green gram (*Vigna radiata*), green peas, yellow peas, ground nut cake (*Arachis hypogaea*), white beans, maize, rice bran (*Oryza Sativa Francais*), long grain rice (*Oryza sativa indica*), sago (*Manihot esculanta*), coffee husk powder, barley (*Hordeum vulgare*), sesame waste (*Sesamum indicum*), jack fruit seed. Among the fifteen different solid substrates tested, maximum lovastatin of 569.55 mg per g of dry solids was obtained from green peas. Green pea was found to be the best substrates for the lovastatin production via SSF process than the remaining substrates. The quantitative analysis of lovastatin was carried out in shimadzu HPLC at 238 nm in Luna C_{18} column of particle size 5µ and (250X4.6) mm I.D, UV detector (SPD 20 A). Binary gradient system was used and the samples were injected manually using Rheodyne Injector of 20 µl.

Keywords: *Aspergillus terreus; lovastatin; solid state fermentation*

I. Introduction

Lovastatin is a secondary metabolite produced by *Monascus species*, an inhibitor for cholesterol biosynthesis. Several types of enzymes are involved in the biosynthesis of cholesterol, in which the statin inhibits the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (E C 1.1.1.34) to mevalonate, which is the rate limiting step in the biosynthesis of cholesterol [1]. The endogenous synthesis of cholesterol is carried out by the mevalonate pathway, in which the rate limiting reaction is the conversion of (S) HMG-CoA to (R) mevalonate, catalyzed by HMG-CoA reductase. Recently, Li and coworkers demonstrated that *M. purpureus* fermented rice (Red Yeast Rice), a natural food product, reduced blood cholesterol in three animal models of hypercholesterolemia, and suggested that suitable prepared *M. purpureus* (RYR) could be effective hypercholesterolemia agent in human [2]. The use of proprietary Chinese yeast as a dietary supplement in human clinical trial has shown significant reductions of cholesterol levels, from 254 ± 36 mg/dl to 208 ± 3 mg/dl in eight weeks, in 83 tested individuals [3].

Lovastatin was the first statin to be approved by the United States Food and Drug Administration in 1987 [4]. Statins produced directly from the fermentations are called as the natural statins (Lovastatin and Pravastatin). In 1980, the production of lovastatin was set up industrially, using the species *A. terreus* (Mevacor, Merck) [5]. Culture homogeneity, effect of various carbon sources, pH, aeration, and agitator design are the different fermentation parameters involved in the process development. Modern SSF systems have a record of successful applications for the production of microbial enzymes and secondary metabolites [6]. In the case of antibiotics and other secondary metabolites, production under such conditions is very often associated with higher yields in shorter time periods compared to the alternative submerged fermentation (SmF) approach [7]. Some antibiotics are only produced in SSF, even though the corresponding producer fungi can be readily cultivated in SmF. The reason for this different physiology in SSF is not fully understood, but it is often called "physiology of solid medium".

Lovastatin can be produced by both submerged fermentation (SmF) and Solid state fermentation (SSF). Fungal products of biotechnological interest, i.e., enzymes, secondary metabolites and spores were developed in moist solid substrate but not in liquids. In recent years, SSF has gained much interest for the production of primary and secondary metabolites. SSF presents a more adequate habitat for fungus, with high productivity in a relatively low cost process by using agro-industrial residues as substrates. The aim of the present study is to estimate the possibility of lovastatin production by *Aspergillus terreus* using various solid substrates and to enhance the productivity by optimizing the environmental parameters.

II. Material and Methods

Chemicals and reagents

Potato dextrose agar (PDA) medium was obtained from the Hi-media, India. HPLC grade acetonitrile and ethanol were purchased from Rankem, New Delhi, India. All the chemicals used were of analytical grade.

Culture maintenance and microorganism

The fungal culture of *Aspergillus terreus* MTCC 279 was obtained from the Institute of Microbial Technology, Chandigarh, India. The culture was maintained on the PDA slants at 4°C, and subcultured every 30 days.

Solid state fermentation

All the sources were purchased from local market and used as the solid substrates in the experiments. 5 g of dry substrate was taken in a 250 mL Erlenmeyer flask covered with a cotton plug and 10 mL of basal medium containing 38.9 g/L dextrose, 9.68 g/L malt extract, 1.96 g/L $MnSO_4.H_2O$, 0.73 g/L was added. The contents of the flasks were mixed and autoclaved at 121ºC at 15 psi for 20 min. Fermentation was carried out at 30ºC with initial moisture content of 40% and inoculum size of 10% (v/v) and pH at 6.0 for 7 days with *Aspergillus terreus*.

Extraction of lovastatin

After fermentation, the harvested broth was homogenized to get the intracellular product. An equal volume of methanol was added and the suspension was kept in an incubated rotary shaker for 1 h at 200 rpm and 30ºC. The suspension was filtered, first through a filter paper and then through a micro filter (Millipore) of 0.22 µm pore diameter.

Analysis of Lovastatin

Analysis of lovastatin was carried out in Shimadzu HPLC (LC 20 AT prominence) at 238 nm in Luna C_{18} column of particle size 5µ and (250 x 4.6) mm I.D, UV detector (SPD 20 A) and the column oven (CTO-10 AS vp) at 45ºC. Binary gradient system was used and the samples were injected manually using Rheodyne injector of 20 µL. The retention time of lovastatin in its beta hydroxyacid form elutes at 6.5 min.

II. Results and Discussion

Screening of various agricultural residues for lovastatin production by A. terreus

In order to screen the best solid substrates for lovastatin production by *A. terreus*, the following substrates were experimented; including besan flour, ragi, baggase, millet, rice flour, wheat bran, black gram, green gram, green peas, yellow peas, ground nut cake, white beans, maize, rice bran, long grain rice, sago, coffee husk powder, barley, sesame waste, jack fruit seed.

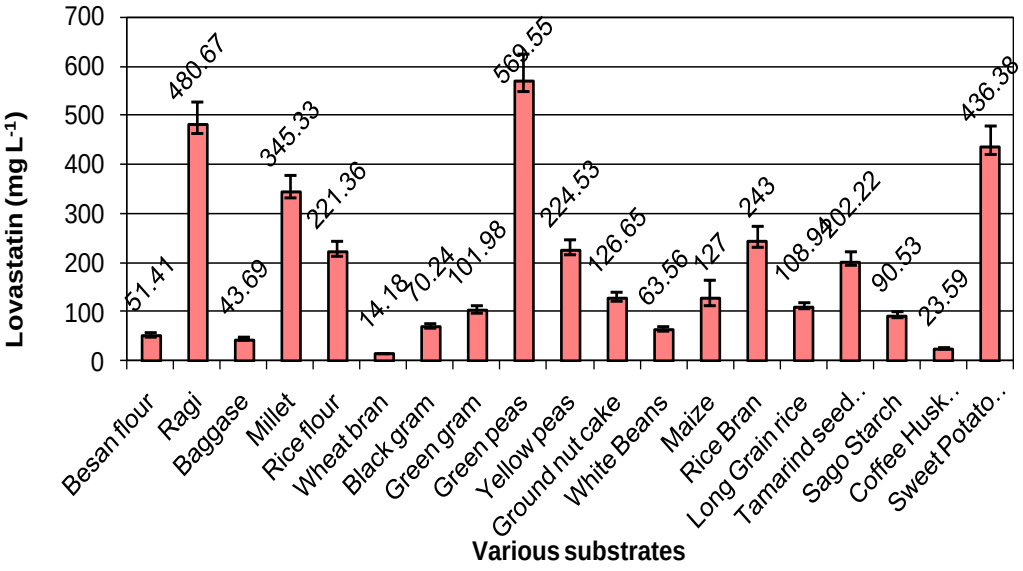


Fig. 1 Lovastatin production from various solid substrates using *A. terreus*

Among the fifteen different solid substrates tested, maximum lovastatin of 569.55 mg per g of dry solids was obtained from green peas. From Fig. 1, it is evident that green pea is the best substrates for the lovastatin production via SSF process than the remaining substrates.

Optimization of environmental condition for lovastatin production in long grain rice by *A. terreus*

In general, lovastatin production in SSF is influenced by the fermentation conditions like incubation temperature, initial moisture content and inoculum size. The parameters incubation temperature, initial moisture and inoculum size were optimized by CCD to enhance the production. Table 1 gives the coded values and the levels of the variables incubation temperature, initial moisture and inoculum size. The experimental and the predicted values were presented along with the CCD experimental design in Table 2.

Table 1 Experimental range and levels of the independent variables

Variables with designate	Coded levels				
	-1.682	**-1**	**0**	**1**	**1.682**
Incubation temperature (°C)	23.3	26	30	34	36.73
Initial moisture (mL)	1.64	2	5	8	8.36
Inoculum size (mL)	0.82	1.5	2.5	3.5	4.2

Multiple regression analysis of the CCD experimental design gives the following quadratic polynomial equation for the biosynthesis

$$Y = 363.24-0.75x_1+15.73x_2-16.24x_3-55.28x_1^2-62.01x_2^2-61.02x_3^2+4.66\,x_1x_2-1.69x_1x_3-25.89x_2x_3 \quad (1)$$

where, Y is the amount of lovastatin produced (mg g^{-1} dry substrate), x_1 is the coded value of variable X_1 (Incubation temperature), x_2 is the coded value of variable X_2 (Initial moisture), and x_3 is the coded value of variable X_3 (Inoculum size) in equation (1).

Table 2 Full factorial central composite design matrix of three variables in coded and actual units along with the observed responses

Run No.	CCD Experimental Design matrix			Lovastatin production (mg L^{-1})	
	x_1 Incubation temperature (°C)	x_2 Initial moisture (mL)	x_3 Inoculum size (mL)	Experimental	Predicted by RSM
1	-1	-1	-1	178.40	163.25
2	1	-1	-1	169.45	155.82
3	-1	1	-1	267.41	237.19
4	1	1	-1	291.50	248.40
5	-1	-1	1	169.26	185.95
6	1	-1	1	167.92	171.73
7	-1	1	1	169.07	156.29
8	1	1	1	171.98	160.72
9	-1.682	0	0	196.22	208.13
10	1.682	0	0	180.18	205.60
11	0	-1.682	0	169.22	161.40
12	0	1.682	0	169.17	214.31
13	0	0	-1.682	170.00	217.96
14	0	0	1.682	173.95	163.31
15	0	0	0	338.83	363.24
16	0	0	0	400.74	363.24
17	0	0	0	300.26	363.24
18	0	0	0	362.65	363.24
19	0	0	0	442.94	363.24
20	0	0	0	340.43	363.24

Table 3 Regression results from the data of central composite designed experiments

Model Terms	Coefficient	Standard error of coefficient	*t*-value	*P*-value
Constant	363.247	19.09	19.027	0.000
x_1	-0.752	12.67	-0.059	0.954
x_2	15.732	12.67	1.242	0.243
x_3	-16.247	12.67	-1.283	0.229
x_1x_1	-55.289	12.33	-4.484	0.001
x_2x_2	-62.008	12.33	-5.029	0.001
x_3x_3	-61.025	12.33	-4.949	0.001
x_1x_2	4.661	16.55	0.282	0.784
x_1x_3	-1.696	16.55	-0.102	0.920
x_2x_3	-25.899	16.55	-1.565	0.149

The results of the regression analysis from the data of central composite design experiments were shown in Table 3. The squared effects of x_1, x_2, x_3 and interactive effects of x_1x_2 were found to be significant as the *P*-value

is less than 0.05 as shown in Table 3 and the linear effect of x_1, x_2 and x_3 are found to be insignificant as the *P*-value is greater than 0.05.

The coefficient of determination R^2 is 0.865, which implied that only 0.135 of the variability in the responses cannot be explained by the model. This value of R^2 justified a good agreement between the experimental and predicted values of lovastatin production from mixed solid substrates fermentation, thus suggesting a high significance of the model. The regression equation (1) obtained using central composite design was maximized using constrain search procedure using MATLAB software and the optimal values of the test variables in the coded units were found to be: x_1 = 0.01, x_2 = 0.153 and x_3 = -0.153 with the predicted maximum lovastatin production of 365.87 mg g^{-1} of dry solids. The real values of the independent variables were incubation temperature = 30.04 °C, Initial moisture = 5.46 mL and Inoculum size = 2.35 mL. The Model F-value of 7.12 implies the model is significant (Table not shown).

From the various solid substrates studied for the lovastatin production barley, long grain rice and sago were found to be the maximum lovastatin producing substrates. Wei et al. [2007] tested the lovastatin production by *A. terreus* using various solid substrates such as wheat bran, rice, soybean cake, rice husk and corn. Rice and wheat bran were found to be the suitable substrates for lovastatin production in SSF process and a maximum yield of 2.9 mg g^{-1} dry substrate was achieved [8]. Szakacs et al. [1998] reported the lovastatin production of 1500 µg g^{-1} dry weight of sweet sorghum pulp supplemented with cheese whey in SSF using *A. terreus* [9]. Gomes et al. [2005] have produced lovastatin by solid state fermentation using *Aspergillus flavipes* and used different solid substrates such as wheat bran, baggase, barley, soybean meal, gram bran, fruits wastes and their combination were tested and reported a maximum yield of 13.49 mg g^{-1} [10]. Gomes et al. [2005] reported that wheat bran was found to be the most suitable substrate for lovastatin production by *Aspergillus flavipes* [10]. Su et al. [2003] used long grain rice as the substrate for monacolin K (lovastatin) production using *Monascus* species [11]. *M. purpureus* was selected as the best strain for monacolin K production in solid culture. The long grain rice and wheat bran were used as solid substrate by Wei et al. [2007] for the lovastatin production using *M. purpureus*, but the productivity obtained in the present study was comparatively higher than the results reported by [8]. Panda et al. [2010] used co-culture of *M. purpureus* and *M. ruber* to produce red mold rice by solid state fermentation and a maximum lovastatin production of 2.3 mg g^{-1} was obtained [12]. Xu et al. [2003] obtained a lovastatin concentration of 6 mg g^{-1} in SSF of *M. ruber* by adding soybean powder, glycerol, sodium nitrate and acetic acid in the production medium [13]. Pansuriya et al. [2010] optimized the lovastatin production using RSM by a strain of *A. terreus* UV 1718 [14]. The response surface plots and their corresponding contour plots for the lovastatin production were generated by the design expert software and are shown in Fig. 2. Therefore in Fig. 2 (A), the contour plot is not elliptical and indicates that the interactions among the independent variables x_1 (incubation temperature) and x_2 (initial moisture content) found to be insignificant and also the *P*-value for this interaction is 0.784 which is greater than 0.05. The response surface plot and the contour plot shown in Fig. 2 (B) depict the effects of x_1 (incubation temperature) and x_3 (inoculum size) on lovastatin production at a fixed value of x_2 (initial moisture content). The interaction effects of the variables incubation temperature and inoculum size is found to be insignificant as the contour plot is not elliptical and the *P*-value for this interaction is 0.920 which is higher than 0.05. The response surface plot and the contour plot shown in Fig. 2 (C) illustrates the effect of interaction between the substrates x_2 (initial moisture content) and (inoculum size) at a fixed level of x_1 (incubation temperature) is statistically insignificant as the contour plot is not elliptical and the *P*-value is also greater than 0.05.

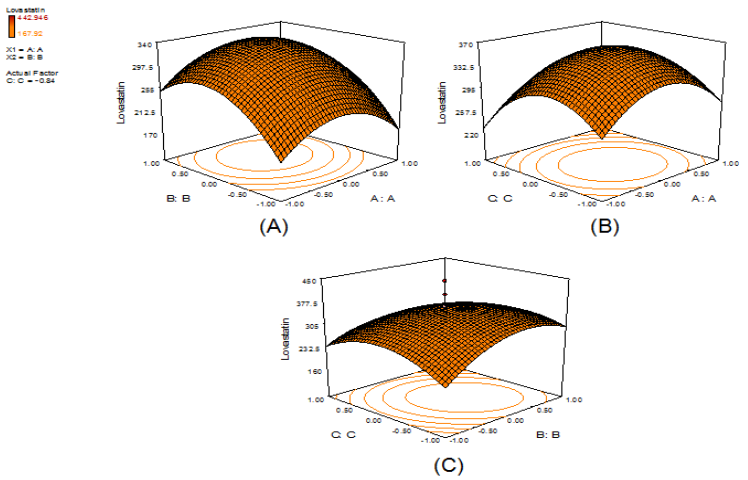


Fig. 2 Response surface plot and the contour plot showing the effects of interaction between the variables (A) Incubation temperature and Initial moisture content (B) Incubation temperature and Inoculum size (C) Initial moisture content and Inoculum size

For the production of lovastatin under solid-state fermentation using *A. terreus*, moisture is also a key parameter to control the growth of microorganisms and metabolite production. For the fermentation conducted in Erlenmeyer flasks, the methods to control the moistures of the solid-state materials are usually done by addition of water to the raw materials and then maintain high humidity level in the incubation room. Xu et al., [2004] the growths of *Monascus* in the solid-state culture with initial moistures being 61.11% and 64.35% were better than others, and the red rice granule is at loose status that favored the mass transfer (intake of oxygen and release of carbon dioxide) increased the lovastatin production [13]. From the mean lovastatin production and experimental range for initial moisture content, the maximum lovastatin production was observed at the middle level of 5 mL. But there was a decrease in the lovastatin production at highest factor setting of initial moisture content which showed that further increase in initial moisture content would decrease the lovastatin production, however the optimum concentration of the parameters x_1 (incubation temperature), x_2 (initial moisture content) and x_3 (inoculum size) can be determined by using the polynomial equation.

As the moisture level increased from 60 to 70%, air present in the void volume was replaced by water, resulting in decrease of available oxygen. Lovastatin content as well as biomass increased when the moisture level increased from 60 to 70%. This may be because of the adequate amount of oxygen and water present within the substrate bed to support good fungal growth and removal of metabolic heat. However, when the moisture level was increased from 80 to 90%, biomass as well as lovastatin content decreased. This is presumably due to poor oxygen availability caused by excessive replacement of air by water in the voidage volume. From the plot of the mean lovastatin production and the experimental range of x_3 (inoculum size), the maximum mean lovastatin production was observed at the middle value of 2.5 mL of inoculum size. Higher inoculum size decreased the lovastatin production marginally.

III. Conclusion

Fifteen different types of solid substrates were tested for production of lovastatin using *Aspergillus terreus*, and a maximum lovastatin of 569.55 mg per g of dry solids was obtained from green peas. Green pea was found to be the best substrates for the lovastatin production via SSF process than the remaining substrates. The adequacy of the predicted model and to confirm the predicted response from the combination of the substrates, validation was carried out by additional independent experiments at the suggested optimal conditions. The validation was carried out in 250 mL Erlenmeyer flask under optimum combination of the substrates predicted by the polynomial model. The lovastatin production of 379.63 mg g^{-1} was obtained which is found to be higher than the predicted lovastatin production of 365.87 mg g^{-1} at 7 days of fermentation validating the proposed model.

References

[1]. M. Manzoni, M. Rollini, S. Bergomi, and V. Cavazzoni, "Production and purification of statins from *Aspergillus terreus* strains," Biotechnol. Tech., 1998, vol. 12, pp. 529-532.

[2]. L.S. Lai, T.H. Tsai, T.C. Wang, and T.Y Cheng, "The influence of culturing environments on lovastatin production by *Aspergillus terreus* in submerged culture." Enzyme Microbial. Technol. 2005, vol. 36, pp. 737-748.

[3]. Y.N. Chang, J.C. Huang, C.C. Lee, I.L. Shih, and Y.M. Tzeng, "Use of response surface methodology to optimize medium for production of lovastatin by *Monascus ruber*. Enzyme Microb Technol 2002; 30: 889-894.

[4]. J.A. Tobert, "New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl coenzyme A reductase." Circulation, 1987, vol. 76, pp. 534-538.

[5]. A.W. Albert, "Discovery, biochemistry and biology of lovastatin." Am. J. Cardiol., 1988, vol. 62, pp. 10-15.

[6]. A. Endo, D. Komagata, and H. Shimada, "Monacolin M, A new inhibitor of cholesterol biosynthesis." J. Antibiot., 1986, vol. 39, pp. 1670-1673.

[7]. J.B. Gonzalez, J.G. Banos, and A.A. Covarrubias, "Lovastatin biosynthetic genes of *Aspergillus terreus* are expressed differentially in solid state and in liquid submerged fermentation." Appl. Microbiol. Biotechnol., 2008, vol. 79, pp. 179-186.

[8]. P.L. Wei, Z.N. Xu, and P.L. Cen, **"**Lovastatin production by *Aspergillus terreus* in solid state fermentation." J. Zhejiang. Univ. Sci. 2007, vol. 8, pp. 1521-1526.

[9]. G. Szakacs, G. Morovjan, and R.P. Tengerdy, "Production of lovastatin by a wild strain of *Aspergillus terreus*." Biotechnol. Lett. 1998, vol. 20, pp. 411-415.

[10]. H.R. Valera, J. Gomes, S. Lakshmi, R. Gururaja, S. Suryanarayan, and D. Kumar, "Lovastatin production by SSF using *Aspergillus flavipes*." Enzyme Microb. Techol. 2005, vol. 37, pp. 521-526.

[11]. Y.C. Su, J.J. Wang, T.T. Lin, and T.M. Pan, "Production of the secondary metabolites *c*-aminobutyric acid and monacolin K by *Monascus*." J. Ind. Microbiol. Biotechnol. 2003, vol. 30, pp. 41-46.

[12]. B.P. Panda, S. Javed, and M. Ali, "Optimization of fermentation parameters for higher lovastatin production in red mold rice through Co-Culture of *Monascus purpureus* and *Monascus rubber*." Food Bioprocess Technol. 2010, vol. 3, pp. 373-378.

[13]. B.J. Xu, Q.J. Wang, X.Q. Jia, and C.K. Sung, "Enhanced lovastatin production by solid state fermentation of *Monascus rubber*." Biotechnol. Bioproc. Eng. 2005, vol. 10(1), pp. 78-84.

[14]. R.C. Pansuriya, and R.S. Singhal, "Response surface methodology for optimization of production of lovastatin by solid state fermentation." Brazilian Journal of Microbiology, 2010, vol. 41, pp. 164-172.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Packed Entrapped Microbial cells for Treatment of Simulated Textile Wastewater

S.Rengadurai[1*], B.Preetha[2]
[1]Assistant Professor, [2]Associate Professor,
Department of Chemical Engineering
Annamalai University
Annamalainagar, Tamil Nadu- 608002
INDIA
rengss97@yahoo.co.in

***Abstract**: Treatment of waste water from textile industries poses a threat to the environment and bio treatment with white rot fungs Trametus hirsuta seems to be a very good option. In this study simulated textile waste water with pH 9.9 was used to treat by 0.2cm Na alginate immobilized Trametus hirsuta beads as the packing materials in packed bed reactor. The reactor was operated continuously at different volumetric flowrates (0.026ml/s, 0.057ml/s,0.102ml/s,0.204ml/s and 0.408ml/s),and different initial COD for simulated textile waste water concentrations(775mg/l, 1550mg/l, 2325mg/l and 3100mg/l). The maximum COD removal efficiency of 71.2% was obtained for low influent COD of 775mg/l simulated textile waste water concentration at the volumetric flow rate of 0.026ml/s (32 min HRT).*

***Keywords**: Trametes hirsuta, Immobilized beads, Simulated Textile Waste Water,Hydralic Retention Time (HRT), COD*

I. Introduction

Textiles dyes are poly-aromatic molecules that give a permanently colour to materials like textile fabrics. In the textile industry, up to 200,000 tons of these dyes are lost to effluents every year during the dyeing and finishing operations, due to the inefficiency of the dyeing process [1]. Unfortunately, most of these dyes escape conventional wastewater treatment processes and persist in the environment as a result of their high stability to light, temperature, water, detergents, chemicals, soap and other parameters such as bleach and perspiration [2]. The presence of very low concentrations of dyes in effluent is highly visible and undesirable [3]. Most fibre-reactive azo dyes are used for dyeing cellulosic materials, such as cotton, and are a major source of dye waste in textile effluents. Auxiliary chemicals such as solvents, detergents, dispersants and wetting agents that are used to aid in the dyeing process add to the treatment complexity of textile wastewaters [4]. Although manufacturers have made considerable efforts to improve dye fixation, the reduction achieved in residual dye concentration in waste effluents has not been sufficient [5]. In particular, the discharge of dye-containing effluents into the water environment is undesirable, not only because of their colour, but also because many of dyes released and their breakdown products are toxic, carcinogenic or mutagenic to life forms mainly because of carcinogens, such as benzidine, naphthalene and other aromatic compounds[6,7]. Without adequate treatment these dyes can remain in the environment for a long period of time. Government legislation is forcing textile industries to treat their waste effluent[8]. Therefore, the removal of colour from textile and dyestuff manufacturing industry wastewater represents a major environmental concern. In this study stimulated textile effluent biodegradation is attempted using different volumetric flowrate and different initial effluent concentrations in upflow packed bed reactor. In this reactor Na alginate immobilized *Trametes hirsuta* beads as the packing materials.

II. Materials and methods

Microorganism and growth conditions

A pure strain of *Trametes hirsuta* (MTCC-136), a white-rot fungi in lyophilized is procured from Institute of Microbial Type Culture Collection Centre, Chandigarh, India. The fungal culture was grown on nutrient agar slant medium containing yeast extract, glucose and agar. Decolorization experiments are carried out in laccase production medium containing wheat bran flakes 4.5 g/l, glucose 1 g/l, ammonium chloride 0.25 g/l, thiamine

dichloride 0.05 g/l, potassium hydrogen phosphate 0.2 g/l, magnesium sulphate 0.05 g/l, calcium chloride 0.01 g/l, photasium chloride 0.05 g/l [9].

Preparation of immobilized cells

For immobilization of microorganism sodium alginate is used as a matrix. *T. hirsuta* suspension is transferred aseptically to a sterile centrifuge tube and centrifuged at 10,000rpm for 10min and the cells are suspended in 4% sodium alginate solution. The slurry is extruded as drops into 2% calcium chloride solution at room temperature in aseptic condition. The insoluble and stable immobilized alginate beads thus formed are hardened in the calcium chloride solution for 1h at room temperature and further used for the degradation studies. The size of the beads can be varied by changing the diameter of the syringe. Plain beads are prepared without the spore suspension and these are used as the reference beads.

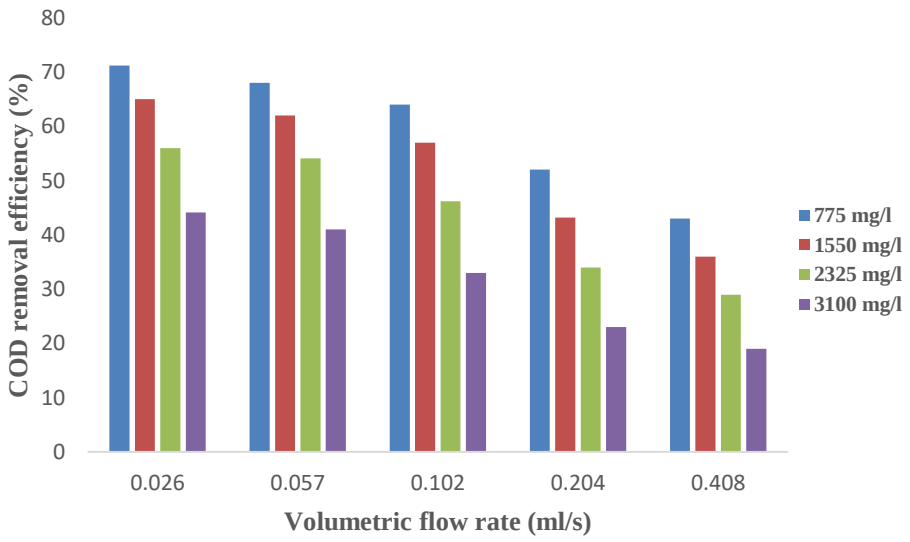
Preparation of Simulated textile wastewater

The simulated textile wastewater is prepared according to the procedure [10]. The composition of simulated textile wastewater used in this study are given in below. The mixture of reactive dyes included Reactive black 5 (RB5), Reactive red 111 (RR111) and Reactive red 251 (RR251) in simulated wastewater. This dye is normally used for dyeing cotton and woollen fabric. Starch solution is hydrolysed by mixing with 4% of sodium hydroxide at 80°C for 2 h and leaving it at room temperature overnight. The simulated wastewater also contains trace metal solution in g/l: $CaCl_2$:5 g/l), $MgSO_4$.$7H_2O$: 5. $FeCl_2$.$4H_2O$: 6, $COCl_2$: 0.88, H_3BO_3: 0.1, $ZnSO_4$.$7H_2O$: 0.1 $CuSO_4$: 0.05, $NiSO_4$. 1. $MnCl_2$: 5, $(NH4)_6$ MO_7O_{24} .$4H_2O$: 0.64 and $CaCl_2$.$2H_2O$: 5.

III. Results & Discussion

Biodegradation studies of simulated effluent by *Trametes hirsuta* are performed in a packed bed reactor, made up of perplex cylindrical column with 2.5 cm diameter and 10 cm height containing a working volume of 64 ml. Initially the column is filled with immobilized biomass beads on to the required bed height. Stimulated effluent solutions at known concentrations along with Laccase production medium are pumped into the bottom of the reactor using a peristaltic pump at a desired flow rate and the experiments are carried out at room temperature. Samples are collected from the outlet connected at the top of the reactor, at fixed intervals of time, for desired experimental conditions. The experiments are repeated for different conditions by changing the liquid flow rates, and initial stimulated effluent concentrations.

Figure 1 COD removal efficiency of simulated textile wastewater verses volumetric flow rate at different initial COD at thed_P of 0.2cm



Simulated textile wastewater with pH 9.9 are treated through up flow packed bed reactor with immobilized *T. hirsuta* beads. The system is operated continuously at different volumetric flow rates (0.026 ml/s, 0.057 ml/s, 0.102 ml/s, 0.204 ml/s and 0.408 ml/s), and different initial COD (775 mg/l, 1550mg/l, 2325 mg/l and 3100 mg/l). The particle diameter is 0.2cm. The Hydraulic Residence Time (HRT) maintained for the above study is 2 min, 4 min, 8 min, 16 min and 32 min. The results of volumetric flow rate, and initial COD on colour and COD

removal efficiencies are shown in Figure 1. COD removal from simulated textile wastewater even at pH=10 could be attributed by immobilized *T. hirsuta.* The same trend of decreasing removal levels with increasing initial COD during each operation is also observed in case of COD. A maximum COD removal of 68 % is obtained at low influent COD of 775 mg/l at a volumetric flow rate of 0.026 ml/s (32 min HRT). This residence time is sufficient for maximum decolorization and it provides enough time for partial mineralization of COD and inter metabolites. The reduction level is quite significant up to 3100 mg/l. It becomes obvious that the removal efficiency of immobilized *T. hirsuta* are lower in the case of simulated textile wastewater, due to the presence of dyeing auxiliaries. The results of these findings provide important insights into the development of effective treatment technology for the decolorization of textile dyes.

IV. Conclusion

Degradation of simulated textile effluent by Na alginate immobilized trametes hirsute is studied in a upflow packed bed reactor . the experimental results obtained indicated that for a particular volumetric flow rate,COD removal efficiency of simulated textile waste water decreases as influent COD decreases. A maximum COD removal efficiency of 71.2% is obtained for low influent COD of 775mg/l simulated textile waste water at at a volumetric flow rate of 0.026ml/s(32 min HRT).

References

[1] C.J.Ogugbue and T.Sawidis, "Bioremediation and Detoxification of Synthetic Wastewater Containing Triarylmethane Dyes by Aeromonashydrophila Isolated from Industrial Effluent". Biotechnology *Research International 2011; DOI 10.4061/2011/967925*.

[2] S.R.Couto , "Dye removal by immobilised fungi". *Biotechnology Advances 2009; 27(3) 227-235.*

[3] P.Nigam,G.Armou,I.M.Banat,D,Singh and R.Marchant. "Physical removal of dyes and solid state fermentation of dye-adsorbed agricultural residues".*Biores.Technol.2000.72,219-226.*

[4] C.J.Wang,S.Thiele, and J,M,Bollag."Intraction of 2,4,6 trinitrotoulene(TNT)4-amino-2,6-dinitro toluene with humic monomers in the presence of oxidative enzymes". *Arch Environ Contam.Texicol,2002,42:1-8.*

[5] J.Pierce."Color in textile effluents: the origin of the problem".*J.Soc.Dyers colorists,1994, 110,131-134*.

[6] D.Suteu,C. Zaharia, D. Bilba, A. Muresan, R. Muresan, and A.Popescu, "Decolorization wastewaters from the textile industry – physical methods, chemical methods". *IndustriaTextila*, 2009a, Vol.60, No.5, pp.254-263

[7] C.Zaharia, D.Suteu,A.Muresan, R.Muresan, and A.Popescu. "Textile wastewater treatment by homogenous oxidation with hydrogen peroxide". *Environmental Engineering and Management Journal, 2009,Vol.8, No.6, pp.1359-1369.*

[8] Rachanapatel and sumathisuresh, "decolorization of drimarene red dye using palladized bacteria.".2008, *J.hazardous material,156(1-3),308-316.*

[9] E.Abadulla, T.Tzanov, S.Costa, K.H.Robra, A.Cavaco-Paulo, and G.M.Gübitz "Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta". Appl. Environ. Microb. 2000.66: 3357-3362.*

[10] C.ONeill,F.R.Hawkes, D.L Hawkes, S.Estves, and S.J.Wilcox. "Anaerobic aerobic biotreatment of simulated textile effluent containg varied ratios of starch and azo dye".*Water Res 2000,34,2355-2361*.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

MICROBIAL PRODUCTION OF XYLITOL IN OPTIMIZED CONDITIONS FROM RICE STRAW

S.Ramesh*[1], S.Subhagar[2], R.Muthuvelayudham[3]
Department of Chemical Engineering, Annamalai University, Annamalainagar-608002, Tamilnadu, India.
Rameshgeetha008@gmail.com

Abstract: Microbial production of xylitol is being researched as an alternative method for xylitol production. The present study evaluated xylitol production from rice straw through batch fermentation. The pretreated hemicellose hydrolysate was fermented by *Candida guillermondii* in submerged fermentation. Screening and optimization were done using statistical methodology based on experimental designs. Various nutrients are designated and screened by Plackett-Burman design. Selected nutrients are optimized to maximize the xylitol production by Box-Behnken design of Response surface methodology (RSM). The optimum medium concentrations and operation conditions for maximum xylitol production are: KH_2PO_4- 3.42 g/L, yeast extract- 4.38 g/L, $MgSO_4.7H_2O$- 1.2 g/L, glucose– 10.42 g/L, temperature- 30.8 ^oC, substrate concentration- 2.93 g/L, pH- 7.18, agitation speed- 168 rpm and inoculum size- 3.41 mL. At this optimized medium concentrations and operation conditions the maximum xylitol production was found to be 0.71 g g^{-1}. These results show that under appropriate level of process variables, experimental analysis reveals that the maximum yield of xylitol for rice straw using *Candida guillermondii*.

Keywords*: Xylitol, Rice straw, Candida guillermondii, Optimization, RSM.*

I. Introduction

Lignocellulosic materials are the most abundant and in expensive material from vegetable residues and agro-industrial by-product could be used as potential high energetic or nutritious sources. The biotechnological approaches use this biomass as a micro-organism substrate for production of several useful feed stocks. Among these products available from lignocelluloses-derived sugars, xylitol has received much attention. It found increasing use in food and pharmaceutical industries due to several advantages. It has outstanding organoleptic and anticariogenic properties [1]. Currently, xylitol market lies between 20 and 40 thousand tons per year with an economic value ranging between 90 and 340 million dollars. As a cheap raw material, agricultural wastes are considered as potential sources for its production.

Among microorganisms, yeast have been shown to possess some desirable properties has a potential xylitol producer [2], [3]. Therefore in the present study, yeast strain of species *Candida guillermondii* was selected for xylitol production. The current work is dedicated to find out the possibilities and optimal process conditions for maximum production of xylitol and the investigations includes the bioconversion of xylitol is influenced by the factors of various ingredients concentration in culture medium. So their screening and optimization study is very important. The Plackett-Burman screening design is applied for knowing the most significant nutrients enhancing xylitol production. Then, Box-Behnken design and central composite design (CCD) was applied to determine the optimum level of each of the significant nutrients and process variables respectively. Response surface methodology (RSM) is a mathematical and statistical analysis, which is useful for the modeling and analysis problems that the response of interest is influenced by several variables [4]. RSM was utilized extensively for optimizing different biotechnological process [5], [6].

II. Materials and methods

A. Microorganisms and maintenance

The yeast strain *Candida guillermondii* (NCIM 3124) was collected from National collection of industrial microorganisms, Pune, India. The lyophilized stock cultures were maintained at 4°C on culture medium supplemented with 20 g agar. The medium composition (g/l) was compressed of the following: Malt extract - 3.0; Yeast extract - 3.0; Peptone - 5.0; Glucose -10.0 and pH - 7. It is sub- cultured every thirty days to maintain viability.

B. Size reduction

Rice straw was collected from agricultural farms at Cuddalore, Tamilnadu, India. The collected raw material are dried in sunlight for 2 days, crushed and sieved for different mesh size ranging from 0.45 mm to 0.9 mm (20–40 mesh) and used for further studies. The rice straw containing (% w/w): cellulose- 43.5, hemicellulose- 22.0, lignin- 17.2, ash- 11.4 which used for xylitol production.

C. Acid hydrolysis

The pretreatment were carried out in 500 ml glass flasks. 2 g of rice straw in solid loading of 10% (w/w) mixed with 1% dilute sulfuric acid (w/w) and pretreated in an autoclave at 120 ˚C with residence time of 1 hour. The liquid fraction was separated by filtration and unhydrolysed solid residue was washed with warm water at 60 ˚C. The filtrate and wash liquid were pooled together.

D. Detoxification

Hemicellulose acid hydrolysate was heated at100 ˚C, and maintained for 15 min to reduce the volatile components. The hydrolysate were overlimed with solid $Ca(OH)_2$ up to pH 10, in combination with 0.1% sodium sulfite and filtered to remove the insoluble materials. The filtrate was adjusted to pH 7 with H_2SO_4. The water phase was treated with activated charcoal.

E. Activated charcoal treatment

Activated charcoal treatment is an efficient and economic method of reduction in the amount of phenolic compounds, acetic acid, aromatic compounds, furfural and hydroxymethylfurfural normally found in hemicellulosic hydrolysates. After centrifugation, the solutions were mixed with powdered charcoal at 5% (w/v) for 30 min and stirred (100 rpm) at 30 ˚C. The liquor was recovered by filtration, chemically characterized and used for culture media.

F. Fermentation Conditions

Fermentation was carried out in 250 ml Erlenmeyer flasks with 100 ml of pretreated rice straw hemicelluloses hydrolysate at pH 7. This is supplemented with different nutrients concentration for tests according to the selected factorial design and sterilized at 120 ºC for 20 min. After cooling the flasks to room temperature, the flasks were inoculated with 1 ml of grown culture broth. The flasks were maintained at 30 ºC under agitated at 200 rpm for 48 hours.

After the optimization of medium composition, the fermentation was carried out with different parameter levels (Table 1) with the optimized media for tests according to the selected factorial design. During the preliminary screening process, the experiments are carried out for 5 days and it was found that the maximum production was obtained in 48 hours. Hence experiments were carried out for 48 hours.

G. Analytical Methods

Xylitol concentrations were determined by high performance liquid chromatography [7].

H. Design of Experiment (DOE)

The RSM has several classes of designs, with its own properties and characteristics. Central composite design (CCD), Box–Behnken design and three-level factorial design are the most popular designs applied by the researchers. A prior knowledge with understanding of the related bioprocesses is necessary for a realistic modeling approach.

III. Results and Discussions

A. Optimization of process variables for the xylitol production by Candida guilluirmondii using rice straw

Under the optimum medium composition the experiments are carried out and the levels of process variables temperature, substrate concentration, pH, agitation speed and inoculum size and the effect of their interactions on xylitol production are determined by central composite design of RSM. Five process variables have been assessed at 5 coded levels. The design matrix of experimental results by tests planned according to the 50 full factorial designs and the central point is repeated eight times. The predicted and observed responses along with design matrix and the results are analyzed by ANOVA.

Table I Ranges of variables used in RSM for the xylitol production

S.No	Variables	Code	Levels				
			-2.37	**-1**	**0**	**1**	**2.37**
1	Temperature (ºC)	A	20	25	30	35	40
2	Substrate Concentration (g/L)	B	1	2	3	4	5
3	pH	C	6	6.5	7	7.5	8
4	Agitation speed (rpm)	D	50	100	150	200	250
5	Inoculum size (mL)	E	1	2	3	4	5

The second order regression equation provided the levels of xylitol production as a function of temperature, substrate concentration, pH, agitation speed and inoculums size, which can be presented in terms of coded factors as in the following equation:

$$Y = 0.70 + 2.560E\text{-}003A + 7.256E\text{-}003B + 0.050C + 0.028D + 0.050E - 0.033AB + 0.032AC + 0.026AD - 0.015AE - 2.188E\text{-}003BC - 5.312E\text{-}003BD - 0.018BE - 0.018CD - 0.020CE - 9.063E003DE - 0.061A^2 - 0.041B^2 - 0.051C^2 - 0.045D^2 - 0.055E^2$$

Where Y is the xylitol yield (g g^{-1}), A, B, C, D and E are temperature, substrate concentration, pH, agitation speed and inoculums size respectively.

ANOVA for the response surface is shown in Table 2. The model *F*-value of 15.18 implies the model is significant. There is only a 0.01% chance that a "Model *F*-value" this large could occur due to noise. Values of "prob > *F*" less than 0.05 indicate model terms are significant. Values greater than 0.1 indicates model terms are not significant. In the present work, linear terms of C, D, E and all the squares effects of A, B, C, D, E and the combination of AB, AC, AD and CE are significant for xylitol production. The co-efficient of determination (R^2) for xylitol production is calculated as 0.9128, which is very close to 1 and can explain up to 91.00% variability of the response. The predicted R^2 value of 0.7733 is in reasonable agreement with the adjusted R^2 value of 0.8527. An adequate precision value greater than 4 is desirable. The adequate precision value of 13.030 indicates an adequate signal and suggests that the model can be to navigate the design space.

The interaction effects of variables on xylitol production are studied by plotting 3D surface curves against any two independent variables, while keeping another variable at its central (0) level. The 3D curves of the calculated response (xylitol yield) and contour plots from the interactions between the variables are obtained. In CCD the Figure 1 shows the dependency of xylitol on temperature and substrate concentration. The xylitol production increased with increase in temperature to about 30.8 ^oC and thereafter xylitol production decreased with further increase in temperature. The same trend is observed in Figures. This is evident from below Figures shows the dependency of pH, substrate concentration, agitation speed and inoculum size on xylitol production. The optimal operation conditions of temperature, substrate concentration, pH, agitation speed, inoculum size for maximum xylitol production are determined by response surface analysis and also estimated by regression equation. The predicted results are shown in Table 2. The predicted values from the regression equation closely agreed with that obtained from experimental values.

Figure 1 3D Plot showing the effect of temperature and substrate concentration on xylitol yield

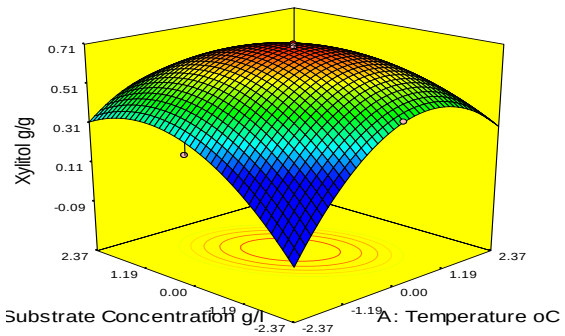


Figure 2 3D Plot showing the effect of temperature and pH on xylitol yield

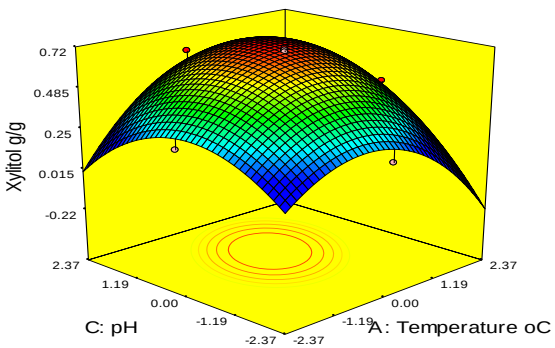


Figure 3 3D Plot showing the effect of temperature and agitation speed on xylitol yield

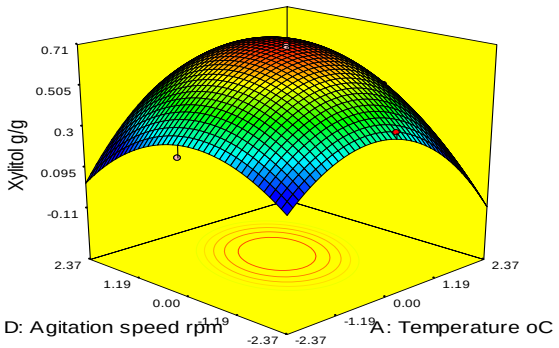


Figure 4 3D Plot showing the effect of pH and inoculum size on xylitol yield

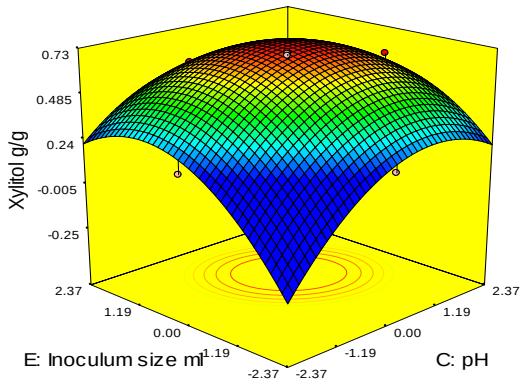


Table 2 Analyses of variance (ANOVA) for response surface quadratic model for production of xylitol

Source	Sum of square	df	Mean square value	F-value	P-value
Model	0.87	20	0.043	15.18	< 0.0001
A-Temperature ^{o}C	2.83E-04	1	2.83E-04	0.099	0.7552
B-Substrate Concentration g/L	2.28E-03	1	2.28E-03	0.8	0.3797
C-pH	0.11	1	0.11	37.14	< 0.0001
D-Agitation speed rpm	0.035	1	0.035	12.2	0.0016
E-Inoculum size ml	0.11	1	0.11	38.46	< 0.0001
AB	0.036	1	0.036	12.51	0.0014
AC	0.032	1	0.032	11.14	0.0023
AD	0.022	1	0.022	7.53	0.0103
AE	7.50E-03	1	7.50E-03	2.62	0.1161
BC	1.53E-04	1	1.53E-04	0.054	0.8187
BD	9.03E-04	1	9.03E-04	0.32	0.5785
BE	0.01	1	0.01	3.55	0.0696
CD	0.01	1	0.01	3.55	0.0696
CE	0.013	1	0.013	4.62	0.0402
DE	2.63E-03	1	2.63E-03	0.92	0.3457
A^2	0.2	1	0.2	71.58	< 0.0001
B^2	0.094	1	0.094	33.03	< 0.0001
C^2	0.14	1	0.14	50.46	< 0.0001
D^2	0.11	1	0.11	38.94	< 0.0001
E^2	0.17	1	0.17	57.72	< 0.0001
Residual	0.083	29	2.86E-03		
Lack of Fit	0.083	22	3.76E-03	91.49	< 0.0001
Pure Error	2.88E-04	7	4.11E-05		
Cor Total	0.95	49			

IV. Conclusions

The nutrients like KH_2PO_4, yeast extract, $MgSO_4.7H_2O$ and glucose are found to be significant for xylitol production by *Candida guillermondii*. The optimum medium concentrations and operation conditions for maximum xylitol production are: KH_2PO_4- 3.42 g/L, yeast extract- 4.38 g/L, $MgSO_4.7H_2O$- 1.2 g/L, glucose- 10.42 g/L, temperature- 30.8 ^oC, substrate concentration- 2.93 g/L, pH- 7.18, agitation speed- 168 rpm and inoculum size- 3.41 mL. At this optimized medium concentrations and operation conditions the maximum xylitol production was found to be 0.71 g g^{-1}. The results show a close concordance between the expected and obtained production level.

.

References

[1] P. Shen, F. Cai, A. Nowicki, J. Vincent and E.C. Reynolds, "Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopepticle-amorphous calcium phosphate". *Journal of Dental Research*, 2001, vol. 80, pp. 2066-2070.

[2] J. M. Domınguez, C. S. Gong, and G. Tsao, "Production of xylitol from D-xylose by Debaromyces hansenii". Applied Biochemistry and Biotechnology, 1997, vol. 63–65, pp. 117–127.

[3] A. Converti, P. Perego, A. Sordi and P. Torre, "Effect of starting xylose concentration on the microaerobic metabolism of Debaromyces hansenii: The use of carbon material balance". Applied Biochemistry and Biotechnology, 2002, vol. 101, pp. 15–29.

[4] D. C. Montgomery, Design and Analysis of Experiments. John Wiley and Sons, New York, 2001.

[5] W. Li, W. Du, D. H. Liu, "Optimization of whole cell-catalyzed methanolysis of soybean oil for biodiesel production using response surface methodology". *Journal of Molecular Catalysis B: Enzymatic*, 2007, vol. 45, pp. 122–127.

[6] B. J. Naveena, M. Atlaf, G. Bhadnah K. Reddy, "Direct fermentation of starch to 1(+) lactic acid in SSF by *Lactobacillus amylophilus GV6* using wheat bran as support and substrate: medium optimization using RSM". *Process Biochemistry*, 2005, vol. 40, pp. 681–690.

[7] S. Ramesh, R. Muthuvelayudham, R. Rajesh Kannan, "Stastical optimization of process variables for corncob hemicellulose hydrolysate to xylitol by Debaryomyces hansenii var hanseii". International Journal of ChemTech Research, 2013, vol. 5, pp.186-196.

Acknowledgments

The authors wish to express their gratitude for the support extended by the authorities of Annamalai University, Annamalainagar, India in carrying out the research work in Bioprocess laboratory, Department of Chemical Engineering. I assure that this research work is not funded by any organizations.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

CHEMICAL COMPOSITION OF ETHANOL EXTRACT OF *DIOSYPROS MONTANA* ROXBERG LEAVES AND ITS BIOLOGICAL ACTIVITES

Saranya.K.S., Karthigai Priya. M and Poonkodi. K
Post Graduate Department of Chemistry
Nallamuthu Gounder Mahalingam College, Pollachi, Tamilnadu, India-642001
E.Mail.ID- poonks.che@gmail.com

Abstract*: The present study examines the nature of phytoconstituents , GC-MS analysis for determining the chemical compostion and in vitro anticancer activity of ethanol extract of Diospyros Montana Roxberg leaves. The preliminary phytochemical screening conducted by usual chemical tests indicated the presence of alkaloids, carbohydrates, fatty acids, flavonoids, phenols, saponins and tannins. GC-MS analysis of the ethanol extract of D. montana. leaves revealed the presence of 14 compounds. The important compounds was Squalene (1.86), phytol (6.09), Adipic acid (2.95), fenchyl acetate (0.69), octadecanoic acid (1.78), 1-octadecane (1.53), nonadecane (1.46), 2-cyclohexen-1-one (1.31) and other compounds. The in vitro anticancer activity was tested against MOLT-3 cell line. The ethanolic extract of leaves of D.montana showed significant dose dependent anticancer activity. The IC_{50} value was determined as 57.54 µl. This is the first kind of report to test the ethanol extract of D.Montana roxb. for anticancer activity.*

Keywords*: Diospyros Montana, GC-MS, in vitro anticancer activity, MTT assay and MOLT-3cell line*

I. Introduction

Diospyros Montana Roxburg belongs to the family Ebenaecea. It is also known as Vakanai in Tamil, Manjaka in Malayalam, Mottled ebony in English. *D. Montana*. is globally distributed in dry evergreen to dry deciduous forests of Indo-Malesia to Austarlia, including the Western Ghats (Singh, 2017). The various parts of *D. Montana* was traditionally used to treat fever, puerperal fever, neuralgia, pleurisy, pneumonia, menorrhagia and dysuria. The fruits of *D. Montana* are applied externally to boil. It is used in the treatment of asthma, abdominal pains, dysentery, leprosy and to cure menstrual troubles (Narayan *et al*., 1978). The bark paste is given with boiled rice for swelling of the body. The extract of fresh stem bark is taken for stomach ache. It is also used as veterinary medicine. The bark is grounded with salt and applied over wounds and also for the broken part of a limb. The stem bark is crushed and given for dysentery. However, the fruits are poisonous when taken orally. The crushed stem bark, leaves and fruits are used as fruit poison (Khare, 2007; Quattrocchi, 2012). The pharmacological activity of *D. Montana* includes analgesic activity, anti-inflammatory activity, anti filarial activity, and free radical scavenging activity (Sudipta Das *et al*., 2011; Rakesh Kumar *et al*., 2009; Puri, 2010).

Cancer is a big threat to the humans today. There are several medicines available for the treatment of cancer, but the drug from plant origin is most welcomed due to its less side effect or nil toxic and cost effective. From the literature survey the presence of free radical scavenging activity is revealed, but the anticancer activity is not reported so far. Hence, the objective of the present work is to screen the nature of preliminary phytoconstituents, the chemical composition was determined by GC-MS and to evaluate the extent of anticancer activity of ethanol extract of *D.Montana* roxb. Leaves using MOLT-3 cancer cell line by MTT assay.

II. Materials and Methods

Plant Material

The leaves of *Diospyros Montana Roxb*.were collected locally in Pollachi, Coimbatore, Tamil Nadu, South India. The plant sample was authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu.

Extraction process

The collected leaves (500g) were dried in shade for 10 days. Air dried leaves of *D. montana* was coarse powdered and was defatted with ethanol by cold maceration for three days with occasional shaking. The extract was then subjected to vacuum distillation and was concentrated to yield a brownish residue (5 g).

Preliminary Phytochemical Screening

The ethanolic extract of leaves of *D. Montana* was subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present (Rakesh Kumar *et al.,* 2009; Puri, 2010; Mangathayaru., 2013).

GC-MS Analysis

GC-MS analysis of the ethanol extract of *D. Montana* Roxb.was carried out in thermo GC-trace ultraversion: 5.0 coupled with thermo MS DSQ II instrument. The compounds were separated on DB-35, MS capillary DSQ II instrument. The MS capillary standard non-polar column (0.25mm), film thickhness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70˚C and held for 2 minutes and the temperature of the oven was raised to 260˚C for 10 minutes, raised 6˚C per minute and the final temperature was 300˚C for 10 minutes.

Identification of phytoconstituents

The components were identified by comparison of their mass spectra with those of National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

***Invitro* anticancer activity by MTT assay**

The *in vitro* anticancer activity of ethanol extract of *D. montana* leaves were tested against MOLT-3 cancer cell line. MOLT-3 cancer cell is the adult T acute lymphoblastic leukemia cell line. In MTT assay the cell viability of cancer cells after incubation with different concentration of the leaf extract was examined.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37˚C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader (T.Mosmann., 1983). The % cell inhibition was determined using the formula

% cell inhibition = 100 – [Abs (sample) /Abs (control)] * 100

IC_{50} value was determined by Nonlinear regression graph was plotted between % cell inhibition and log_{10}concentration.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

From the chemical tests, the nature of phytochemical constituents present in the ethanolic extract of *D.Montana* was given in the table 1. The result agrees well with the literature report (Rakesh Kumar *et al.,* 2009; Puri, 2010; Mangathayaru., 2013, Maridass et al., 2008).

Table 1: Preliminary phytochemical screening of ethanolic extract of *D. montana* leaves.

S.No	Phytoconstituents	phytoconstituent
1.	Alkaloids	++
2.	Carbohydrates	+
3.	Fatty acids	+
4.	Flavanoids	++
5.	Phenolic compound	+
6.	Saponins	+
7.	Steroids	++
8.	Tannins	+

++ -Highly presence of phytochemicals, + presence of phytochemicals.

GC-MS Analysis

The chemical constituents especially volatile compouds, long chain htdrocarbons, branched hydrocarbons etc., can be easily identified by GC-MS chromatogram. GC-MS analysis of the ethanol extract of *D. montana* leaves revealed the presence of 14 chemical constituents. The compounds were characterized and identified by comparing the GC-MS spectra of the constituents matching with the NIST library along with the parameters which includes the retention time, peak area, molecular weight. Among the 14compounds identified the most prevailing compound is ethyl linoletae (0.67) and the minor compounds include phytol (6.09), octadecanoic acid (1.78), hexadecanoic acid (1.93), 4-methyl-N-hydroxyphthalamide (1.00), Nonadecane (1.46), squalene (1.86), fenchyl acetate (0.69), 2-cyclohexen-1-one (1.31), adipic acid (2.95), (2-(2'-formylethyl) benzaldehyde (1.63), 1-octadecene(1.53), 3-tetradecene (1.48). The GC-MS chromatogram was shown in the figure 1 and the characterized compounds were shown in table 2.

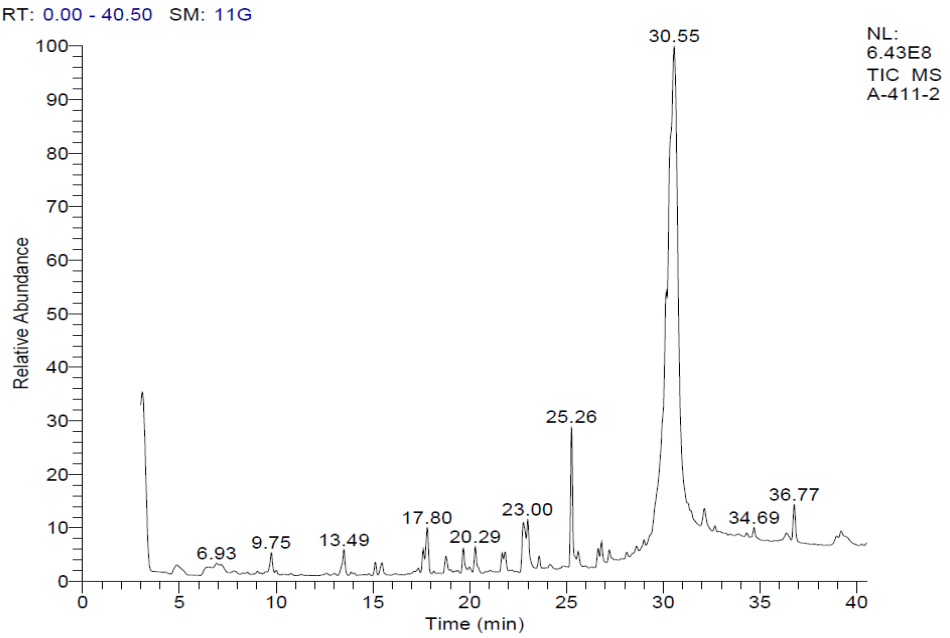


Figure 1: GC-MS chromatogram of ethanolic extract of *D. Montana* roxb.leaves

Table 2: Compounds present in ethanol extract of *D. montana* roxb.

S.No	RT	% of compound	Compound
1	36.77	1.86	Squalene
2	36.36	0.69	Fenchyl acetate
3	27.21	0.67	Ethyl linoleolate
4	26.81	1.78	Octadecanoic acid, ethyl ester
5	25.26	6.09	Phytol
6	23.59	0.50	Heneicosane
7	23.00	1.93	Hexadecanoic acid, ethyl ester
8	22.77	2.95	Adipic acid, 4-methoxy phenyloctyl ester
9	21.81	1.64	2-(2'-formyl ethyl)benzaldehyde
10	20.29	1.31	2-cyclohexen-1-one
11	19.68	1.46	Nonadecane
12	18.78	1.00	4-methyl-N-hydroxyphthalimide
13	13.49	1.53	1-Octadecene
14	9.75	1.48	3-Tetradecene

***In vitro* anticancer activity by MTT assay**

MTT assay is a reliable method for screening the anticancer activity by measuring the cell viability. The present study examined the *in vitro* anticancer activity of ethanol extract of *D. montana* leaves using MOLT-3 cell line against MTT assay. The incubation with different concentration of the extract affected the viability of MOLT-3 cell line in dose dependent pattern. The extract was diluted to 5µl, 25µl, 50µl, 75µl, 100µl. The cell viability of MOLT-3 cancer cells after incubation in different concentration of ethanol extract of leaves of *D. montana* was depicted in figure2. The IC_{50} value of cell viability was 57.54µl. The result revealed that the ethanol extract of *D. Montana* showed potent *in vitro* anticancer activity against the MOLT-3 cancer cell line. So far There is no report in the literature on *in vitro* anticancer activity ethanol extract of leaves of *D. Montana* plant.

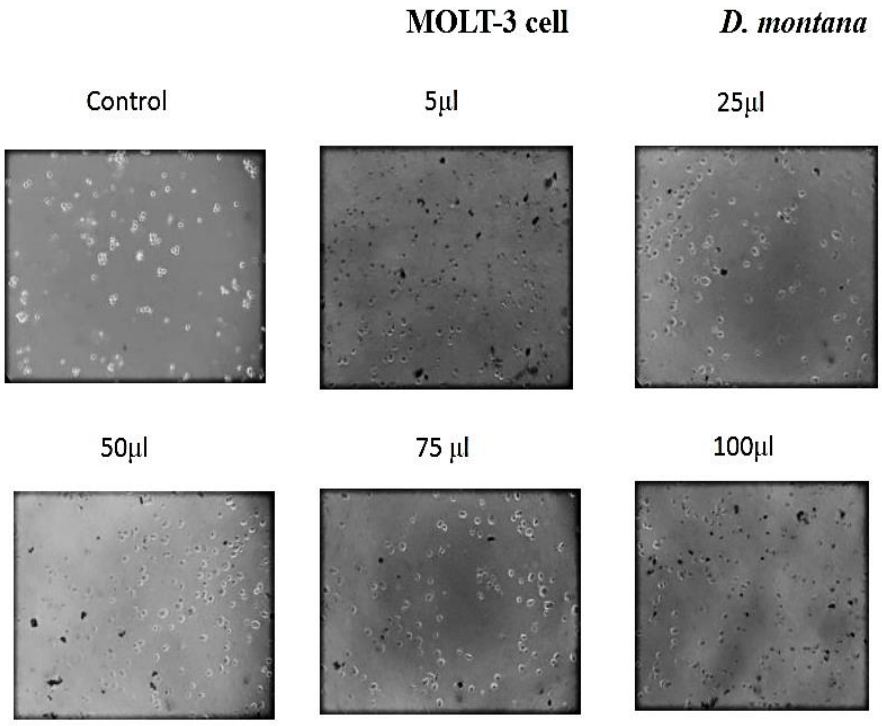
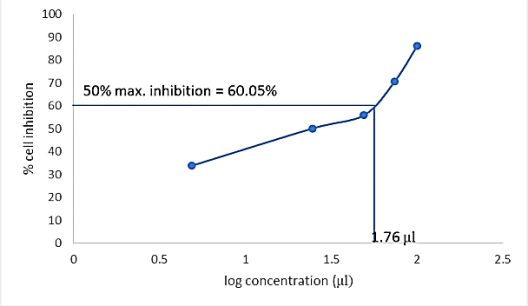


Figure 2: The cell viability of MOLT-3 cancer cell line after incubation in different concentration of ethanol extract of leaves of *D. montana.*

Table 3 : % Cell inhibition in different concentration of ethanol extract of leaves of *D. montana.*

S.No	Concentration (µl)	% Cell inhibition
1	5	33.9
2	25	50.1
3	50	55.9
4	75	70.7
5	100	86.2

Figure 3: Plot of log concentration Vs % of incubation



CONCLUSION

The present study revealed that the ethanol extract of *D. montana* leaves has significant anticancer activity against MOLT-3 Cell line, this may be due to the active phytoconstituents present in the plant material which was confirmed in preliminary study. The isolation of active phytoconstituents is under process.

REFERENCES

1. K. Singh. Wild relatives of Cultivated Plants in India: A reservoir of alternative genetic resources and more. 1st ed. Singapore: Springer; 2017.
2. G. K. A. S. S. Narayan, L. R. Row and P. Satyanarayana, Curr. Sci., 47, 345 (1978).
3. Khare CP. Indian medicinal Plants: An illustrated Dictionary. 1st ed. New York USA: Springer; 2007.
4. U. Quattrocchi. CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms and etymology. 1st ed. New York: CRC Press; 2012.
5. Sudipta Das, Pallab K, Goutam Pramanik, Siva P Panda, Samit Bera. Evaluation of analgesic and anti-inflammatory activity of *Diospyros Montana*. Afr J Trad Complement Altern Med. 2011; 8(1):11-14.
6. Rakesh Kumar, Waseem Rizvi MD, Anil Kumar MD, Mehtab Parveen. Antifilarial potential stem wood of *Diospyros Montana*. Pharmacology online. 2009; 3:285-291.
7. Rakesh Kumar, Mehtab Praveen, Anil Kumar, Waseem Rizvi MD. Screening of fruit of *Diospyros Montana* for antifilarial activity. Journal of Natural Remedies. 2009; 9(1):56-61.
8. Abhijeet Puri. Preliminary phytochemical investigation and free radical scavenging activity of *Diospyros Montana*. Indian Drugs. 2010; 47 (12):48-52.
9. Mangathayaru K. Pharmacognosy: an Indian perspective. Delhi: Dorling Kindersley (India) Pvt. Ltd; 2013.
10. T. Mosmann ., Journal of lmmunological Methods, 65 (1983) 55-63
11. Maridass M, Ghanthikumar S, Raju G. Preliminary phytochemical anlaysis of Diospyros species. Ethonobotanical leaflets. 2008;12 :868-72.
12. K. Kokila, E. Nagaraj, V. Sujatha. *Diospyros montana* leaf extract - mediated synthesis of selenium nanoparticles and its biological importance. New Journal of Chemistry. 2017:1-27.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

EVALUATION OF PHYTOCONSTITUENTS OF *FERONIA LIMONIA* AND ITS *IN VITRO* CYTOTOXICITY

Karthigai Priya M, Saranya. K .S and Poonkodi. K
Post Graduate Department of Chemistry
Nallamuthu Gounder Mahalingam College, Pollachi, Tamilnadu, India-642001
E.Mail.ID- poonks.che@gmail.com

Abstract*: For the continuation of phytochemical research and isolation of active constituents, the present investigation evaluated the preliminary phytochemical screening and in vitro cytotoxicity of ethanol extract of the tree Feronia limonia which belongs to family Rutaceae and have many traditional uses. Phytochemical screening revealed the presence of flavonoids, steroids, coumarins, phenolics and terphenoids. The ethanol extract of F. limonia was subjected to GC-MS analysis, a total of 17 compounds were identified and the major compounds of F. limonia were 6-methyl-1-(1-naphthyl)dihydrouracil (9.12), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (5.99), Eicosane (5.08), Nonadecane (5.52), Pentadecane, 2,6,10,14-tetramethyl (5.05), Eicosane (5.08) and other compounds. Invitro cytotoxicity was performed using MOLT-3 cancer line by MTT assay, which showed IC_{50} value of 63.24µl. The ethanol extract of this plant has significant cytotoxicity against tested cell line.*

Keywords: *F. limonia, MOLT-3, coumarins, flavonoids and MTT assay.*

I.Introduction

Feronia limonia belongs to the family Rutaceae has many synonyms include *Feronia elephantum* Correa *and Limonia acidissima* Linn. is a small tree found in India and South east Asia. The plant has a long history of traditional usage, tocure asthma, tumors, opthalmia, leucorrhoea, scurvy and sore throat. The fruit pulp is sour, sweet, edible, stimulant and astringent. The bark is aromatic, having cool sensation and is useful in vitiated conditions of pitta. The bark is occasionally prescribed for biliousness and useful in liver disease, The fruit is used in India as a liver and cardiac tonic, and when unripe, as an astringent means of halting diarrhoea and dysentery and effective treatment for hiccough, sore throat and diseases of the gums. The pulp is poulticed onto bites and stings of venomous insects, as is the powdered rind. Juice of young leaves is mixed with milk and sugar candy and given as a remedy for biliousness and intestinal troubles of children. The powdered gum, mixed with honey, is given to overcome dysentery and diarrhea in children. Oil derived from the crushed leaves is applied onto the itch and the leaf decoction is given to children as an aid to digestion. Leaves, bark, roots and fruit pulp are all used against snakebite. The bark is chewed with that of Barringtonia and applied on venomous wounds (Nandkarni, 1976 ; Kirtikar and Basu, 1998; Dymock *et al*., 2005).

The different parts of the plant have been investigated by several workers and found to contain coumarins, furanocoumarins, lignans, alkaloids, steroids and flavonoids. The unripe fruits contain Stigmasterol (Anonymous). The pharmacological studies, like antioxidant, antibacterial Activity (Garg, 2001; Momin *et al*., 2013; Nithya and Saraswathi, 2010; Mehta *et al*., 1983; Panda), anti tumor Activity (Saima *et al*, .2000) cytotoxicity (Sampandan and David., 2014; Sadia Shermin *et al*., 2012; Hossain, 2013), anti inflammatory (Ahamed *et al*., 2008) and hepatoprotective (Illango and Chitra, 2009)

Cancer is a dangerous disease producing abnormal cell growth which affects various parts of the body. Like different types of cancer, cancer curing synthetic drugs also available with side effects. The plant based drugs are tested against cancer since from ancient times, in this view every medicinal plant is tested for its anticancer potential. Our present study focused to evaluate preliminary screening and it's *in vitro* cytotoxic study of ethanol extract of *F.limonia* using MOLT-3 cancer cell line by MTT assay.

II. Materials and Methods

Plant material

Feronia limonia under the plant family Rutaceae was collected from Pollachi, Tamil Nadu, South India. The plant sample was authenticated by Department of Botany and the voucher specimens have been kept for further reference.

Extraction Process

The leaves of 500g of *F.limonia* were dried in shade for 10 days and tightly packed for further process. Air dried leaves of *F.limonia* was chopped into small pieces. The coarse material was subjected to maceration and Soxhlet extraction by using different solvents used based on their increasing order of polarity, i.e. petroleum ether. The defatted plant leaves were again extracted with ethanol using Soxhelt apparatus. The extract was subjected to vacuum distillation and was concentrated to yield brownish residues of 80g.

Preliminary phytochemical screening

Ethanol extracts of *F.limonia* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it. The test for steroids, phenol, tannis, fatty acid, alkaloids, flavonoids, saponins and coumarins was performed.

GC-MS analysis

GC-MS analysis of the phytochemical of *F.limonia* carried out using thermo GC – trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. The compounds were separated on DB-35, MS capillary DSQ II instrument. The MS capillary standard non-polar column (0.25mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70˚C and held for 2 minutes and the temperature of the oven was raised to 260˚C for 10 minutes, raised 6˚C per minute and the final temperature was 300˚C for 10 minutes.

Identification of Phytoconstituents

The components were identified by comparison of their mass spectra with those of the National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

***In vitro* cytotoxicity by MTT assay**

The *in vitro* anticancer activity of ethanol extract of *F.limonia* leaves were tested against the MOLT-3 cancer cell line. MOLT-3 cancer cell is the adult T acute lymphoblastic leukemia cell line. In MTT assay the cell viability of cancer cells after incubation with different concentration of the leaf extract was examined. 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37˚C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader (T.Mosmann., 1983). The % cell inhibition was determined using the formula

% cell inhibition = 100 – [Abs (sample)/Abs (control)] * 100

IC_{50}value was determined by Nonlinear regression graph was plotted between % cell inhibition and log_{10}concentration.

III. RESULT AND DISCUSSION

Preliminary Phytochemical Screening

Ethanol extracts of *F. limonia* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituent present in it. alkaloids, terpenoids ,steroids, phenolics and excess amount of coumarins were present in the ethanol extract of leaves of *F. limonia* which was shown in table.1.

Table 1: Phytochemical constituents of *F. limonia*

S.No	Phytoconstituents	Phytoconstituents
1	Fatty acid	-
2	Alkaloids	+
3	terpenoids	+
4	Flavonoids	+
5	Steriods	+
6	Saponins	-
7	carbohydrate	-
8	Phenolics	+
9	Comarins	+

+ presence of phytochemicals, - Absence of phytochemicals

GC-MS ANALYSIS

The ethanol extract of *F. limonia* was subjected to GC-MS analysis, a total of 17 compounds were identified and

was given in table2. and figure2.The major compounds of F. limonia were 6-methyl-1-(1-naphthyl)dihydrouracil (9.12), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (5.99), Eicosane (5.08), Nonadecane (5.52), Pentadecane, 2,6,10,14-tetramethyl (5.05), Eicosane (5.08), -Caryophyllene oxide (4.82), Hexadecane (3.95), Myrtenol (3.44), á-Cubebene (1.63), GERMACRENE-D (2.13), 1-Hexadecene (1.8), other compounds were Tricosane (1.29), docosane (1.30), noncosane (1.14) etc,.

Figure.1.GC-MS chromatogram of ethanol extract of *F.limonia*

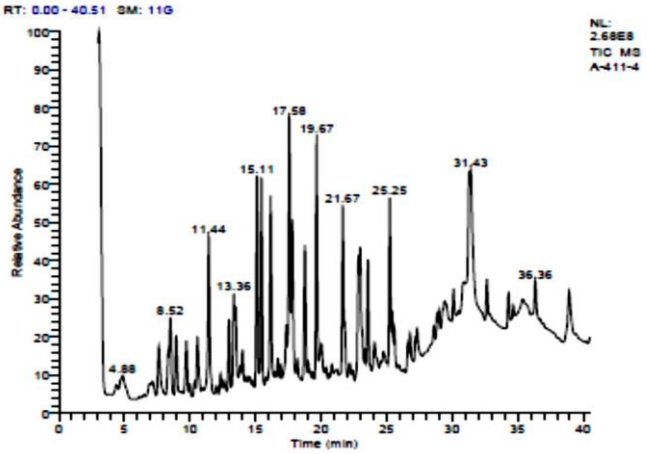


Table.2 GC-MS Analysis of ethanol extract of *F.limonia*

S.NO	NAME OF THE COMPOUND	RETENTION TIME (min)	(%) of the compound
1	Iso-pinocampheol	4.88	1.25
2	1,3-O-Benzylidene-1,3-dihydroxy-4-	7.63	1.78
3	Myrtenol	8.52	3.44
4	1-Hexadecene	9.74	1.8
5	Germacrene-D	10.53	2.13
6	á-Cubebene	12.98	1.63
7	Hexadecane	13.36	3.95
8	Pentadecane, 2,6,10,14-tetramethyl	15.11	5.05
9	-Caryophyllene oxide	16.18	4.82
10	6-methyl-1-(1-naphthyl)dihydrouracil	17.58	9.17
11	Nonadecane	19.67	5.52
12	3-(4-Methyl-1,3-cyclohexadienyl)butanoic acid	20.04	1.47
13	Eicosane	21.67	5.08
14	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	25.25	5.99
15	Tricosane	27.35	1.29
16	Docosane	32.67	1.30
17	Nonacosane	36.36	1.14

In vitro Cytotoxicity

The present study examined *in vitro* cytotoxicity of ethanolic extract of *F.limonia* leaves against MOLT-3 cells (adult T acute lymphoblastic leukemia cell line) by MTT assay which measures the % inhibition of cancer cells. The ethanol extract of leaves of *F.limonia* was diluted in different concentrations and the result, was shown in the Table (5). The extract was diluted to 5µl, 25µl, 50µl, 75µl, 100µl. The viability of cancer cell after incubation was given in the figure (3). The ethanol leaves extract of *F.limonia* showed moderate to severe cytotoxicity on MOLT-3 cells in dose dependent pattern. The IC_{50} value of cell viability was 63.24µl. The result revealed that the ethanol extract of *F.limonia* showed potent *in vitro* cytotoxicity against the MOLT-3 cancer cell line. The obtained results are comparable with results of (Shermin *et al*., 2012). There are few reports only available for *in vitro* cytotoxicity of this plant.

Figure 3. – Plot of % cell inhibition of ethanol extract of *F.limonia* leaves.

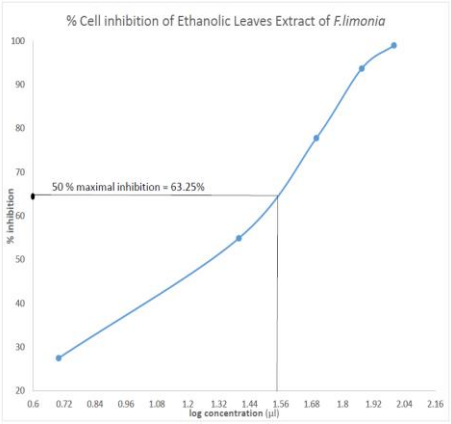
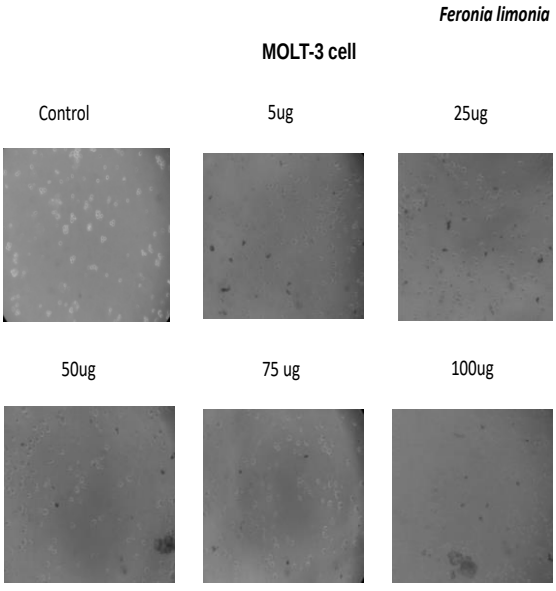


Figure 4. The cell viability of MOLT-3 cancer cell line of ethanol extract of leaves of *F.limonia*



IV. Conclusion

The present investigation revealed that the ethanol extract of *F.limonia* showed the presence of many active phytochemicals like, alkaloids, flavonoids, coumarins and steroids . *In vitro* cytoxicity study using MOLT-3

cancer cell line by MTT assay was performed and have the IC_{50} of 63.24 µl, which showed the extract has significant cytotoxicity against cancer cells.

NO Conflict of interests

References

[1] Anonymous, the wealth of India, A dictionary of Indian raw materials and industrial products. Raw material, shastri B.N (Ed.) Vol,IV, council of scientific and industrial research (CSIR) publications, New Delhi, 1995.18.

[2] K. R. Kirtikar and B. D. Basu, Indian medicinal plants, 2nd ed. vol. 1. Dehra Dun: Bishen Singh, Mahinder Pal Singh, 1998.

[3] W. Dymock, C. J. H. Warden, and D. Hooper, Pharmacographia indica vol. 1. New Delhi: Shrishti Book Distributors, 2005.

[4] K. M. Nandkarni, Indian materia medica vol. 1. Mumbai, India: Popular Prakashan, 1976.

[5] V .Neelamadhab Panda, Jagannath Patro V, Basanta Kumar Jena, Panda P K, Evaluation of phytochemical of antimicrobial activity of Ethanolic extract of limonia acidissima Leaves, International journal of herbal medicine, 2013, 1(1), 2321-2187.

[6] K. Ilango, Chitra V. Hepatoprotective and antioxidant activities of fruit pulp of Limonia acidissima Linn. Int J Health Res 2009; 2(4): 361-367.

[7] S C.Garg, Antimicrobial activity of essential oil of feronia elephantum Correa, Indian Journal of Pharmaceutical Sciences, 2001, 155-157.

[8] M. A. M. Momin, M. R. Khan, J. Rayhan, A. Afrose, S. Rana, A.Ara Begum, Evaluation of Antibacterial and Antidiarrhoeal Activities of Feronia limonia Leaf Extract, American Journal of Plant Sciences, 2013, 4, 2181-2185.

[9] N. Nithya, Saraswathi U, In vitro antioxidant and antibacterial activity of feronia elephantum Correa fruit, Indian Journal of Natural Products and Resources, 2010, 1(3), 201-205.

[10] Mehta P, Chopra S, Mehta A, Antimicrobial properties of some plant extract against bacteria. Folia Microbiology, 1983, 28(6), 467-469.

[11] Saima Y, Das A K, Sarkar K K, Sen A K, Sur P, An antitumor pectic polysaccharide from *Feronia limonia,* Bio. Macromol., 2000, 27, 333-335.

[12] R.T. Sampandan, D. David, In *vitro* antioxidant and cytotoxic activities of *Feronia elephantum* correa, Asian Pacific Journal of Tropical Biomedicine, 2014, 4(4), 290-293.

[13] S. Shermin, F. Aktar, Monira Ahsan and Choudhury, Hasan. M, Antioxidant and Cytotoxic Activitiy of *Limonia acidissima* L. *J. Pharm. Sci*. June2012, *11*(1), 75-77.

[14] M. S. Hossain, Evaluation of antimicrobial and cytotoxic activity of the plant extracts of *Feronia limonia* (Linn), *Journal of Scientific and Innovative Research,* 2013, *2*(5), 859-863.

[15] S.M. Ahamed, K.S. Swamy, Jayaverra KN, Venketeshwara Rao J ,Vijay Kumar S, Anti-inflammatory, Antipyretic and Analgesic activity of Methanolic extract of Feronia Limonia fruit Pulp, *Pharmacologyonline* , 2008, 3: 852-857.

[16] A.A.Rahuman, Gopalakrishnan G., Ghouse B.S. Arumugam S and Himalayan B: Effect of *feronia limonia* on mosquito larvae**,** Fitoterepia 2000, 71(5), 553-555.

[17] M.M.Rahaman and Gray A.I., Antimicrobial constituents from the stem bark of *feronia limonia* Phytochemistry 2002,59 (1) 73-77.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Statistical screenings of medium components for the production of Chitinase by *Trichoderma viride*

SUDHAKAR.P[1], NAGARAJAN.P[2]

Assistant Professor[1], Associate Professor[2] Department of Chemical Engineering,

Annamalai University

India

psudha211@ yahoo.co.in

Abstract: Statistics based experimental designs were used to optimize the medium components for chitinase production. Optimization studies were performed on strains of *T.viride*, which produce Chitinase enzyme using sugarcane bagasse as substrate. Preliminary studies on the factors enabled identification of eight variables important with respect to chitinase production. The CCD contains a total of 31 experimental trials involving the replications of the central points. The dependent variables selected for this chitinase yield (U/gds). The most important factors with respect to each strain were then identified using fractional factorial. In general, colloidal chitin, KH_2PO_4, yeast extract and $MgSO_4.7H_2O$ affected Chitinase production. CCD was used for model building and chitin and yeast extract were found to have an interactive effect on chitinase production in *T.viride* whereas yeast extract alone had a quadratic effect on chitinase production in the strain. Similarly, colloidal chitin and yeast extract had a quadratic effect on chitinase production in *T.viride*. The optimal values of important variables were determined by response surface methodology (RSM) and numerical optimization. This optimization strategy led to an increase in Chitinase production is 33.02 U/gds in the strain using Sugarcane bagasse as substrate.

Keywords*: Chitinase, Trichoderma viride, Optimization,* Plackett-Burman, *Sugarcane bagasse*.

I. Introduction

Chitin is one of the most abundant biopolymers widely distributed in the marine and terrestrial environments. Chitinases are the enzymes that degrade chitin, which is a polymer of N-acetylglucosamine linked by -1,4 glycosidic bond. Endochitinase and N-acetylhexosaminidase are two major enzymes of the chitinase system. Chitinase enzyme has received increased attention due to its wide range of biotechnological applications, especially in agriculture for biocontrol of phytopathogenic fungi and harmful insects [4], medicine [6], food technology and waste management [1]. Fermentation involves the use of microorganisms, like bacteria and yeast to produce the enzymes [5]. There are two methods of fermentation used to produce enzymes. These are submerged fermentation and solid-state fermentation. Submerged fermentation involves the production of enzymes by microorganisms in a liquid nutrient media. Solid-state fermentation is the cultivation of microorganisms, and hence enzymes on a solid substrate [4]. Carbon containing compounds in or on the substrate are broken down by the microorganisms, which produce the enzymes either intracellularly or extracellularly. The enzymes are recovered by methods such as centrifugation, for extracellularly produced enzymes and lysing of cells for intracellular enzymes. The important medium components identified by initial screening method of Plackett-Burman design. Plackett-Burman Pareto chart illustrates the order of significance of the variables affecting the cellmass production. Central composite response surface methodology was performed to evaluate the screened media components on production of chitinase by Trichoderma viride was studied using agro industrial waste under solid state fermentation [8],[9], [10].

2. Materials and Methods

2.1. Micro-organism and inoculum preparation

Trichoderma viride (167) is obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The aerobic culture is transferred to agar slants containing Potato Dextrose Agar (PDA), The pH is adjusted to 5 and incubated at 30°C for 6 days.

2.2. Chitinase Assay

Chitinase activity was determined by a dinitrosalicylic acid (DNS) method [3]. To determine the chitinase activity from crude SSF extract, the reaction mixture contained 0.5 ml protein extract, 0.5 ml of 1% colloidal chitin in

phosphate buffer pH 5.5 and 1ml distilledwater. The solutionwas incubated at 50◦Cfor 10 min with continuous shaking. The reactionwas terminated by 3ml 3,5-dinitrosalicylic acid then the solutionwas mixed and kept in boiling water for 5 min before reading at 575 nm. One unit of chitinase was defined as the amount of enzyme releasing 1mol N-acetyl-d-glucosamine equivalent per minute from colloidal chitin under the given assay conditions.

2.3. Optimization of nutrient supplements

The medium components were evaluated using Plackett-Burman statistical design [2]. This is a fraction of a two-level factorial design and allows the investigation of 'n-1' variables with at least 'n' experiments. The main effect was calculated as the difference between the average of measurements made at the high setting (+1) and the average of measurements observed at low setting (−1) of each factor. This model describes no interaction among factors and it is used to screen and evaluate the important factors that influence enzyme production. The factors that have confidence level above 95% are considered the most significant factors that affect the enzyme production..

2.4. Experimental designs

A response surface design is a set of advanced design of experiments (DOE) techniques that help you better understand and optimize your response. Response surface design methodology is often used to refine models after you have determined important factors using screening designs or factorial designs [7]. Central composite designs are a factorial or fractional factorial design with center points, augmented with a group of axial points (also called star points) that let you estimate curvature. Central Composite designs can fit a full quadratic model. They are often used when the design plan calls for sequential experimentation because these designs can include information from a correctly planned factorial experiment. CCD is used to determine the optimum conditions for the chitinase production by *T.viride* using sugarcane bagasse. The range and levels of nutrients (colloidal chitin, KH_2PO_4, yeast extract and $MgSO_47H_2O$) are given in Table 2.1. Thirty one experiments are performed at different combinations. The predicted and observed responses along with design matrix are presented in Table 2.1

Table 2.1 Ranges of the independent variables used in RSM for the production of chitinase by *T.viride* using sugarcane bagasse

Variables		Levels, g/gds				
	Code	-2	-1	0	1	2
Colloidal chitin	x_1	2.0	4.0	6.0	8.0	10.0
KH_2PO_4	x_2	0.4	0.5	0.6	0.7	0.8
Yeast extract	x_3	0.5	1.0	1.5	2.0	2.5
$MgSO_4.7H_2O$	x_4	0.03	0.06	0.09	0.12	0.15

3. Results and discussion

3.1. Chitinase activity

Trichoderma Viride gave maximum chitinase activity of 34.03 U/gds for Sugarcane bagasse after incubation for 5 days.

3.2. Screening of important media components

The effect of eight medium components of the fermentation for chitinase production by *Trichoderma Viride* was examined using Plackett-Burman statistical design [8]. Experiments are conducted in 12 runs to screen the media components for the production of chitinase by *T.viride* using sugarcane bagasse as substrate.

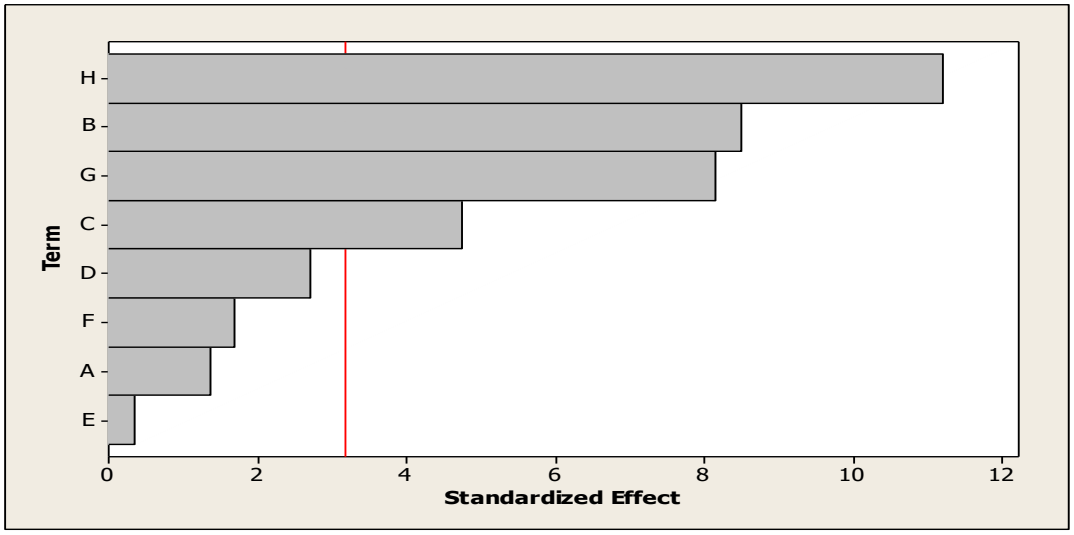


Fig.3.1 Pareto chart for screening of nutrients for the production of chitinase by *T.viride* using sugarcane bagasse

Where A-$(NH_4)_2SO_4$, B-KH_2PO_4, C-$MgSO_47H_2O$, D-urea, E-$CaCl_2$, F-NaH_2PO_4, G-yeast extract, H-colloidal chitin. The components are screened at the confidence level of 90% based on their effects. The equation obtained from PBD is Y= 17.333-0.333A+4.167B-2.333C-1.333D+0.167E-0.833F-4.000G+5.500H - (3.1)
On analysis of regression coefficient of eight medium components $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4{\cdot}7H_2O$,Urea, $CaCl_2$, NaH_2PO_4, yeast extract and colloidal chitin, From the equation 3.1 it is found that the nutrients KH_2PO_4, $MgSO_47H_2O$, yeast extract, colloidal chitin showed positive effect whereas $(NH_4)_2SO_4$, urea, NaH_2PO_4 and $CaCl_2$ showed negative effect of chitinase production**.** The Pareto chart offers a convenient way to view the results obtained by Plackett-Burman design. The Pareto chart Fig. 3.1 illustrates the order of significance of the variables affecting the chitinase production is colloidal chitin, KH_2PO_4, yeast extract, $MgSO_47H_2O$, urea, NaH_2PO_4, $(NH_4)_2SO_4$ and $CaCl_2$. From the Pareto chart it is inferred that the nutrients, colloidal chitin, KH_2PO_4, yeast extract and $MgSO_4.7H_2O$ have significant effect on chitinase production. Thus these four variables colloidal chitin, KH_2PO_4, yeast extract and $MgSO_47H_2O$ are selected and they are optimized using response surface methodology.

3.3. Optimization of Process Parameters for Chitinase Production using Sugarcane bagasse as Substrate

In this study, agro industrial waste Sugarcane bagasse collected from sugarmill was used as main substrate under solid state fermentation. In order to obtain optimum levels of chitinase by *Trichoderma Viride*. Optimization of cultivation conditions variables that had a significant impact on chitinase production was necessary. It can be seen there was a considerable variation in the chitinase production depending on the four chosen variables. The maximum chitinase production (34.30U/gds) was achieved in run number 9, while the minimum chitinase production (10.50 U/gds) was observed in run number 21. The former was much higher than the latter, which adequately indicated that choosing appropriate cultivation conditions could evidently enhance the yield of chitinase. In order to estimate the error, the centre point in the design was repeatedly carried out for three times. By applying multiple regression analysis on the experimental data, the following second order polynomial equation was found to explain the chitinase production by only considering the significant terms and was shown in equation 3.2.

$$Y = 32.2 - 1.877x_1 - 2.110x_2 + 2.348x_3 + 0.685x_4 - 0.888x_1^2 - 5.013x_2^2 - 4.357x_3^2 - 3.044x_4^2 + 0.934x_1x_2 + 1.466x_1x_3 + 0.647x_1x_4 + 5.003x_2x_3 - 3.503x_2x_4 - 1.534x_3x_4 \quad (3.2)$$

Table 3.1 ANOVA for response surface quadratic model for the production of chitinase by *T.viride* using sugarcane bagasse

Source	Coefficient factor	Sum of squares	DF	F	P
Model	32.2	2301.33	14	68.7	0.0001
x1	-1.8771	84.56	1	35.34	0.0001
x2	-2.1104	106.89	1	44.68	0.0001
x3	2.34792	132.31	1	55.3	0.0001
x4	0.68542	11.28	1	4.71	0.0453
x1*x1	-0.888	22.55	1	9.42	0.0073
x2*x2	-5.013	718.62	1	300.35	0.0001
x3*x3	-4.3568	542.79	1	226.86	0.0001
x4*x4	-3.0443	265.01	1	110.76	0.0001
x1*x2	0.93438	13.97	1	5.84	0.028
x1*x3	1.46563	34.37	1	14.36	0.0016
x1*x4	0.64688	6.7	1	2.8	0.1138
x2*x3	5.00313	400.5	1	167.39	0.0001
x2*x4	-3.5031	196.35	1	82.06	0.0001
x3*x4	-1.5344	37.67	1	15.74	0.0011
Residual		38.28	16		
Lack of fit		38.28	10		
Pure Error		0	6		
Total		2339.61	30		

Where Y is the chitinase activity (U/gds), x_1 - colloidal chitin, x_2 - KH_2PO_4, x_3 - yeast extract and x_4 - $MgSO_47H_2O$. Equation 3.2 can be used to predict the chitinase production within the limits of the experimental factors. Anova table 3.1 The Model F-value of 68.7 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of P less than 0.05 indicate model terms are significant. Values greater than 0.1 indicate the model terms are not significant. In the present work, all the linear effect of x_1, x_2, x_3 and x_4, interactive effects of x_1x_2, x_1x_3, x_2x_3, x_2x_4 and x_3x_4 and square effects of x_1, x_2, x_3 and x_4 are significant for chitinase production. The coefficient of determination (R^2) for Chitinase activity is calculated as 0.9836, which is very close to 1 and can explain up to 98.36% variability of the response. The predicted R^2 value of 0.9058 is in reasonable agreement with the adjusted R^2 value of 0.9693. An adequate precision value greater than 4 is desirable. The adequate precision value of 24.75 indicates an adequate signal and suggests that the model can be used to navigate the design space. The model significance (*F*-value) indicates the level of confidence that the selected model cannot be due to experimental error (Henika, 1972). Linear and quadratic terms were significant at the 1% level. Therefore, the quadratic model was selected in this optimization study

4. Conclusion

The significant factors identified by Plackett-Burman design are considered for the next stage of the medium optimization using response surface optimization technique for the future study, Using the above optimized nutrient solution, Maximum chitinase activity of Colloidal chitin - 4g, yeast extract - 1.0g, KH_2PO_4 - 0.5g, and $MgSO_4.7H_2O$ - 0.12g. The predicted response for chitinase production is 33.02 U/gds, while the actual response is 34.30 U/gds

5. References

[1] K.Madhavan Nampoothiri, T.V.Baiju, C.Sandhya, Ashok Pandey. Process optimization for antifungal chitinase production by Serratia marcescens. Process biochemistry, 39, 1583-1590, (2004).
[2] Rajiv vaidya, Pranav vyas, chhatpar.H.S .Statistical optimization of medium components for the production of chitinase, by Alcaligenes xylosoxydans. Enzyme and Microbial Technology, 33, 92-96, (2003).
[3] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem, 31, 426–8, (1959)
[4] Nawani.N.N, Kapadnis B.P. Optimization of chitinase production using statistics based experimental designs. Process biochemistry, 40, 651-660, (2005).
[5] Fenton D.M., and Eveleigh D.E., Purification and Mode of Action of a Chitinase from Penicillium islandicus, J Gen Microbiol , 126, 151–154, (1981).
[6] Sudhakar.P, Nagaraan.P. Production of Chitinase by Solid State Fermentation from Rice bran. International Journal of Environmental Science and Development, Vol.1, No.5,435-430, (2010)
[7] Montgomery DC. Design and analysis of experiments, 4th ed. NewYork: John Wiley and Sons; (1997).
[8] Sudhakar.P, Nagaraan.P. Process optimization for chitinase production by Trichoderma harzianum. As. J. Food Ag-Ind. 2011, 4(02), 91-102.
[9] Chahal DS. Solid State fermentation in Trichoderma reesie for cellulose production. Appl Environ Microb, 499, 205–10, (1985)
[10] Eman Fathi Sharaf, Abd El-Aziz Qablan El-Sarrany and Mai El-Deeb, Biorecycling of shrimp shell by Trichoderma viride for production of antifungal chitinase. African Journal of Microbiology Research , Vol. 6(21), pp.4538-4545, (2012).

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Evaluation of phytoconstituents of *Andrographis paniculata* and its *IN VITRO* antioxidant activity against DPPH and ABTS assay

Jeevitha N[1], Nandha kumar K [2], Vasantha Mohan S[3], Pradeep K[4] and Velliangiri Prabhu[5*]
Post Graduate Department of Chemistry
Nallamuthu Gounder Mahalingam College, Pollachi, Tamilnadu, India-642001
E.Mail.ID- prabhunmr@gmail.com

Abstract*: GC-MS study of chloroform extract of Andrographis paniculata revealed the presence of 26 compounds. The major compounds are Bis (2-ethyl hexyl) phthalate(53.608%), dibutyl phthalate(10.537%) and phenol, 2,5-bis (1,1-dimethyl ethyl)(5.079%) and the minor compounds are phthalic acid, butyl-tetrdecyl ester (1.00%), octadecane, 3-ethyl-5-(2-ethyl butyl)- (1.022%) and heptadecane, 9-hexyl (1.034%). In-vitro antioxidant activity carried out for chloroform extract of Andrographis paniculata by DPPH and ABTS assay. The extract shows moderate activity against DPPH and ABTS. It's IC_{50} values are11.17 (µg/ml) and Ic_{50}= 58.74 (µg/ml) respectively.*

Keywords: *A. paniculata, DPPH and ABTS assay.*

I. Introduction

Plants have been an important source of medicine for thousands of years. Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. The use of medicinal plants in developing countries is increasing, which offer a new source of antibacterial, antifungal and antioxidants agents. The higher plants are used to treat a number of infectious diseases around the world. Almost all organisms are well protected against free radical damage by the presence of enzymes or compounds such as ascorbic acid, tocopherols and glutathione. When the mechanism of antioxidant protection becomes unbalanced by several internal and external factors, deterioration of physiological functions may occur requiring the system to depend on exogenous antioxidants from natural sources. Several synthetic antioxidants such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT) have restricted use in foods as they are suspected to be carcinogenic [1]. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years.

Andrographis paniculata Nees. belongs to the family *Acanthaceae* and is an important medicinal plant in India, China and Thailand. It is an annual, branched, erect herb whose aerial parts (stem and leaves) are used in traditional medicine. The plant is claimed to possess immunological, antibacterial, anti-inflammatory, antithrombotic, hepatoprotective, hypoglycaemic and hypotensive properties [2]. The importance of search and exploitation of natural antioxidants of plant origin has greatly increased in recent years. Though, in traditional medicine *A. paniculata* has been claimed to possess antioxidant properties, very few studies have shown the antioxidant activity. For all biological activities phytoconstituents is essential. So the aim of the present study was to investigate the chemical constituents by GC-MS and its antioxidant activities for chloroform extract of *A. paniculata* leaves.

II. Materials and methods

Plant sample

Fresh leaves of *A. paniculata* was collected in Aug, 2018, from Pollachi, Tamil Nadu and the plant species was authenticated in the Department of Botany, NGM college, Pollachi. Voucher specimen was preserved in our Department (No. NGM18CHE2018).

Extraction of crude extracts

Fresh leaves were washed thoroughly with water to remove the earthy matters and freed from debris. And subjected to chloroform extraction using Soxhlet's apparatus. Extracts were concentrated under vacuum, in a rotary evaporator, dried and stored in vacuum desiccators for further analysis.

Gas Chromatography-Mass Spectrum Analysis (GC-MS)

GC-MS analysis of *A. paniculata* chloroform extract was performed using Agilent technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped withHP-5MS column (30 m in length x 250 µm in diameter x 0.25 µmin thickness of film). Spectroscopic detection by GC-MS involved an electron ionization system which utilized high energy electrons (70ev). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1mL/min. The initial temperature was set at 50-150ºC with increasing rate of 3ºC/min and holding time about 10 min. Finally the temperature was increased to 300°C for 10 min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in an splitless mode. Relative quantity of the chemical compounds present in each of the extracts of *A. paniculata* was expressed as percentage based on peak area produced in the chromatogram. Compounds were identified by comparing the mass spectral data with NIST library.

Antioxidant activity

DPPH (Diphenyl-2-picrylhydrazyl) Radical Scavenging Activity

The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical method. The DPPH solution (0.1mM) in ethanol was made, and 1.0ml of this solution was added to 3.0 ml of extracts solution (or standard) in water at different concentrations. After 30 minutes, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

% of DPPH of radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

Where control was the absorbance of the control reaction and test was the absorbance in the presence of extracts. The mean values were obtained from triplicate experiments.

$ABTS^+$ radical scavenging assay

$ABTS^+$ radical scavenging activity was determined according to Re et al (13). ABTS+ radical was freshly prepared by adding 5 ml of 4.9 mM ammonium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with ethanol (99.5%) to yield an absorbance of 0.70±0.02 at 734 nm and the same was used for the assay. To 950 µl of ABTS radical solution, added 50 µl of extract solutions (25-500 µg/ml) and the reaction mixture was vortexed for 10 sec. After 6 minutes the absorbance was recorded at 734 nm and compared with the control ABTS solution. Percentage inhibition was calculated from the formula

Percentage inhibition= [1−(absorbance of test/absorbance of control)]×100.

III. Result and Discussion

Compounds present in the extracts

The compounds present in chloroform extracts obtained from *A. paniculata* leaves are shown in (Table1).

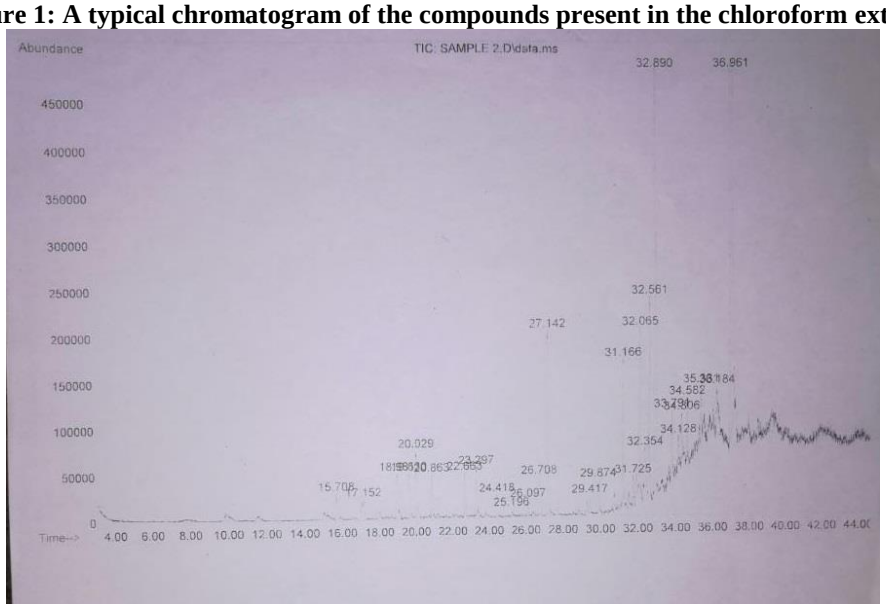
Table 1: Chemical compositions of chloroform extract from *A. paniculata* leaves.

Sl.No	Name of compounds	Molecular formula	Retention time (min)	%
1	Cetane	$C_{18}H_{32}$	15.708	0.955
2	Hexadecane, 2,4,10-trimethyl	$C_{19}H_{40}$	17.152	1.173
3	Octadecane	$C_{18}H_{38}$	18.981	0.878
4	1-octadecane	$C_{18}H_{36}$	19.630	1.138
5	Benzylalcohol	C_7H_8O	20.029	1.661
6	Nonadecane	$C_{19}H_{40}$	20.683	0.960
7	Nonadecane-9-methyl	$C_{20}H_{42}$	22.663	1.064
8	Behenic alcohol	$C_{22}H_{46}O$	23.297	1.077
9	Heneicosane	$C_{21}H_{44}$	24.418	0.469
10	Selina-3,7-(II)-diene	$C_{15}H_{24}$	25.196	0.199
11	Tetracosane	$C_{24}H_{50}$	26.097	0.397
12	1-nonadecane	$C_{19}H_{38}$	26.708	0.939
13	Phenol,2,5-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	27.142	5.079
14	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	29.417	0.430
15	N-tetracosanol-1	$C_{24}H_{50}O$	29.874	0.840
16	Phthalic acid, hept-4-yl isobutyl ester	$C_{19}H_{28}O_4$	31.166	3.526
17	Phthalic acid, butyl-2-ethyl butyl ester	$C_{18}H_{26}O_4$	32.065	3.113
18	Phthalic acid, butyl-tetradecyl ester	$C_{26}H_{42}O_4$	32.654	1.000
19	Phthalic acid, butyl hex-3-yl ester	$C_{18}H_{26}O_4$	32.561	3.722
20	Dibutyl phthalate	$C_{16}H_{22}O_4$	32.890	10.537
21	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	33.791	2.843
22	Tert-hexadecanethiol	$C_{16}H_{34}S$	34.128	0.505
23	1-(+)-ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	34.306	0.803
24	Octadecane,3-ethyl-5-(2-ethyl butyl)-	$C_{26}H_{54}$	34.582	1.022
25	9,10-secocholesta-5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl)oxy],(3β,5Z,7E)-	$C_{30}H_{52}O_3$ Si	36.184	1.961

26	Bis (2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	36.961	53.608

The retention time, molecular formula and the percentage composition of these compounds were also presented. The GC chromatograms of the chloroform extract are presented in Figure 1.

Figure 1: A typical chromatogram of the compounds present in the chloroform extract



Antioxidant activity:-

Antioxidant activities for chloroform extract of *A. paniculata* leaves are shown in table 2, 3 and figure 2 and 3.

Table 2: Shows % inhibition of DPPH radical scavenging activity of chloroform extract and standard (ascorbic acid)

Sl.No	Concentration	Ascorbic acid		AP	
		Absorbance	Percentage inhibition	Absorbance	Percentage inhibition
1	10	0.264	50	0.416	21
2	20	0.248	53	0.388	26
3	40	0.232	56	0.342	35
4	60	0.214	59	0.314	40
5	80	0.184	65	0.268	49
6	100	0.154	71	0.224	57
	Ic_{50} values	**Ic_{50}= 8.29 (µg/ml)**		**Ic_{50}= 11.17 (µg/ml)**	

Table 3: Shows % inhibition of *$ABTS^+$* radical scavenging activity of chloroform extract and standard (ascorbic acid)

Sl.No	Concentration	Ascorbic acid		AP	
		Absorbance	Percentage inhibition	Absorbance	Percentage inhibition
1	10	0.692	22	0.520	41
2	20	0.648	27	0.506	43
3	40	0.592	33	0.482	46
4	60	0.422	52	0.444	50
5	80	0.388	56	0.412	53
6	100	0.322	64	0.364	59
	Ic_{50} values	**Ic_{50}= 17.16 (µg/ml)**		**Ic_{50}= 58.74 (µg/ml)**	

Fig 2: Shows % inhibition of DPPH radical scavenging activity

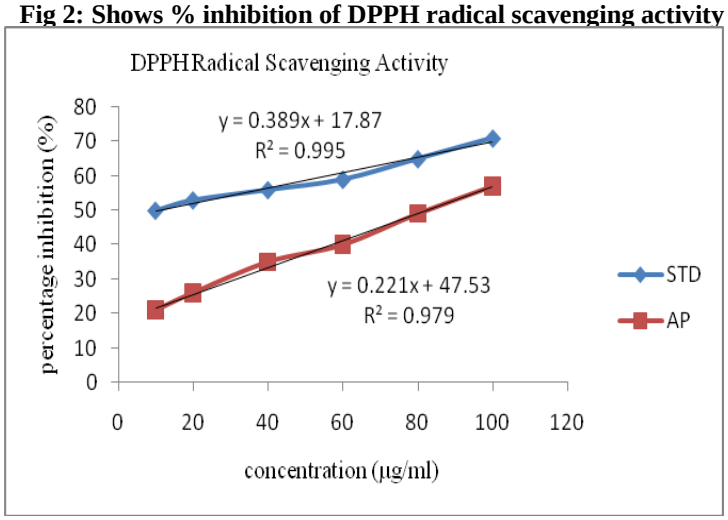
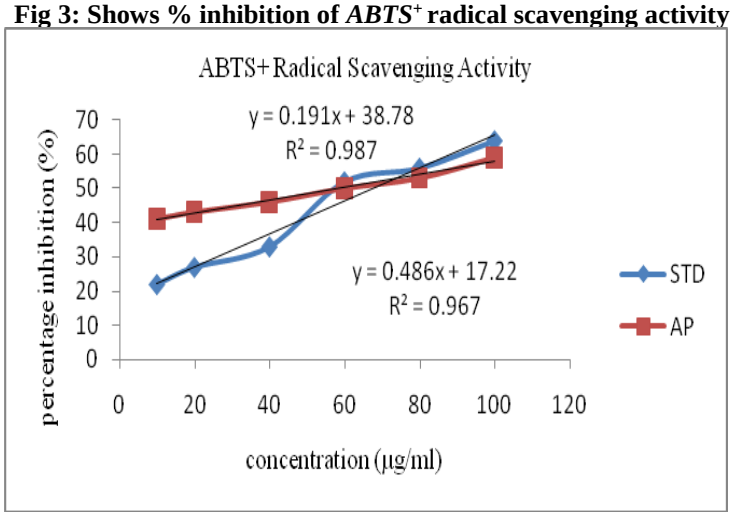


Fig 3: Shows % inhibition of *ABTS*$^{+}$ radical scavenging activity



GC-MS

Mathan Kumar S *et al.*, carried out GC-MS analysis for ethanolic extract of whole plant *A. paniculata* and proved 42 compounds were present in the extract, the major compounds are Citronellyl isobutyrate, 11-Dodecene-1-yl acetate, Bicyclo[2.2.2]octane-1,4-diol, β – Citronellol Methyl ester [3] . Udobre *et al*., identified only six compounds from ethanolic extract of *A. paniculata* leaves namely, 6,10-dimethyl undecanone, 3-pentadecyl phenol, 1-methyl-4-/2-pentenyl-oxy-benzene, Bicyclo-hex-3-en-one and p-methyl phenyl acetate, 4-phenylbut-3-en-1-yne [4]. Kalaivani *et al*., reported that the 13 different compounds from the ethanolic extract of *A.paniculata* leaves and presence of 1,2-benzenedicarboxylic acid(1.17%) and other major compounds namely Retinoic acid, methyl ester [5],[6]-[8], Androstan-17-one,3-ethyl-3-hydroxy-,(5α), Phytol. Kalaiselvan *et al*., noted that the 19 different phytochemical compounds from the ethanolic extract of plant bark of *Andrographis paniculata* and presence of 1,2-benzenedicarboxylic acid, diundecylestero(1.52%), other major compounds 2,5-octadecadiynoic acid, methyl ester, 10-12-pentacosadiynoic acid, 1,2,3,5-cyclohexanetetrol,(1a,2a,3a,5a) [9]. Palaniyandi krishnamoorthy *et al*., carried out GC-MS analysis for methanolic extract of leaves of *A. paniculata* and proved 30 compounds were present in the extract, the major compound are phytol (58.3%) and also identified the 1,2-Benzenedi carboxylic acid (97.44%) from the fraction of solvent methanol-acetone (6:4ratio) [10]. Nnabuk O. Eddy *et al.*, identified 17 compounds by GC-MS from which the major compound is methyl stearate [11]. Sivakumar *et al*., noted that 11 different compounds were identified from ethanolic extract of whole plant of *A. paniculata* and the major compounds namely tetratetracontane(18.732%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (14.820%) [12]. In our present study revealed 26 compounds from the chloroform extract of *A. paniculata* leaves. From which the major compounds are bis (2-ethyl hexyl) phthalate (53.608%), dibutyl phthalate (10.537%) and phenol,2,5-bis(1,1-dimethyl ethyl) (5.079%). Minor compounds are phthalic acid, butyl-tetrdecyl ester (1.00%), octadecane, 3-ethyl-5-(2-ethyl butyl)- (1.022%) and heptadecane, 9-hexyl (1.034%). n-tetracosanol-1 (0.840%), Selina-3,7-(II)-diene (0.199%) and tetracosane (0.397%). The obtained major and minor compounds are entirely different from the others report. Since chemical constituents varied accordingly climatic condition and soil nature.

Antioxidant activity

Ramya premanath *et al*., proved ethanolic extract of *A. paniculata* leaf showed the highest scavenging ability of 53.16% at 0.5 mg/ml (IC50 value) and methanol extract showed a scavenging ability of 53.86% at a higher concentration of 0.9 mg/ml (IC50 value). For the case of lipid peroxidation ethanol extract had an IC50 value as low as 0.1 mg/ml and showed a inhibition of 58.73% which was followed by methanol with an IC50 value of 0.7 mg/ml. BHA showed a very strong lipid peroxidation inhibitory activity with an IC50 of 0.012 mg/ml. There was a scavenging of 51.06% of superoxide anions with ethanol extract at a concentration of 0.9 mg/ml (IC50 value). Quercetin was found to be more potent having an IC50 value 0.155 mg/ml [13]. Akowuah *et al*., studied free radical scavenging activity of *A. paniculata* extracts using DPPH assay. Analyzed methanol extracts exhibited free radical scavenging activity ranging from 45.67% to 53.82%, however water extracts revealed lower activity (25.29% to 28.77%) [14]. Antioxidant action of the aqueous extract of AP against mice's lymphoma was analyzed and compared with doxorubicin (DOX) and results confirm significant effect on lymphoma level in mice[15]. Moreover, the antioxidant activity of AP has been found greater than DOX. In present study *in-vitro* antioxidant activity carried for chloroform extract of leaves of *A. paniculata* by DPPH and ABTS assay. In our study both assay ascorbic acid used as a standard compound. In DPPH assay standard compound showed Ic_{50}= 8.29 (µg/ml) and extract showed Ic_{50}= 11.17 (µg/ml). In ABTS assay the extract showed Ic_{50}= 58.74 (µg/ml) and standard showed Ic_{50}= 17.16 (µg/ml). The above result we concluded that chloroform extract of *A. paniculata* leaves is purely dose dependent manner. And also extract exhibit temperate activity against both assays due to synergistic effect of phytoconstituents.

References

[1] P.K. Basniwal, M. Sutha, G.S. Rathore, R. Gupta, V. Kumar, A. Pareek, and D. Jain, "*In-vitro* antioxidant activity of hot aqueous extract of *Helicteres isora Linn*. Fruits. Natural Product Radiance, 2009; 8(5): 483-487.

[2] K.S. Mishra, S.N. Sangwan, and S.R. Sangwan, " *Andrographis paniculata* (kalmegh): A review". Pharmacognosy Reviews, 2007; 1(2): 283-298.

[3] S. Mathan Kumar, and A. Mohamed Salem Gani, "Phytochemical Investigation, GC- MS Analysis, Invitro Antimicrobial and Antioxidant Property of Andrographis Paniculata". International Journal of Current Trends in Science and Technology, 2017;7(12): 20171-20177.

[4] A.S. Udobre, A.E. Udoh, E.I. Etim, U.F. Umoh, and E.C. Johnson, "GC-MS Analysis of Six Bioactive Compounds Isolated From The Ethanol Leaf Extract of *Andrographis Paniculata*(Acanthaceae). Chemistry Research Journal, 2016;1(2):6-10.

[5] C.S. Kalaivani, N. Janakirman , and M. Johnson, " GC-MS analysis of the leaves of *A.paniculata"*. International Journal of Medicine and Aromatic Plant, 2012; 1(2): 69-74.

[6] L. Wu, H. Gao, X. Wang, J. Ye, J. Lu, and Y. Liang, " Analysis of chemical composition of *Chrysanthemum indicum* flowers by GC-MS and HPLC."Jornal of Medicinal Plants Research, 2010; 4(5**)**: 421-426.

[7] A. Vohra, and H. Kaur, " Chemical investigation of Medicinal plant *Ajugabracteosa*". Journal of Natural Product Plant Research, 2011; 1(1): 37-45.

[8] M. Holt, M.D. Stephen, and C. Linda, " Miracle Herb: How Herbs combine with modern medicine to treat cancer heart disease and Aid". Caroblushing Group, New York. 1998.

[9] A. Kalaiselvan," GC-MS Analysis of bioactive components of the ethanol extract of *Andrographis paniculata"*. Journal of Pharmacy and Biomedical science, 2012; 20(15).

[10] Palaniyandi Krishnamoorthy, and Dhanabal Kalaiselvan, "Isolation of Plasticizer Compound 1, 2- Benzenedicarboxlyic acid in Leaf Extract of *Andrographis paniculatas."* International Journal of Innovative Research in Science,Engineering and Technology,2016;5(4): 4985-4991.

[11] Nnabuk O. Eddy, Femi E. Awe, Abdulfatai A. Siaka, Ladan Magaji, and Eno E. Ebenso," Chemical Information From GC-MS Studies of Ethanol Extract of Andrographis Paniculata and Their Corrosion Inhibition Potentials on Mild Steel in HCl Solution." International Journal of electrochemical science , 2011; 6: 4316-4328

[12] V. Sivakumar, and S. Rajeshkumar, " Protective effect of *Andrographis paniculata* on hyperglycemic mediated oxidative damage in renal tissues of diabetic rats." The Journal of Phytopharmacology, 2015; 4(6): 287-294.

[13] Ramya Premanath, and N. Lakshmi Devi, "antibacterial, antifungal and antioxidant activities of *andrographis paniculata* nees. Leaves." International journal of pharmaceutical sciences and research. 2011; 2(8): 2091-2099.

[14] G.A. Akowouah, I. Zhari, I. Norhayati, and A. Mariam, "HPLC and HPTLC densitometric determination of andrographolides and antioxidant potential of *Andrographis paniculata"*. Journal of Food Composition and Analysis, (2006); 19: 118-126.

[15] N. Verma, and M. Vinayak, "Antioxidant action of *Andrographis paniculata* on lymphoma." Molecular Biological Report, (2008); 35: 535-540.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

PHYTOCHEMICAL ANALYSIS AND *INVITRO* ANTICANCER ACTIVITY OF ETHANOL EXTRACT OF *WRIGHTIA TINCTORIA*

Mini.R[1] and Vimaladevi.K[2]

Assistant Professor[1, 2]

PG Department of Chemistry

Nallamuthu Gounder Mahalingam College, Pollachi, Coimbatore- 642001

Tamilnadu, INDIA

E-mail ID: minichem1990@gmail.com

Abstract*: The present study examines the nature of phytoconstituents and in vitro anticancer activity of ethanol extract of Wrightia Tinctoria leaves. The preliminary phytochemical screening conducted by usual chemical tests indicated the presence steroids, alkaloids, terpenoids, flavonoids, glycosides and phenolic compounds. GC-MS analysis of Wrightia Tinctoria ethanolic extract revealed the presence of eighteen compounds. In the present study, the major chemical constituents are 2,5-Dimethoxybenzaldehyde oxime (9.52%), 5,5''-Dibromo-4'-methoxymethoxymethyl-2,2',6',2''-terpyridine (6.31%), 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole (4.68%) and 2-Ethyl-5-propylpyridine (4.23%). The in vitro anticancer activity was tested against lung cancer (NH40) cell line using MTT assay. The ethanol extract of W. Tinctoria showed dose dependent anticancer activity. The IC_{50} value was 100µg/ml. The result revealed that W. Tinctoria plant has significant anticancer activity.*

Keywords*: Wrightia Tinctoria, GC-MS, in vitro anticancer activity, NH40 cell line, MTT assay*

I. Introduction

Wrightia Tinctoria belongs to the family Apocynaceae is a small deciduous tree with pale grey, smooth bark, distributed in tropical Africa and Asia. The plant is commonly known as Paalai and generally called "Sweet Indrajao".[1] The juice of the tender leaves is used an effective drug for treatment of jaundice.[2] The crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. The leaves are a fodder for the cattle, goat and sheep. In south India the plant is used for green manuring rice fields.[3] The leaves of this tree yield a blue dye called pala indigo.[4] *W. Tinctoria* leaves were soaked in coconut oil for few hours and applied for eczema psoriasis and other skin diseases.[5] The *W. tinctoria* flower has been reported to have a good anti-inflammatory activity.[6] Bark and seeds are used to cure bilious infections, psoriasis, leprosy, asthma and various skin diseases.[7] Many authors reported pharmacological activity of *W. Tinctoria* plant like antiulcer activity, anti-inflammatory activity, wound healing activity, anthelmintic activity and antimicrobial activity.[8-11]

There is limited work on anticancer activity. So our main objective of the present work is to evaluate the preliminary phytochemical screening and the anticancer activity against NH40 cancer cell line using MTT Assay.

II. Materials and Methods

Plant Material

Fresh leaves of *W. tinctoria* were collected from area near Udumalpet, Tamilnadu, South India between the periods of September to October. The plant sample was authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu.

Extraction process

The collected leaves (500 kg) were dried in shade for 10 days. Air dried leaves of *W. tinctoria* was chopped into small pieces. The crude powered material was defatted with ethanol by cold macerated for three days with occasional shaking. The extract was then subjected to vacuum distillation and was concentrated to yield a greenish residue (5 g).

Preliminary Phytochemical Screening

The ethanol extracts of leaves of *W. tinctoria* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present.[12]

GC-MS Analysis

GC-MS analysis of the ethanol extract of *W. tinctoria* was carried out in thermo GC-trace ultra version: 5.0 coupled with thermo MS DSQ II instrument. The compounds were separated on DB-35, MS capillary DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non-polar column (0.25mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70ºC and held for 2 minutes and the temperature of the oven was raised to 260ºC for 10 minutes, raised 6ºC per minute and the final temperature was 300ºC for 10 minutes.

Identification of phytoconstituents

The components were identified by comparison of their mass spectra with those of National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

***In vitro* anticancer activity by MTT assay**

The *in vitro* anticancer activity of ethanol extract of *W. tinctoria* leaves were tested against NH40 lung cancer cell line using MTT assay. The ethanol extract was diluted to different concentrations via 5µl, 25µl, 50µl, 75µl, 100µl. The cell viability of cancer cells after inhibition was noted.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % cell inhibition was determined using the formula

$$\% \text{ cell inhibition} = 100 - [\text{Abs (sample)}/\text{Abs (control)}] * 100$$

IC_{50}value was determined by Nonlinear regression graph was plotted between % cell inhibition and $\log_{10}$ concentration.

III. Results and Discussion

Preliminary phytochemical screening

From the chemical tests, the nature of phytochemical constituents present in the ethanolic extract of *Wrightia tinctoria* was given in the table 1. The result agrees well with the literature report.[12]

Table 1: Preliminary phytochemical screening of ethanolic extract of *W. tinctoria* leaves

S.No	Phytoconstituents	phytoconstituent
1.	Alkaloids	++
2.	Phenolic Compound	++
3.	Flavanoids	++
4.	Tannins	++
5.	Terpenoids	++
6.	Steroids	++
7.	Glycosides	++

GC-MS Analysis

The GC – MS is used to identify the compounds like Terpenes, hydrocarbons, fatty acids and branched chain hydrocarbons. The GC-MS analysis of the ethanol extract of *W. tinctoria* leaves showed the presence of 18 chemical constituents. The compounds were characterized and identified by comparing the GC-MS spectra of the constituents with the NIST library along with the parameters which includes the retention time, peak area, molecular weight. Among the 18 compounds identified the most popular compound is 2, 5-Dimethoxybenzaldehyde oxime (37.52) and the minor compounds include 5,5”-Dibromo-4’-methoxymethoxymethyl-2,2’:6’,2”-terpyridine (34.28), 3-Dodecen-1-al (20.42), 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole (31.53), 2-Ethyl-5-propylpyridine (30.91), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (23.97), 2-Methyl-6-Styrylpyridine-3,4-Dicarboxylic Acid (26.62),

Methyl 3-(pentafluoro phenyl)acrylate (32.14), 2-Methyl-7-nonadecene (21.36), 1-Phenyl-1-(1'-naphthyl)-1,2-hexadiene (22.99). The GC-MS chromatogram is shown in the figure 1 and the characterized compounds were shown in table 2.

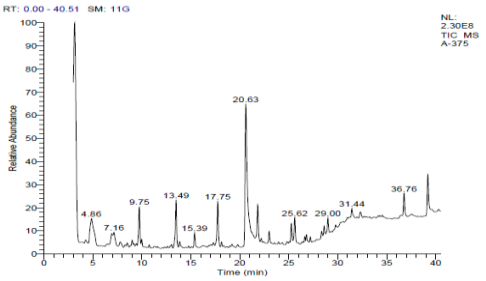


Figure 1: GC-MS chromatogram of ethanol extract of *Wrightia tinctoria* leaves

Table 2: Chemical composition of ethanol extract of *Wrightia tinctoria* leaves

S.NO	COMPOUND NAME	RETENTION TIME	AREA
1.	3-Dodecen-1-al	20.42	2.35
2.	2-Methyl-7-nonadecene	21.36	2.03
3.	1-Phenyl-1-(1'-naphthyl)-1,2-hexadiene	22.99	2.88
4.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	23.97	3.90
5.	6-Amino-5-benzylamino-3-methylsulfonyl-1,2,4-triazine-1-oxide	25.98	3.30
6.	N-Ethyl-5,8-difluoro-1,4-dimethyl-1,2,3,4-tetrahydropyrido[3,4-b]pyrazin-7-amine	26.35	3.06
7.	2-Methyl-6-Styrylpyridine-3,4-Dicarboxylic Acid	26.62	1.24
8.	6-{[(1',1'Dimethylethyl) dimethylsilyl]oxy]-à-4-[(phenyl methoxy) phenyl]naphthalene-2-methanol	29.96	1.01
9.	2-Ethyl-5-propylpyridine	30.91	4.23
10.	4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole	31.53	4.68
11.	Dimethyl-2-[(ethoxycarbonyl)(3,4-dihydro-2H-1,4-benzothiazin-3-ylidene)methyl] fumate	31.83	3.39
12.	Methyl 3-(pentafluoro phenyl)acrylate	32.14	1.87
13.	5,5"-Dibromo-4'-methoxymethoxymethyl-2,2':6',2"-terpyridine	34.28	6.31
14.	2,5-Dimethoxybenzaldehyde oxime	37.52	9.52
15.	[4'-(3"-Ethyl-5"-methyl-2"-pyrrolyl)-2',3'-dichlorophenoxy]-acetic acid	38.23	2.02
16.	Methyl 2,12-Dibromo-7-phenyl-5,6,8,9-tetrahydrobenz[a,j]-anthracene-14-carboxylate	39.47	1.25
17.	(3-Fluoropyridin-2-yl)-[5-hydroxy-1-(4-methoxybenzyl)-1H-pyrazol-4-yl]methanone	39.88	1.91
18.	Ethyl-1[(5,6-difluoro-1-methyl-2-benzdiazolyl)amino]-6,7,8,9-tetrafluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate	40.25	1.06

In vitro anticancer activity by MTT assay

MTT assay is a reliable method for screening the anticancer activity by measuring the cell viability. The present study examined the *in vitro* anticancer activity of ethanol extract of *W. tinctoria* leaves against NH40 cell line using MTT assay. The incubation with different concentration of the extract affected the viability of NH40 cell line in dose dependent pattern. The extract was diluted to 5µl, 25µl, 50µl, 75µl, 100µl. The cell viability of NH40 cancer cells after incubation in different concentration of ethanol extract of leaves of *W. tinctoria* is depicted in figure 2. The IC_{50} value was 100µl and results showed that the ethanol extract posses significant anticancer activity.

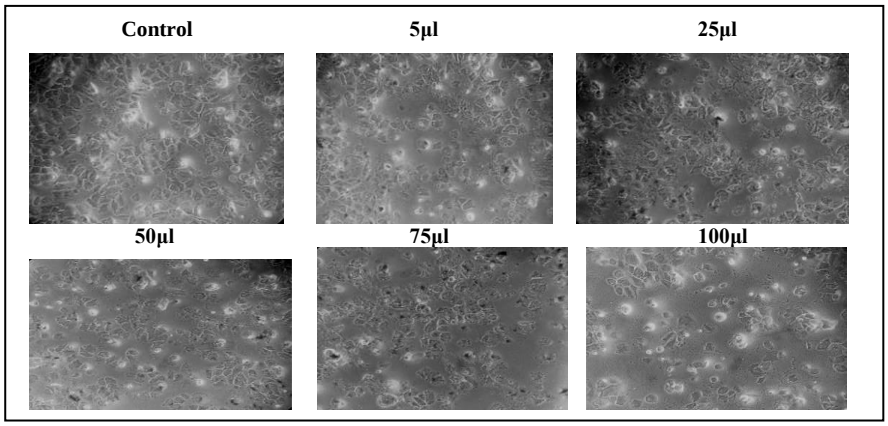


Figure 2: The cell viability of NH40 cancer cells after incubation in different concentrations of ethanol extract of leaves of *W. tinctoria.*

Table 3: % Cell inhibition in different concentration of ethanol extract of leaves of *W.tinctoria*.

S.No	Concentration (µl)	% Cell inhibition
1.	5	15
2.	25	21
3.	50	34
4.	75	39
5.	100	45

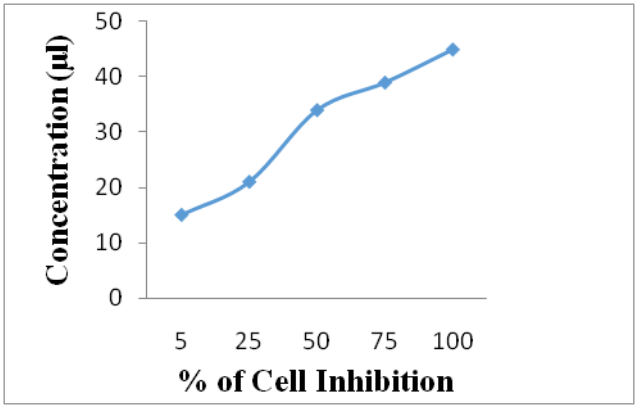


Figure 3: The IC_{50} value is determined by the plot of concentration Vs % incubation

IV. Conclusion

The present study reveals that the ethanol extract of *Wrightia tinctoria* leaves has shows that slight anticancer activity against NH40 Cell line. The isolation of active phytoconstituents is under process.

References

[1] Sivarajan VV and Balachandran I. Ayurvedic Drugs and Their Plant Sources, Oxford and IBH Publishing Co. PVT.Ltd. New Delhi. 1999. 267-269.
[2] Joshi, S.G.; Medicinal plants. New Delhi: Oxford and IBH publishing Co Pvt Ltd. 2000, 51-52.
[3] Veerapur VP, Palkar MB, Srinivasa H, Kumar MS, Patra S, Rao PGM, Srinivasan KK. The effect of ethanol extract of *Wrightia tinctoria* bark on wound healing in rats. J Nat Rem 2004; 4(2):155-159.
[4] Muruganadam AV, Bhattacharya SK, Ghosal S. Indole and flavonoid constituents of Wrightia tinctoria and W. tomentosa and W. coccinea. Ind J Chem 2000; 39B(2):125-131
[5] Ganesan S, Venkateshan G, and Banumathy N. Medicinal plants used by ethnic group thottianaickans of semmalai hills (reserved forest), hiruchirappalli district, Tamil Nadu. Indian J. of Trad. Knowledge. 5(2), 2006, 245-252.
[6] The Treatise on Indain Medicinal Plant, pp. 125-127.
[7] M.C. Divakar, S. Lakshmi Devi. Antiulcer activity of *Wrightia tinctoria* (Roxb) R.Br. Der Pharmacia Sinica. 2011; 2 (2): 355-360.
[8] P. R. Tharkar et al. Anti-inflammatory Study of Wrightia tinctoria R.Br Stem Bark in Experimental Animal Models. International Journal of Pharm Tech Research. 2010; 2(4):2434-2437.
[9] M.C. Divakar, S. Lakshmi Devi. Wound Healing Activity of the leaves of *Wrightia tinctoria*. Indian Drug, 2012; 49(3):40-46.
[10] Rajalakshmi, G. R., Jyoti Harindran, In – vitro Anthelmintic activity of Wrightia tinctoria. Int. Pharm Tech. Res. 2013, 5(2), 308-310.
[11] Vijayaragavan thangaraj.; Pharmacognostical and anti microbial studies on the leaf of *Wrightia tinctoria*. Int *J Pharm Tech*. **2013**, 5(1), 5326-5336.
[12] Jenilarani, D., Archana, H., Theriappan, P., Dineshkumar, R., Nirmal kumar, P., Phytochemical Analysis and Antioxidant Potentials of Endophytic Fungi Isolated From Leaves of Wrightia tinctoria, International Journal of Ethnomedicine and Pharmacological Research. 2013, 1(3), 179-183.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Recent Trends and Prediction of Long Intergenic Non-coding RNA – a Review

Cashlin Anna Suveetha G[1], Abirami SK[1], Jeyapriya Rachana S[1] and Ram Kothandan[1]*
Bioinformatics Laboratory, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, INDIA, ram.k.bt@kct.ac.in

Abstract*: Long non-coding RNAs (lncRNAs) are group of transcripts synthesized within the cells of all organisms. They play a crucial role in the various cellular processes viz., transcription regulation, chromatin remodelling. At present, only a small number of lncRNAs have been studied experimentally giving some important insights on the function they play in regulating the gene expression and other cellular processes. Due to its profound involvement in the cellular process, the identification of lncRNAs have emerged to be cutting-edge trend in biology. Several computational methods and tools are available for the prediction of lncRNAs. Here, in this review the current computational tools available for prediction of lncRNA along with their features and algorithms mentioning their accuracy been discussed. In addition, various databases containing the annotated lncRNAs are been highlighted. Further, the limitations of the tools and methods with the perspective towards developing new tools for prediction were also discussed.*

Keywords*: Long non-coding RNA, transcriptional regulation, computational tools*

I. Introduction

Among 20,000 protein-coding genes of the mammalian genome; mostly were dispelled between repetitive and largely non- transcribed sequence [1] . LincRNAs (Long intergenic noncoding RNAs) play diverse role in gene regulation and numerous cellular processes. They also play an important role in genetic mutations of human lincRNAs affiliate with diseases and several pathophysiological conditions [2] . Because of these important roles, over past decades study of non-protein-coding genes have been in the limelight particularly in mammalian genome and certain plant genomes. Structurally, RNAs can be classified into mRNAs (messenger RNAs), which are translated into proteins and ncRNAs (non-coding RNAs), which are functional RNAs does not encode a protein [3]. ncRNAs longer than 200 nucleotides were classified as lncRNA, emerged as potent regulators in mammals [4]. LincRNAs have been distinguished from lncRNA, are transcribed ncRNAs were not similar to annotated coding genes [5].

Structurally, RNAs can be classified into mRNAs (messenger RNAs), which are translated into proteins and ncRNAs (non-coding RNAs), which are functional RNAs does not encode a protein [3]. ncRNAs longer than 200 nucleotides were classified as lncRNA, emerged as potent regulators in mammals [4]. LincRNAs have been distinguished from lncRNA, are transcribed ncRNAs were not similar to annotated coding genes [5].

LincRNAs of animals especially in mammals have shorter conserved regions in comparison with plants like rice [4]. Functionally categorized, plant lincRNAs are largely similar to animals. Despite the fact that plant microRNAs were similar to mammals, it contains minimal long non-coding RNAs when compared to mammals [6]. The larger differentiated pattern and lower level of expression at lincRNA were encountered in mammalian and Arabidopsis, an plant species [2]. Many studies reveal that conserved regions in Protein Coding Transcripts (PCTs) are higher than lincRNA, this feature is reflected in CPC (a Support Vector Machine based predictor) with high sensitivity and low specificity [7]. The expression of lincRNAs are tissue-specific and thus suggests that mammalian lincRNA were transcribed by RNA polymerase-II and process using 3‘poly(A)addition and 5‘-capping [2].

Computational approaches employed in identification of lincRNAs intensifies with the types of dataset available, followed by the feature extraction and ranking for accurate identification. The dataset construction varies with the algorithm employed in identification process for instance CPC eliminates de-novo assembled transcripts; on the other hand PhyloCSF employs statistical comparison for distinguishing coding and non-coding regions and requires a higher time interval for prediction [7]. Considering, all these algorithmic facts and the molecular basis of lincRNA-mediated gene regulation is still found to be as mystery and only the regulatory roles of certain lincRNAs being recognized.

Experimental methods are time-consuming and expensive process, hence different computational methodologies were introduced to organize lincRNA for future experimental validation. Machine learning methods necessitate pre-process of input datasets and selection of features. With the available datasets, the identification of lincRNAs has become a challenging process; computational tools are largely involved in the prediction of lincRNAs in both plants and animals.

II. TOOLS AND ALGORITHMS ON PREDICTION

Prediction and annotation of lincRNAs studies involve numerous challenges which in turn attract the development of computational approaches. In addition, lack of experimental studies and unambiguous classification of lincRNA also raises the uncertainty in this process. Computational approaches such as Neural Network model (NN), Support Vector Machines (SVM), Genetic Algorithms (GA), Random Forest (RF), Hybrid Classifiers, Bayesian Networks (BNs) and Hidden Markov Model (HMM) have been widely used in prediction of ncRNAs. Among them SVM, RF and neural network are widely used approaches. The computational methods which involved in lincRNA studies have been discussed below in Table I.

A. Databases in prediction of ncRNA

Continuous increase in development of transcripts and sequencing techniques leads to enhance the available non-coding RNA data and also introduces various computational approaches especially in the study of lincRNA. This requires a proper repository to pull through the available non-coding RNA in various databases. Diverse resources (such as LNCipedia, ChiPBase, NONCODE, lncRNAdb and the functional lncRNA database) containing plant and mammalian datasets provide their functional annotation along with their interactions with other proteins and RNAs [8]–[11]. Available databases differ in coverage and quality of datasets which acquired from various plants and animals. Comparatively databases such as NONCODE, PLncDB, PNRD, PncRNADB provide datasets which acquired from plant have been higher than mammalian species [12]–[14]. Despite lncRNAdb provides only a few plant species, it is considered to be reliable for it offer datasets for functional characterization has been done by knock-down experiments [13]. Other databases include CHIPBase, DIANA-LncBase, LNCipedia, lncRNADisease, lncRNome and Functional lncRNA Database are provide transcripts of experimentally obtained and computationally annotated datasets of noncoding RNAs [8], [9], [14]–[18]. Plant dataset obtained from *Arabidopsis thaliana* have been majorly available in databases providing plant datasets (such as NONCODE and PlncDB) [12], [14]. Functional ncRNA Database contains datasets extracted manually with a parallel database of protein-coding RNAs of plants and mammals.

B. Current approaches towards prediction

Molecular studies on lincRNA can reveal their regulatory roles, however they fail to classify them as coding and non-coding. Machine learning studies have been emerged to identify lincRNA in various mammals and plants. Preprocessing of input dataset and selection of features is a time-consuming process during the identification of lincRNA and feature selection process differs based on datasets that have been taken from previous studies. Features may range from ORF (Open Reading Frame) identification, ORF conservation, frame length, experimental strategies, combinational strategies, interaction with ncRNAs to biogenesis of these non-coding RNAs. Biogenesis is the most fundamental and primary feature in identification of ncRNA from mRNA, further its ease of calculation and discriminative power. To add on, the ORF length also discriminates these non-coding from coding part [18], [19].

Table I describes algorithms, features used in prediction tools and its accuracy that were obtained using sample dataset. Deep Neural Network (DNN), SVM and Random forest are the most used computational algorithms in numerous prediction tools (includes iSeeRNA, PlncPRO, LncRNA-ID, COME, PLEK, PlantRNA-Sniffer, lncRScan-SVM, CPC and DeepLNC) to distinguish noncoding RNAs from coding RNAs [20]–[27]. SVM, a non-parametric method creates models for regression and classification and not limited by the size of datasets obtained either from mammals or plants [24]. SVM construct a classification hyper-plane to separate the transformed data. Based on its good performance and high accuracy in classification it is not only used in ncRNA classification but also in bioinformatics analysis especially as prediction of sub-cellular localization, microarray based cancer classification and prediction of protein function [28], [29] . Among the various prediction tools, PlantRNA-Sniffer, a lincRNA prediction tool particularly for plants shows 99% accuracy incase of maize plants [24] . Based on the availability of datasets and approaches used computational methods differ in their accuracy and sensitivity of prediction. Comparatively SVM, random algorithm and DNN shows reasonable results than the other methods (includes alignment free logistic model, logistic regression model, phylogenetic codon model, melting-temperature and primer-dimer prediction algorithm) [30]–[34]. Next to PhyloCSF, DNN (a non-coding RNA prediction tool build using neural network algorithm) has showed an accuracy of 98% by experiencing the features ORF length, sequence length, interaction with other RNA and biogenesis [26] . Explanation of some prediction tools, its methods and description were explained in Table I. Then again DNN, have been arise to control the problems faced during the pre- and post-preparing steps during feature selection from huge raw

dataset [35] . Using mammalian datasets (obtained from mouse and human), CPC shows higher accuracy of 95%. While using plant datasets, PlantRNA-Sniffer is the only available tool for prediction of lincRNA with an accuracy of 99% [24], [25] . Using k-mer scheme along with SVM, PLEK delivers >90% in simulated data and paired de-nova assembled transcriptome datasets [23] . Feature selection includes fickett score and hexamer score were identified while using logistic regression model [31], [32], but these methods deliver lower accuracy when compared to other approaches. For identifying bimolecular secondary structure of input RNAs, autodimer have been used and ORF and FF-score can be identified using framefinder [20], [34]. While using DNN algorithm, biogenesis were identified along with other features (includes ORF length, sequence length and interaction with other ncRNA) in the cases of DeepLNC [26] . Others features such as HSP's (High-scoring Segment Pairs) and ribosome interaction (to neglect false results) have been used along with ORF and protein conservation for distinguishing coding and non-coding RNAs [21], [25].

Tools for prediction and annotation of lncRNA and lincRNA	**Computational approach**	**Features involved**	**Outcomes**	**comments**
PLEK	Improved k-mer scheme and SVM algorithm	lncRNA transcripts and k-mer scheme	Accuracy - >90%	Distinguish lncRNA from mRNA without the involvement of annotations or genomic sequences
PlantRNA Sniffer	Support vector machine learning algorithm	Sequence length, ORF length and nucleotide pattern frequencies	Accuracy - 99% (in maize)	
PlncPRO	Random forest algorithm	FF-score and ORF coverage	Accuracy - 94%	
LncRNA-ID	Balanced random forest algorithm	Ribosome interaction, ORF and protein conservation	Accuracy - 94%	
lncRScan-SVM Support vector machine learning algorithm		Transcript length, exon length, exon count, standard deviation, CDS score and conservation scores	Accuracy - 91% , Specificity - 94%	It shows high specificity and low sensitivity, which implies that it may predict many false negatives
COME	Decompose-compose method and Random forest algorithm	Multiplescorematrixwithmultiple features and transcripts	-	It annotate and characterize every predicted lncRNA transcripts with multiple lines of characteristics is unique feature when compared with other tools
CPAT	Alignment-free logistic model	ORF size, ORF coverage, hexamer usage bias and Fickett TESTCODE statistic	Specificity - 97% Sensitivity – 96%	
lncScore	Logistic regression model algorithm	ORF length, coverage, hexamer score, Fickett score, coding score, GC content	Accuracy - 89%, specificity - 94%, sensitivity - 84%	
CPC	Support vector machine learning algorithm	Log-odds score, Coverage of predicted ORF, Integrity of predicted ORF, Number of hits, HSPs (High-scoring Segment Pairs) and frame	Accuracy - 95.77%	Comparatively shows higher performance than CONC

		score		
PhyloCSF	Phylogenetic codon models	Standard dN/dS test in empirical codon models and genome wide training data	Correlates scores with CSF	PhyloCSF theoretically attractive than the CSF, which distinguish noncoding and protein coding regions on formal statistical comparison.
LncTAR	Melting-temperature and primer-dimer prediction algorithm	bimolecular secondary structure of input RNAs using AutoDimer	Accuracy 80%	Binding regions between paired RNA can be predicted using Nearest-Neighbor method provides an advantage of more reliable and effective than matching score method.
DeepLNC	Deep neural network	ORF length, sequence length, interaction with other ncRNA and biogenesis	Accuracy 98.07%, Specificity 97.19%, Sensitivity 98.98%	It also used to test human genome dataset with an accuracy of 99%
linc-SF	Support Vector Machine learning algorithm (SVM)	CPC value, optimal feature subsets, ratio of MFE and sequence length, minimum free energy of secondary structure and ratio of MFE and content of G and C.	Accuracy 96.19%	It is effective on the prediction of lincRNA in human datasets since identification occurs based on sequence and structural features.

III. CONCLUSION

DNN have arisen to control the risks emerged during the feature selection and dataset preparation while using large size of mammalian and plant datasets [26] . Even though methods includes SVM and random algorithm have been used in larger number of tools in the identification of lincRNA (datasets obtained from mammalian and plant datasets), DNN were used for its easier feature selection. In lincRNA identification iSeeRNA delivers higher accuracy of 96.1% using mammalian datasets (obtained from humans), whereas using plant dataset, PlantRNA-Sniffer delivers larger accuracy of 99% (in case of maize) [24], [36]. Feature selections (includes ORF size, ORF length, ORF proportion, exon length and count, conservation score, sequence length and nucleotide pattern frequencies) have been majorly identified for identification of lincRNA. Among the various features, exon conservation and ORF proportion were delivered highest discrimination power for identification of lincRNA [7]. Since lincRNA takes part in numerous gene regulatory diseases, study on lincRNA should participate on both plants as well as mammals. Comparatively, in plants there are limited findings due to shortage of datasets and problems such as feature selection involved in it. In future approaches, a large number of computational prediction is needed to bring around the gene regulatory diseases associated along with lincRNAs.

REFERENCES

[1] I. Ulitsky and D. P. Bartel, "lincrnas: genomics, evolution, and mechanisms," Cell, vol. 154, no. 1, pp. 26–46, 2013.
[2] J. Liu, C. Jung, J. Xu, H. Wang, S. Deng, L. Bernad, C. Arenas-Huertero, and N.-H. Chua, "Genome-wide analysis uncovers regulation of long intergenic noncoding rnas in arabidopsis," The Plant Cell, vol. 24, no. 11,
pp. 4333–4345, 2012.
[3] J. Liu, J. Gough, and B. Rost, "Distinguishing protein-coding from non-coding rnas through support vector machines," PLoS genetics, vol. 2, no. 4, p. e29, 2006.
[4] Y.-C. Zhang, J.-Y. Liao, Z.-Y. Li, Y. Yu, J.-P. Zhang, Q.-F. Li, L.-H. Qu, W.-S. Shu, and Y.-Q. Chen, "Genome-wide screening and functional analysis identify a large number of long noncoding rnas involved in the

sexual reproduction of rice," Genome biology, vol. 15, no. 12, p. 512, 2014.
[5] J. D. Ransohoff, Y. Wei, and P. A. Khavari, "The functions and unique features of long intergenic non-coding rna," Nature Reviews Molecular Cell Biology, 2017.
[6] Q.-H. Zhu and M.-B. Wang, "Molecular functions of long non-coding rnas in plants," Genes, vol. 3, no. 1, pp. 176–190, 2012.
[7] K. Sun, X. Chen, P. Jiang, X. Song, H. Wang, and H. Sun, "iseerna: identification of long intergenic non-coding rna transcripts from transcriptome sequencing data," BMC genomics, vol. 14, no. 2, p. S7, 2013.
[8] P.-J. Volders, K. Helsens, X. Wang, B. Menten, L. Martens, K. Gevaert, J. Vandesompele, and P. Mestdagh, "Lncipedia: a database for annotated human lncrna transcript sequences and structures," Nucleic acids
research, vol. 41, no. D1, pp. D246–D251, 2012.
[9] J.-H. Yang, J.-H. Li, S. Jiang, H. Zhou, and L.-H. Qu, "Chipbase: a database for decoding the transcriptional regulation of long non-coding rna and microrna genes from chip-seq data," Nucleic acids research, vol. 41, no. D1, pp. D177–D187, 2012.
[10] Y. Zhao, H. Li, S. Fang, Y. Kang, W. Wu, Y. Hao, Z. Li, D. Bu, N. Sun, M. Q. Zhang et al., "Noncode 2016: an informative and valuable data source of long non-coding rnas," Nucleic acids research, vol. 44, no. D1, pp. D203–D208, 2015.
[11] P. P. Amaral, M. B. Clark, D. K. Gascoigne, M. E. Dinger, and J. S. Mattick, "lncrnadb: a reference database for long noncoding rnas," Nucleic acids research, vol. 39, no. suppl 1, pp. D146–D151, 2010.
[12] C. Liu, B. Bai, G. Skogerbø, L. Cai, W. Deng, Y. Zhang, D. Bu, Y. Zhao, and R. Chen, "Noncode: an integrated knowledge database of non-coding rnas," Nucleic acids research, vol. 33, no. suppl 1, pp. D112–D115, 2005.
[13] G. Bhatia, N. Goyal, S. Sharma, S. K. Upadhyay, and K. Singh, "Present scenario of long non-coding rnas in plants," Non-Coding RNA, vol. 3, no. 2, p. 16, 2017.
[14] S. Fritah, S. P. Niclou, and F. Azuaje, "Databases for lncrnas: a comparative evaluation of emerging tools," Rna, vol. 20, no. 11, pp. 1655–1665, 2014.
[15] M. D. Paraskevopoulou, G. Georgakilas, N. Kostoulas, M. Reczko, M. Maragkakis, T. M. Dalamagas, and A. G. Hatzigeorgiou, "Diana-lncbase: experimentally verified and computationally predicted
microrna targets on long non-coding rnas," Nucleic acids research, vol. 41, no. D1, pp. D239–D245, 2012.
[16] G. Chen, Z. Wang, D. Wang, C. Qiu, M. Liu, X. Chen, Q. Zhang, G. Yan, and Q. Cui, "Lncrnadisease: a database for long-non-coding rna-associated diseases," Nucleic acids research, vol. 41, no. D1, pp. D983–D986, 2012.
[17] D. Bhartiya, K. Pal, S. Ghosh, S. Kapoor, S. Jalali, B. Panwar, S. Jain, S. Sati, S. Sengupta, C. Sachidanandan et al., "lncrnome: a comprehensive knowledgebase of human long noncoding rnas," Database, vol. 2013, 2013.
[18] M. E. Dinger, K. C. Pang, T. R. Mercer, and J. S. Mattick, "Differentiating protein-coding and noncoding rna: challenges and ambiguities," PLoS computational biology, vol. 4, no. 11, p. e1000176, 2008.
[19] M. C. Frith, T. L. Bailey, T. Kasukawa, F. Mignone, S. K. Kummerfeld, M. Madera, S. Sunkara, M. Furuno, C. J. Bult, J. Quackenbush et al., "Discrimination of non-protein-coding transcripts from protein-coding
mrna," RNA biology, vol. 3, no. 1, pp. 40–48, 2006.
[20] U. Singh, N. Khemka, M. S. Rajkumar, R. Garg, and M. Jain, "Plncpro for prediction of long non-coding rnas (lncrnas) in plants and its application for discovery of abiotic stress-responsive lncrnas in rice and chickpea," Nucleic Acids Research, vol. 45, no. 22, pp. e183–e183, 2017.
[21] R. Achawanantakun, J. Chen, Y. Sun, and Y. Zhang, "Lncrna-id: Long non-coding rna identification using balanced random forests," Bioinformatics, vol. 31, no. 24, pp. 3897–3905, 2015.
[22] L. Hu, Z. Xu, B. Hu, and Z. J. Lu, "Come: a robust coding potential calculation tool for lncrna identification and characterization based on multiple features," Nucleic acids research, vol. 45, no. 1, pp. e2–e2, 2016.
[23] A. Li, J. Zhang, and Z. Zhou, "Plek: a tool for predicting long non-coding rnas and messenger rnas based on an improved k-mer scheme," BMC bioinformatics, vol. 15, no. 1, p. 311, 2014.
[24] L. M. Vieira, C. Grativol, F. Thiebaut, T. G. Carvalho, P. R. Hardoim, A. Hemerly, S. Lifschitz, P. C. G. Ferreira, and M. E. M. Walter, "Plantrna sniffer: a svm-based workflow to predict long intergenic non-coding rnas in plants," Non-Coding RNA, vol. 3, no. 1, p. 11, 2017.
[25] L. Kong, Y. Zhang, Z.-Q. Ye, X.-Q. Liu, S.-Q. Zhao, L. Wei, and G. Gao, "Cpc: assess the protein-coding potential of transcripts using sequence features and support vector machine," Nucleic acids research, vol. 35,
no. suppl 2, pp. W345–W349, 2007.
[26] R. Tripathi, S. Patel, V. Kumari, P. Chakraborty, and P. K. Varadwaj, "Deeplnc, a long non-coding rna prediction tool using deep neural network," Network Modeling Analysis in Health Informatics and
Bioinformatics, vol. 5, no. 1, p. 21, 2016.
[27] L. Sun, H. Liu, L. Zhang, and J. Meng, "lncrscan-svm: a tool for predicting long non-coding rnas using support vector machine," PloS one, vol. 10, no. 10, p. e0139654, 2015.
[28] K. M. Borgwardt, C. S. Ong, S. Sch¨onauer, S. Vishwanathan, A. J. Smola, and H.-P. Kriegel, "Protein function prediction via graph kernels," Bioinformatics, vol. 21, no. suppl 1, pp. i47–i56, 2005.

[29] T. S. Furey, N. Cristianini, N. Duffy, D. W. Bednarski, M. Schummer, and D. Haussler, "Support vector machine classification and validation of cancer tissue samples using microarray expression data," Bioinformatics,
vol. 16, no. 10, pp. 906–914, 2000.
[30] Z. Lei and Y. Dai, "An svm-based system for predicting protein subnuclear localizations," BMC bioinformatics, vol. 6, no. 1, p. 291, 2005.
[31] L. Wang, H. J. Park, S. Dasari, S. Wang, J.-P. Kocher, and W. Li, "Cpat: Coding-potential assessment tool using an alignment-free logistic regression model," Nucleic acids research, vol. 41, no. 6, pp. e74–e74, 2013.
[32] J. Zhao, X. Song, and K. Wang, "lncscore: alignment-free identification of long noncoding rna from assembled novel transcripts," Scientific reports, vol. 6, p. 34838, 2016.
[33] M. F. Lin, I. Jungreis, and M. Kellis, "Phylocsf: a comparative genomics method to distinguish protein coding and non-coding regions," Bioinformatics, vol. 27, no. 13, pp. i275–i282, 2011.
[34] J. Li, W. Ma, P. Zeng, J. Wang, B. Geng, J. Yang, and Q. Cui, "Lnctar: a tool for predicting the rna targets of long noncoding rnas," Briefings in bioinformatics, vol. 16, no. 5, pp. 806–812, 2014.
[35] Y. Zhang, H. Huang, D. Zhang, J. Qiu, J. Yang, K. Wang, L. Zhu, J. Fan, and J. Yang, "A review on recent computational methods for predicting noncoding rnas," BioMed research international, vol. 2017, 2017.
[36] Y. Wang, Y. Li, Q. Wang, Y. Lv, S. Wang, X. Chen, X. Yu, W. Jiang, and X. Li, "Computational identification of human long intergenic non-coding rnas using a ga–svm algorithm," Gene, vol. 533, no. 1, pp.94–99, 2014.

.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Studies on the antioxidant activity of the fern *Hemionitis arifolia* (Burm.) Moore

Dr. R. Rakkimuthu[1], P. Naveenraj[2], M. Susila[3], A. Sindhusha[4] and R. Indu[5]

[1, 2, 3, 4,5] PG and Research Department of Botany

Dr. Mahalingam Center for Research and Development, Nallamuthu Gounder Mahalingam College Pollachi-01, TAMILNADU, INDIA, Email id: biorakki@gmai.com, rakkimuthu@ngmc.org

Abstract*: In the present work, antioxidant ability of extracts obtained from the fern Hemionitis arifolia (Burm.) Moore, which is endemic to India, was investigated. Fresh fronds were used in enzymatic assay and dried fronds were used in non-enzymatic assay. Antioxidant contents of the fronds of the fern H. arifolia was determined by adopting three common enzymatic assay (Catalase, Ascorbic acid oxidase and Polyphenol oxidase) and antioxidant contents of the methanol extract from the fern fronds were evaluated by adopting non enzymatic methods like DPPH radical scavenging activity, ABT's radical scavenging activity, Nitric oxide radical scavenging activity, total antioxidant assay and FRAP assay methods. The fronds contains the enzymes catalase, ascorbic acid oxidase and polyphenol oxidase of 32.69 Units/g tissues, 0.001 Units /g tissues and 0.0421 Units /g tissues respectively. DPPH, ABT's, Nitric oxide scavenging activity exhibited an IC_{50} value of 47.84mg/ml, 36.73 mg/ml and 125 mg/ml respectively. Total antioxidant activity and FRAP assay exhibited significant activity and its absorbance increased with increase in concentration. The present study suggests that H. arifolia has significant antioxidant activity and may be useful for the discovery of novel antioxidant agents of fern origin and used in pharmaceutical industry.*

Keywords*: Enzymatic assay; DPPH; ABT's; Nitric oxide; Total antioxidant activity; FRAP assay*

I. Introduction

Antioxidants are defined as molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Naturally occurring antioxidants are needed to replace the synthetic antioxidants which are restricted due to their inherent carcinogenicity [1].

The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. Pteridophytes have a vital role in the earth's biodiversity. In the world flora of pteridophytes 12,000 species has been identified among which 1,000 species into 70 families and 191 genera are occur in India. *H. Arifolia*, family Hemionitidaceae is one of the endemic and widely distributed species on Tirumala hills of Tirupathi, Andhra Pradesh, India. Ethno medicinally, the genus is important and popularly known as Ramabanum. This fern acts an excellent antibacterial agent because it has a more secondary metabolite [2].

The leaf juice has been used to cure burns and diabetes. Though the recent ethnobotanical, pharmacological and biological searches have revealed medicinal, pharmaceutical and phytochemical attributes of Pteridophytes, which have valuable potential applications in health and industry. Still many species of Pteridophytes are yet to be explored for their potential applications to isolate new active principles. Till now there is no literature on antioxidant activity of the fern *H. arifolia*. Thus the present study was to investigate the enzymatic and non-enzymatic antioxidant contents of the fern, *Hemionitis arifolia* (Burm) Moore.

II. Materials and Methods

Plant material collection

Hemionitis arifolia (Burm.) Moore was collected from the Valparai hills, Western Ghats of Tamilnadu, South India.

Sample preparation for enzymatic antioxidant assay

One gram of fresh fronds of *H. arifolia* were weighed and grounded in a mortar and pestle under chilled condition with buffer solution pH-7.0, 6.5 and 6.5 for catalase, ascorbic acid oxidase and polyphenoloxidase

activity respectively. The extract was centrifuged at 4˚C for 10 min at 10,000 rpm. The supernatant obtained was used for further enzymatic assays.

Assay of Catalase

The method of Luck (1974) as mentioned by Sadasivam and Manikam [3] was adopted to measure the activity of catalase**.** The fern frond extract (0.1ml) was added to the reaction mixture containing 3 ml of H_2O_2 and 0.01M phosphate buffer (pH 7.0) separately and OD was taken at 240 nm. The time taken for decrease in the absorbance from 0.45 to 0.4 is noted as ΔT. The activity of the enzyme is expressed in the terms of µ mole of H_2O_2 consumed/ min/ mg protein.

Units in the assay mixture= 17/ΔT

ΔT is the time in seconds.

Assay of Ascorbic acid oxidase

Assay of ascorbic acid oxidase activity was carried out by following the procedure of oberbacher and vines [4]. The 3.0 ml of the substrate solution (8.8 mg of ascorbic acid in 300ml phosphate buffer, pH(5.6). 0.1ml of the frond extract was added separately and the absorbance change was observed at 265 nm for every 30 seconds for 5 minutes. One enzyme unit is equivalent to 0.01 OD change per minutes.

Assay of polyphenol oxidase

Polyphenol oxidases activity was assayed by the procedure of Esterbauer method [5]. The frond extract 0.2 ml was taken in cuvette and added with reaction mixture containing 2.5 ml of phosphate buffer and 0.3 ml catechol solution. The change in the absorbance was recorded every 30 sec for 5 minutes. One unit of either catechol oxidase or laccase is defined as the amount of enzyme that transforms one F mole of dihydrophenol to one F mole of quinine/minute. The enzyme activity is expressed as u/g tissue. The enzyme activity can be calculated using the formula,

Enzyme unit= k × (A/minute)

K for catechol oxidase= 0.272

A= Average Absorbance of 5 minutes

Sample Extraction

The fern (*Hemionitis arifolia*) was collected and shade dried at room temperature. The fern material was chopped and ground to fine powder using a pulverizer. Dried powder was packed in a soxhlet apparatus and extracted with methanol at 60-65 ℃ for 3-4 h. The extract was filtered through a whatman filter paper No.1 and the filtrate was collected and concentrated under reduced pressure at 40 ℃. The extract was dried and stored at 4 ℃ in storage vials for experimental use.

In vitro Free Radical Scavenging activity

Sample preparation

The methanolic extract of *H. arifolia* 100.0 mg was dissolved in 1 ml of DMSO. The concentration viz, 12.5, 25.0, 50.0 and 100 mg/ml concentration of the sample was prepared and used for different assays. Ascorbic acid was used as standard for comparing the activities.

DPPH radical scavenging activity

The free radical scavenging capacity of the evaporated sample of *H. arifolia* extract was determined using DPPH [6]. DPPH solution was prepared in 95% methanol. Different concentration (12.5-100mg/ml) of methanol extract of *H. arifolia* was reacted with freshly prepared 3.0 ml DPPH solution (0.1mM) and incubated at room temperature. After 30minutes, the absorbance was taken at 517 nm using a spectrophotometer (Labman UV-Visible spectrophotometer). Ascorbic acid was used as a reference standard. The Control was devoid of the sample. Standard ascorbic acid was treated similar to the extracts.

ABT's radical scavenging assay

ABT's (2, 2'-azinobis-3-ethylbenzothiozoline- 6-sulphonic acid) assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process that is associated with a change in absorption. The relatively stable ABT's radical was green and it was quantified spectrophotometrically at 734nm. Fresh ABT's solution was prepared for each assay. Different concentration (12.5-100 mg/ml) of *H. arifolia* extract was allowed to react with 1 ml of the ABT's solution and the absorbance was taken at 734nm after 7 minutes using a spectrophotometer [7].

Nitric oxide scavenging activity

The Nitric oxide scavenging capacity of the extract of *H. arifolia* was determined using nitric oxide generated by oxidation of sodium nitroprusside [8]. Sodium nitroprusside (10 mM, 2ml) in phosphate buffer saline was incubated with different concentration of methanol extract (12.5-100 mg/ml) at room temperature for 30 minutes. After 30 minutes, 0.5 ml of the solution was mixed with 1ml of Griess reagent. The absorbance was measured at 546 nm. Ascorbic acid was used as a reference standard.

Total antioxidant capacity by phosphomolybednum assay

The spectrophotometric measurement of Total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo (V) by antioxidant compound and the formation of green phosphate / Mo (v) complex at acidic pH [9]. Different concentrations (12.5-100 mg/ml) of methanolic leaf extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction mixture was incubated at 95˚C for 90 min. Then the absorbance of the solution was measured at 695 nm. Methanol (0.3 ml) was used as blank and Ascorbic acid was used as reference standard.

FRAP assay

The reducing power of methanolic extracts of *H. arifolia* was determined according to the method previously described by Oyaizu [10]. Different concentrations of methanolic extracts of *H.arifolia* was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%) separately. The mixtures were incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixtures and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and FeCl3 (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank

III. Results and Discussion

Enzymatic assays

The fronds of fern showed 32.69 Units/g tissues, 0.001 Units /g tissues and 0.042 Units /g tissues for catalase, ascorbic acid oxidase and polyphenol oxidase content respectively. Catalase is antioxidant enzyme which plays an important role in the body defence mechanism against the harmful effects of the reactive oxygen species (ROS) and free radicals in biological systems [11]. Tingentsai and Taatsai leaves (leafy vegetables) showed the ascorbic acid oxidase activity of 0.008 and 0.049 U/gm of tissue respectively [12]. In the current report the ascorbic acid oxidase activity is lower than both the leafy vegetables. The activity of PPO in the present study is lower than *Terminalia bellirica* leaves and fruits [13].

Non Enzymatic assays

DPPH radical scavenging activity

The table (I) indicates the free radical scavenging ability of the fern *H. arifolia* methanol extract and standard ascorbic acid. The *H. arifolia* extract scavenged DPPH radical of 78.54% at 100mg/ml concentration with an IC_{50} value of 47.84mg/ml. The activity was not significant when compared to standard ascorbic acid which had an IC_{50} value of 4.84 mg/ml. The efficacy of extract of *Azolla pinnata* and *Azolla rubra* showed the IC_{50} value of 7.32 µg/ml) and14.47 µg/ml respectively [14]. The methanol extract of *H. arifolia* showed less antioxidant activity when compared with *A. rubra* and *A. pinnata.*

DPPH radical scavenging activity was observed in the extracts of *Adiantum iunulatum* (IC_{50} 0.4± 0.3 mg/ml) and *Diplazium esculentum* (0.32± 0.12 mg/ml). The methanol extract of *H. arifolia* showed less DPPH scavenging activity than *Adiantum iunulatum* and *Diplazium esculentum* [15].

Nishika Jaishee and Usha Chakraborty [16] investigated the *Pteris biaurita L*. The antioxidant activities of hot water, methanol, ethanol extracts were evaluated by DPPH free radical scavenging activity. The DPPH was higher in Hot water extract. DPPH inhibition activity compared to methanol and ethanol. The IC_{50} values for hot water, methanol, ethanol and ascorbic acid were 1.107, 1.84, 5.09 and 0.142 mg/ml respectively. The methanol extract of *H. arifolia* showed less antioxidant activity than *Pteris biaurita L*.

ABT's radical scavenging activity

Effective ABT's radical scavenging process was exhibited by the fern methanol extracts of *H. arifolia* with 98.71 % inhibition at 100 mg/ml concentration and IC_{50} value of 36.73 mg/ml. The ascorbic acid had an IC_{50} value of 3.57 mg/ml (**Table-II**). Hassan *et al*. [17] studies the ABT's radical scavenging activity of eight fern species. Among the tested extracts, *Athyrium filix-femina* was the strongest ABT's radical cation scavenger with TEAC value about 29.85µmol Trolox/g followed by *Polystichum aculeatum* aerial parts extract (22.45 ± 2.14 µmol Trolox/g). The methanol extract of *H. arifolia* showed less antioxidant activity than *Athyrium filix-femina* and *Polystrichum aculeatum.*

NO radical scavenging activity

The methanol extract of *H. arifolia* directly competes with oxygen to react with the nitric oxide and thereby inhibits nitrite formation. The *H. arifolia* methanol extract showed scavenging action against nitric oxide (NO) induced release of free radicals and the value was 44 at 100 mg/ml concentration with an IC_{50} value of 125 mg/ml. The IC_{50} value for ascorbic acid was 14.72 mg/ml (**Table-III**).

Tsun thai chai [18] to determine the NO activities of the leaf and rhizome extracts of *Phymatopteris triloba*. NO scavenging activity of both extracts was about 30% lower than that of ascorbic acid. In the present study the NO activities of the *H. arifolia* showed lower activity compare to the ascorbic acid.

Nishika Jaishee and Usha Cakraborty [16] studied the Nitric oxide scavenging activity of *Pteris biaurita* L. Nitric oxide scavenging activity, methanol extract exhibited slightly higher activity than ethanol extract with IC_{50} values of 694.825µg/ml and 708.007µg/ml respectively. Hot water extract showed very less activity with

IC_{50} value of 915.137µg/ml. The methanol extract of *H. arifolia* shows less antioxidant activity than ethanol, methanol and hot water extract of *Pteris biaurita* L.

Total antioxidant capacity by Phosphomolybednum

Increase in absorbance indicates increase in total antioxidant capacity. The *H. arifolia* methanol extract exhibited significant activity and its absorbance increased with increase in concentration and the values were in comparison with ascorbic acid. The methanol extract exhibited significant absorbance of 0.282 for 100 mg/ml. Ascorbic acid had an absorbance of 0.009 at 10 mg/ml concentration (**Table-IV**).

Selvaraj *et al*. [19] to study the antioxidant effects of methanol extract of *Azolla microphylla*. The assay was based on the reduction of Mo (VI) to Mo (V) by various concentrations of green gold nanoparticles (1, 2, 3, 4 and 5 mg/mL). The *H. arifolia* methanol extract exhibited significant activity and its absorbance increased with increase in concentration and the values were in comparison with ascorbic acid. Saleha *et al*. Investigated the total antioxidant activity of *Diplazium esculentum.* The total antioxidant capacity (TAC) of two extracts DECH and DEM showed higher antioxidant activity compared to standard ascorbic acid at all the concentrations [20]. The *H. arifolia* methanol extract exhibited significant activity and its absorbance increased with increase in concentration and the values were in comparison with ascorbic acid.

FRAP assay

The reducing power of *H. arifolia* methanol extract was shown in **table-V**. The *H. arifolia* methanol extract exhibited significant activity and its absorbance (0.343 to 1.633) increased with increase in concentration at 12.5 mg/ml to 100 mg/ml. The *H. arifolia* extract showed potent ferric reducing power at 100 mg/ml concentration. Ascorbic acid had significant ability to reduce the Fe^{3+} with the absorbance of 0.386 at 10 mg/ml concentration. Increases in absorbance value indicate increase in Reducing Power capacity.

Jasim *et al*. [21] studied the ferric reducing ability of *B. orientale*. To evaluate the ferric reducing potential expressed as Trolox equivalent capacity (TE). Analysis of the different parts extracts showed that the plant extracts exhibited a significantly different ($p<0.05$) reducing ability to ferric compounds. In the present study, the results indicate that the reducing ability of the extract of *Hemionitis arifolia* was increased with the absorbance. Maneesha *et al*. [22] revealed the ferric reducing ability of *Pteris vittata*. All the extracts of *Pteris vittata* were determined from distinct color changes (yellow, green, and blue) at 3000 rpm. The high absorbance of the reaction mixture indicates high reducing power in Rajpur road sample. In the present study, the results indicate that the reducing ability of the extract of *Hemionitis arifolia* was increased with the absorbance.

Table: I DPPH radical scavenging activity of *H. Arifolia*

DPPH radical scavenging activity of *H. arifolia*					
Concentration	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**IC_{50} mg/ml**
Methanol extract (% of inhibition)	53.32	71.01	71.76	78.54	47.84
Concentration	**1.25 mg/ml**	**2.5 mg/ml**	**5 mg/ml**	**10 mg/ml**	**IC_{50} mg/ml**
Ascorbic acid (% of inhibition)	28.85	55.83	70.89	84.19	4.84

Table: II ABT's radical scavenging activity of *H. Arifolia*

ABT's radical scavenging activity of *H. arifolia*					
Concentration	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**IC_{50} mg/ml**
Methanol extract (% of inhibition)	80.28	93.71	97.42	98.71	36.73
Concentration	**1.25 mg/ml**	**2.5 mg/ml**	**5 mg/ml**	**10 mg/ml**	**IC_{50} mg/ml**
Ascorbic acid (% of inhibition)	97	98	98.42	99.85	3.57

Table: III NO radical scavenging activity of *H. Arifolia*

NO radical scavenging activity of *H. arifolia*					
Concentration	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**IC_{50} mg/ml**
Methanol extract (% of inhibition)	1.84	7.83	14.05	44.00	125
Concentration	**1.25 mg/ml**	**2.5 mg/ml**	**5 mg/ml**	**10 mg/ml**	**IC_{50} mg/ml**
Ascorbic acid (% of inhibition)	0.46	5.06	11.98	37.78	14.72

Table :IV Total antioxidant capacity by Phosphomolybednum

Total antioxidant capacity by Phosphomolybednum of H. arifolia				
Concentration	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
Methanol extract (Absorbance)	0.006	0.037	0.075	0.282
Concentration	**1.25 mg/ml**	**2.5 mg/ml**	**5 mg/ml**	**10 mg/ml**
Ascorbic acid (Absorbance)	0.004	0.006	0.007	0.009

Table : V Reducing power assay of *H. arifolia*

FRAP assay of *H. arifolia*				
Concentration	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
Methanol extract (Absorbance)	0.343	0.443	1.424	1.633
Concentration	**1.25 mg/ml**	**2.5 mg/ml**	**5 mg/ml**	**10 mg/ml**
Ascorbic acid (Absorbance)	0.093	0.160	0.220	0.386

IV. Conclusion

Our findings suggest that *H. arifolia* has significant antioxidant activity and it could be very useful in the discovery of novel antioxidant agents of fern origin. Further phytochemical studies are required to determine and isolate compounds responsible for the antioxidant effects of this species. *H. arifolia* might be considered as a potential source of metabolites which could be developed as precursor for antioxidants drugs.

V. Acknowledgement

The authors are grateful to the Tamilnadu State Council for Science and Technology, Chennai for providing financial support to complete the part of M.Sc., Botany dissertation.

VI. References

[1] E.S. Abdel Hameed, "Total Phenolic contents and free radical scavenging activity of certsain Egyptian Ficus species leaf samples", Food Chemistry, 114, 1271-1277, 2009.
[2] R. Rakkimuthu, B. Naveenraj, L. Benitta, K. Dhanya, "Phytochemical screening and its antibacterial activity of the fern *Hemionitis arifolia* (Burm.) Moore", WJPR, (7) 13,603-609, 2018.
[3] S. Sadasivam and A. Manikam, Biochemical methods for agricultural science, Wiley Eastern Limited, New Delhi,1992.
[4] M.F. Oberbacher and Vines H.M, "Spectrophotometric assay of ascorbic acid oxidase", Nature, 1203 – 1204,1963.
[5] H. Esterbauer, E. Schwarzl and M. Hayn, " A rapid assay for catechol oxidase and lactase using 2-nitro-5-thiobenzoic acid", Anal. Biochem, 77, 486-494, 1977.
[6] M.S. Blois, "Antioxidant determinations by the use of a stable free radical", Nature, 29,1199- 1200, 1958.
[7] Arnao M, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem 2001; 73:239–44.
[8] C. A. Soler-evens and G. Paganga, "Antioxidantt properties of phenolic compounds", Trends plant sci, 2, 152-159, 1997;
[9] P. Prieto, M. Pineda and M. Anguilar, Spectrophotometric antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal. Biochem 1999; 269: 337-341.

[10] Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr 1986; 44: 307-315.
[11] B. Halliwell and J.M.C. Gutteridge. 2nd ed, Clarendron Press, Oxford, 1989.
[12] Y Shimada and K. Sanae, "Ascorbic acid and ascorbic acid oxidase in vegetables", Chugokuga kuen J. ,7, 7-10, 2008.
[13] Mary shoba Das, T.N. Manila and S. Gayathri Devi, "Evalution of Antioxidant and *in vitro* free Radical Scavenging Activity of *Terminalia bellirica*" In: Parimelazhagan, T. (Ed.) Turning plants into medicines- Novelapproaches. Vol.1. New India publishing agency, New Delhi, India,. P. 59-77, 2013.
[14] A.S. Noor Nawaz, N. Syed Junaid, K. Dillep, N. Rakesh and T.R. Prashithkekuda, " Antioxidant activity of *Azolla pinnata* and *Azolla rubra* – A comparative study", Sch. Acad. J. Biosci, 2(10), 719-723, 2014.
[15] Amit Semwal, Mamta S Farswan , Kamalesh Upreti, SP Bhatt and Kumud Upadhyaya, " Evaluation of Antioxidant Activity of Some Pteridophytes", Int. J. Herbal Medicine, 1 (1), 1-5, 2103.
[16] Nishika Jaishee and Usha Chakraborty, " Evaluation of *In-vitro* antioxidant activities of *Pterisbiaurita* L." Int. J. Pharm. Pharm Sci, 6(2), 413-421, 2014.
[17] Hassan Valizadeh, Ali Sonboli, Fatemeh Mahmoodi Kordi, Hossein Dehghan and Mir Babak Bahadori, " Cytotoxicity, antioxidant activity and phenolic content of eight fern species from North of Iran", Pharmaceutical Sciences, 21, 18-24, 2015.
[18] Tsun – Thai Chai, Sanmugapriya Elamparuthi, Ann- Li young and Yixian Quah, " Antibacterial, anti- glucosidase and antioxidant activities of selected highland ferns of malasia", Botanical studies, 54 (1), 1-7, 2013.
[19] Selvaraj Kunjiappan, Chiranjib Bhattacharjee and Ranjana Chowdhury, "Hepatoprotective and antioxidant effects of *Azolla microphylla* based gold nanoparticles against acetaminophen induced toxicity in a fresh water common carp fish (*Cyprinuscarpio L.*)", Nanomedicine Journal, 2(2), 88-110, 2015.
[20] Saleha Akter, Md. Monir Hossain, Ismot Ara and Parvez Akhtar, "Investigation of *in vitro* antioxidant, antimicrobial and cytotoxic activity of *Diplazium esculentum* (RETZ). SW", IJAPBC, 3(3), 723-733, 2014.
[21] S. Hind Jasim, J. Nissreen Hayawi, Mushrifah Idris and Aminah Abdullah, "Determination of antioxidant activity, total phenolic content in different parts of medicinal fern, *Blenchum orientale",* WJPR, 4(12), 393-402, 2015.
[22] Manesha Singh, Kanaga Malhotra and Chhaya Singh, "Phytochemical Evaluation and antioxidant activity of different samples of *Pteris Vittata* in Doon valley, Uttarakhand region", International Journal of Pure and Applied Bioscience,3(4), 296-304, 2015.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM DIFFERENT PLANTS AND IDENTIFYING ITS ANTI-BACTERIAL ACTIVITY

S.Kiruthika[1] and Dr. Lali Growther[2]

PG and Research Department of Microbiology, Hindusthan college of Arts and Science

Avinashi Road, Behind Nava India, Coimbatore – 641028, INDIA, Kiruthi649@gmail.com

Abstract: Nanotechnology and Nanoscience is the emerging field in today's research world. It has wider application for the field of medicine, cosmetics, electrical, electronics and so on. In the field of medicine it is used for drug delivery, preparation of new drugs, anti-microbial activity and also used in cancer therapy. There are many metals based nanoparticles, among those silver nanoparticles have more potential anti-microbial and anti-cancer properties. Plant mediated synthesized silver nanoparticles have less or non-toxicity when compared to chemically synthesized silver nanoparticles. Green synthesis of silver nanoparticles is very cheap and easy to produce. They are eco-friendly in nature. This study reveals that green synthesis of silver nanoparticles from various plants (*Cassia auriculata, Murraya koenigii, Piper betle, Mentha piperita, Azadirachta indica, Emblica officinalis, Psidium guajava, Syzygium cumini, Ocimum sanctum, Musa paradisiaca*) with the help of silver nitrate solution. Then undergo characterization (UV-Visible spectroscopy, XRD analysis, FTIR analysis, SEM analysis etc) for the conformation of synthesized silver nanoparticles. Finally check the anti-bacterial efficiency of the synthesized silver nanoparticles by agar well diffusion method

Keywords: Green synthesis; silver nanoparticle; plant; anti-bacterial

I. Introduction

Nanotechnology is defined as the study and use of structures between 1 nanometer and 100 nanometers in size. The ability to see nano-sized materials has opened up a world of possibilities in a variety of industries and scientific endeavors. Nanotechnology is essentially a set of techniques that allow manipulation of properties at a very small scale. Nanoscience involves the study of chemical and physical changes that happen at the nanoscale. Nanotechnology is applied in the field of Medicine, Electronics, Food, Fuel cells, Solar cells, Batteries, Space, Better air quality, Cleaner water, Chemical sensors, Sporting goods, Fabrics etc. In the field of Medicine it is applied for Drug delivery, Protein and Peptide delivery. It is used to treat neuro degenerative disorders such as Parkinson's disease, Alzheimer's disease and Tuberculosis. It is also used in cancer therapy and it contains effective Anti-microbial properties.

There are many metals based nanoparticles such as Gold, Platinum, Silver, Titanium etc. Among that silver nanoparticles have more potential anti-microbial and anti-cancer properties. Synthesis of nanoparticles is done by Physical, Chemical and Biological methods. In physical methods there are two basic steps one is evaporation and condensation. Electrical arc discharge, RF plasma method, Pulsed laser method, Iron sputtering, Chemical vapor deposition, the ball milling method, Laser ablation are some of the method used for physical synthesis of nanoparticles. Chemical synthesis of nanoparticles includes chemical reduction method. In these method chemicals such as sodium citrate, ascorbate, sodium borohydride, tollenes etc are used to reduce silver ions and polymeric compounds such as poly vinyl alcohol, poly ethylene glycol are used to stabilize nanoparticles. In biological method microorganisms and plants are used for the synthesis of nanoparticles. In these methods physical synthesis are very expensive and time consuming process. Chemical synthesis is toxic and cause pollution to environment. Biological synthesis, especially green synthesis of nanoparticles is less or non toxic and they are eco-friendly in nature.

This study reveals that green synthesis of silver nanoparticles from different plants and identifying its anti-bacterial efficiency. For these studies, we take 10 different plants namely *Cassia auriculata, Murraya koenigii, Piper betle, Mentha piperita, Syzygium cumini, Azadirachta indica, Emblica officinalis, Psidium guajava, Ocimum sanctum, Musa paradisiaca. Cassia auriculata* is commonly known as avaram. It belongs to legume family called Fabaceae. It occurs in dry regions of India and Srilanka. It tolerates drought and dry conditions. The roots of *Cassia auriculata* are used against diabetes, constipation and urinary system diseases. It also has good anti-bacterial activity. *Murraya koenigii* is commonly known as curry plant. It belongs to the family Rutaceae. It is subtropical and tropical tree; leaves are used in many dishes in Indian subcontinent. It is rich in vitamin A, B, C & B_2. It helps to reduce weight and deals with digestive issues. *Piper betle* is commonly known as betle leaves. It belongs to Piperaceae family. It is present in the regions of India and Srilanka and mostly consumed in Asia. It improves digestion, reduces gastric pain and has anti-septic and anti-fungal properties. *Mentha piperita* is commonly known as mint leaves. It belongs to the family Lamiaceae and is native to Europe & Middle East. Now it is widely cultivated in many regions and it has more than 25 species. It is used to add flavor to foods, cosmetics, soaps, toothpastes and mouth washes.

It is used to treat nausea, skin irritations, headaches, cold, flu, gastrointestinal problems, nerve & muscle pain etc. *Azadirachta indica* is commonly known as neem. It belongs to the family Meliaceae and it is native to Indian subcontinent. It grows in tropical and semi-tropical regions. It has anthelmintic, anti-fungal, ant-bacterial, anti-diabetic and anti-viral properties. It is used to treat skin diseases, fever reduction, dental treatments, cough, asthma, ulcers, piles, urinary diseases and it improves liver function.

Syzygium cumini is commonly known as black plum or jamun tree. It belongs to Myrtaceae and is Indian subcontinent; adjoining regions of south East Asia, china and Queensland. It has carminative and astringent properties. The seeds are used to treat diabetes, dysentery. The juice is used to treat wounds and enlargement of the spleen and bark is used to treat mouth ulcers. *Emblica officinalis* is commonly known as Indian goose berry or phyllanthus emblica. It contains excellent source of vitamin C; it also prevents cancer, prevent hair loss, glowing skin, burn fat, prevents cold, cough, mouth ulcers, control diabetes and relives asthma. *Psidium guajava* is commonly known as guava. It is an evergreen shrub native to the Caribbean, Central America & South America. It is easily pollinated by insects. Guava leaves contains anti-inflammatory, anti-microbial, hepatoprotective and anti-oxidant activities and it is used to treat diarrhea, constipation, diabetes and hepatitis. *Ocimum sanctum* is commonly known as holy basil or tulasi. It belongs to the family Lamiaceae and it is native to Indian subcontinent. It has ant-cancer, anti-fungal, anti-bacterial, cardio protective and analgesic properties. In traditional system of medicine it is used to treat bronchitis, asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, and chronic fever etc. *Musa paradisiaca* is commonly known as banana tree. It belongs to the family Musaceae and it is mostly cultivated in tropical regions. The flowers are used to treat bronchitis, dysentery and ulcers; plant sap is used to treat leprosy, fevers and hemorrhages; the roots are administered in digestive disorders; the leaves are used to serve foods traditionally and it is placed as poultius on burns and other skin diseases. It also acts as anti-fungal agent.

Leaves of these 10 plants contain both silver nanoparticles and anti-bacterial efficiency. The major aspect of this study is to synthesis silver nanoparticles with the help of silver nitrate solution and checks their anti-bacterial efficiency by agar well diffusion method.

II. Materials and methods

Materials

Fresh and green leaves of *Cassia auriculata, Musa paradisiaca, Emblica officinalis, Azadirachta indica, Psidium guajava, Ocimum sanctum & Syzygium cumini* are collected from Pothum Palayam of Avinashi; Tirupur district Tamil Nadu. Other leaves such as *Murraya koenigii, Piper betle and Mentha piperita* are collected from local market. Silver nitrate solution and media (Muller Hinton Agar) for agar well diffusion method is taken from the college laboratory.

Preparation of aqueous extract

10 grams of fresh leaves from *Cassia auriculata, Murraya koenigii, Piper betle, Mentha piperita, Azadirachta indica, Syzygium cumini, Emblica officinalis, Psidium guajava, Ocimum sanctum & Musa paradisiaca* are taken

and washed thoroughly with running tap water and then rinsed with distilled water to remove surface impurities. Then finely cut the leaves and placed in a flask containing 100 ml distilled water. Boil them for 20 minutes, then cooled the extract and filter it through whatmann filter no.1; finally store the extract at refrigerator for Test organisms such as *Klebsiella, Pseudomonas, Bacillus, Staphylococcus aureus* are isolated and taken from the college laboratory. Prepare Muller Hinton Agar for anti-bacterial susceptibility testing. The test is done by agar well diffusion method. The media were prepared and sterilized. After attaining bearable warm it is poured in a sterile petriplate then allows it to solidify. After solidification, swab the test organisms on the surface of further use.

Synthesis of silver nanoparticles

Synthesis of silver nanoparticles is done by adding 10ml of (1mM) silver nitrate ($AgNo_3$) solution in 90ml of plant extract. $AgNo_3$ (1mM) was prepared by adding 0.116 gram of silver nitrate into 100ml distilled water. After adding silver nitrate solution, kept the extract at dark condition for color change. Changes in color after adding $AgNo_3$ solution indicates the presence of silver nanoparticles.

Characterization

Different techniques are used to characterize silver nanoparticles such as UV-Visible spectroscopy (shimaduz) 1601 used to know the band at nanometer. X-ray diffraction (XRD) shimaduz 6000 is used to determine the crystalline structures of synthesized silver nanoparticles. Fourier Transform Infrared (FTIR) is also used to characterize synthesized silver nanoparticles. SEM analysis is used to find out the morphology of synthesized silver nanoparticles.

Anti-bacterial activity

Muller Hinton Agar (MHA) and allow it for 5 minutes to dry, and then cut the well on the agar with the help of well puncture. After cutting wells the plant extract containing synthesized silver nanoparticles are poured into the well by using micro pipette. Then incubate the plates at 37℃ for 24 hours. Then observe the plate for zone formation. Measure the size of zone and predict the results.

III. Results and discussion

The synthesis of silver nanoparticles in 10 different plants is done by adding 1mM silver nitrate in plant extract. It reduces the silver ions in the extract and makes color change in the extract. Changing of color was observed after 6hrs for *Cassia auriculata, Musa paradisiaca, Murraya koenigii, Azadirachta indica, Psidium guajava* and 24 hrs for *Mentha piperita, Emblica officinalis, Syzygium cumini* and 48hrs for *Ocimum sanctum* and *Piper betle*. It indicates the presence or formation of silver nanoparticles.

Anti – bacterial activity:

Anti – bacterial susceptibility test for 10 different plants has to done by agar well diffusion method. Four different cultures (*Klebsiella, Pseudomonas, Bacillus, staphylococcus aureus*) are taken as test organisms. Test organisms are swabbed on the surface of MHA agar plate and then cut wells on the agar plate. Then 150µl of plant extract containing silver nanoparticles are added into the well. Incubate it and the zone was observed.

Zone of inhibition

Plant Extract	***Pseudomonas* (zone size)**	***Staphylococcus aureus* (zone size)**	***Klebsiella* (zone size)**	***Bacillus* (zone size)**
Cassia auriculata	10mm	7.5mm	7mm	6mm
Murraya koenigii	7.5mm	6mm	6.5mm	7mm
Piper betle	13.5mm	6.5mm	7.5mm	7.5mm
Mentha piperita	8mm	7mm	6mm	7.5mm
Azadirachta indica	7.5mm	6mm	5mm	No zone
Emblica officinalis	10mm	6mm	6mm	7mm
Psidium guajava	13.5mm	10.5mm	7.5mm	10mm
Syzygium cumini	12mm	6.5mm	9mm	7.5mm
Ocimum sanctum	8.5mm	6.5mm	7.5mm	8mm
Musa paradisiaca	9mm	5.5mm	No zone	6mm

Figure 1 Zone formations against *Pseudomonas*



Figure 2 zone formations against *Staphylococcus aureus*



Figure 3 Zone formations against *Klebsiella*

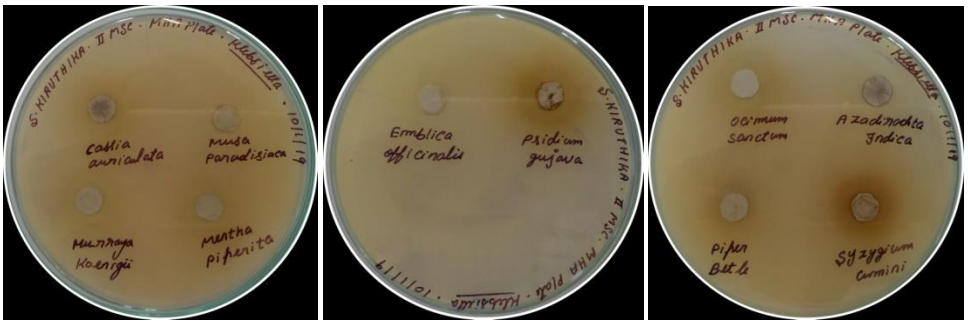
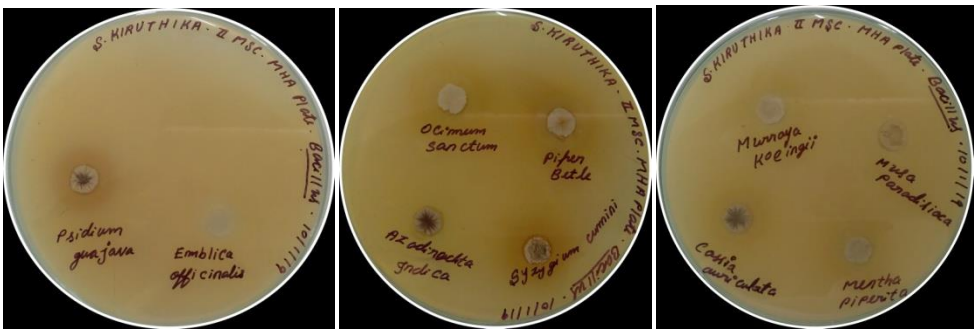


Figure 4 Zone formations against *Bacillus*



Among those plants extract containing silver nanoparticles, *Psidium guajava* (13.5mm) & *Piper betle* (13.5mm) are more effective against *Pseudomonas*. *Psidium guajava* (10.5mm) is effective against *Staphylococcus aureus*. Syzygium cumini (9mm) is effective against *Klebsiella*. Psidium guajava (10mm) is effective against *Bacillus*.

Characterization

Further characterizations are to be done.

IV. Conclusion

We have demonstrated a simple, cost-effective, safe and eco-friendly technique for the synthesis of silver nanoparticles from 10 different plants and comparing their antibacterial efficiency. Among those plants *Psidium guajava* commonly called as guava is more effective against most bacteria when compared to others. It has most potent anti-bacterial activity. By using this plant for the preparation silver nanoparticles is a cheap, cost effective and eco-friendly technique. Now the plant is mostly used to produce silver nanoparticles and coated them in breast cancer drugs to improve the efficiency of the drug and reduce the side effects. This green synthesis technique also has less or non-toxicity when compared to chemical and physical synthesis of nanoparticles.

References

1. Imran L. Patel, Hatim Kara, Joash Mathew, Jayant Londhe; journal of medicinal chemistry and drug discovery 2015.
2. P. Shanmuga Praba, J. Jeyasundari and Y. Brightson Arul Jacob; Eur. Chem. Bull., **2014.**
3. C.Udayasoorian, K. Vinoth kumar, R. M. Jayabalakrishnan; Digest Journal of Nanomaterials and Biostructures Vol. 6, 2011.
4. Nisha K. Raju and C. Initha Lebanon Ebency; European Journal Of Pharmaceutical And Medical Research 2017.
5. Krishnaveni and Priya; International Journal of Pharmaceutical & Biological Archives 2014
6. K. Satheesh Kumar and P. Kathireswari; World Journal of Pharmacy and Pharmaceutical Sciences 2016.
7. Wisam J. Aziza and Haneen A. Jassimb; World News of Natural sciences 2018.
8. Sayeeda Sultana, S. Anusha; International Journal of Chemical Concepts 2017.
9. Sajesh Kumar N.K., Prem Jose Vazhacharickal, Jiby John Mathew and Jyothimol Joy; Cib tech Journal of Biotechnology ISSN: 2319–3859 2015.
10. Garima Singhal, Riju Bhavesh, Kunal Kasariya, Ashish Ranjan Sharma, Rajendra Pal Singh; Springer 2011.
11. Birendra K Bindhani and Ashok K Panigrahi; Journal of Nanomedicine and Nanotechnology 2015
12. T.Sriram and V. Pandidurai; International Journal of Applied Sciences 2014.
13. Grewa Deepak Kumar Malik and Rajesh Kumar; International Journal of Pharmaceutical Sciences and Researches 2013.

Acknowledgements

The authors are thankful to the Management, Principal, Head of the Department and college for their encouragement and necessary laboratory facilities.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Effect of silver nanoparticles of *Ulva fasciata* in germination of selected pulses

D. Sowmiya*[1], R. Rakkimuthu[2], P. Sathishkumar[3], A. M. Anandakumar[4], S. Sinju[5] and B. Vanisree[6]
[1,2,3,4,5,6] PG and Research Department of Botany
Nallamuthu Gounder Mahalingam College, 90, Palghat Road, Pollachi, Tamil Nadu- 642001, IINDIA
*Corresponding author Email id: d. sowmiya22@gmail.com

Abstract*: In this study, an eco friendly approach was applied for synthesis of silver nanoparticles from the seaweed of Ulva fasciata aqueous extract. SNPs of Ulva fasciata used as reducing agent and tested their effects on seed germination. Biologically synthesized Ag NPs were applied to evaluate the effect on seed germination and seedling growth of Vigna unguiculata, Vigna radiata and Cicer arietinum. Seeds were treated with different concentrations of Ag NPs (50%, 75% and 100%) and compared with control. The results of above experiment revealed that the Ag NPs enhanced the considerable germination in Vigna unguiculata, Vigna radiata and Cicer arietinum as 50%, 75% and 75% respectively and the parameter seedling growth was found to be higher at 50% conc. AgNPs as 3.6 cm in Vigna unguiculata and the seedling growth of Cicer arietinum was higher at 100% concentration of AgNPs as 2.52 cm. So the present study is useful to explore the potential of green synthesized silver nanoparticles on germination and seedling growth of selected pulses.*

Keywords*: Silver nanoparticles; Ulva fasciata; Seed germination; seedling growth*

I. Introduction

Nanotechnology is an innovative interdisciplinary research. It has wide range of applications in various fields like pharmaceutical, electronics, medicine, food processing, textile, agriculture and it also has tremendous benefits. Basically nanoparticles are the atomic or molecular aggregates that possesses size dimension between 1 to 100 nm (Nel *et al*., 2006).

Among different NPs, silver nanoparticles (AgNPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro- scaled counterparts (Sharma *et al*., 2009). Green synthesis of silver nanoparticles using organisms and plants or plant extracts has emerged as a cost effective, nontoxic and ecofriendly method for synthesis of silver nanoparticles which does not need high temperatures or pressures and toxic chemicals (Lansdown 2006).

At present, several researches have been carried out to study the positive effects of silver nanoparticles on the growth and development in various plants. Biologically synthesized silver nanoparticles promoted seed germination and seedling growth of Boswellia ovalifoliolata (Savithrammae *et al*., 2012). AgNPs application increased growth attributes such as root and shoots length and leaf area of Zeyamays, Brassica juncea and Phaseolus vulgaris (Salama 2012; Sharma *et al*., 2012).

Seed germination in plant growth is crucial stage and it is also an important process in agriculture because it is regarded as thread of life of plants that ensure its survival (Zaka *et al*., 2016). The advances in nanotechnology and its applications and utilization in the field of agriculture is tremendously increasing; so it is quite persuading to study or explore the role of AgNPs in the germination of seeds.

Ulva fasciata (Ulvaceae), also known as Limu palahalaha and sea lettuce, is common green algae that are used for consumption in many parts of the world. High nutrients and fresh water are often indicated by its presence and it also possesses many medicinal properties (https://www.ncbi.nlm.nih.gov/pubmed?term=Marine+alga). The objectives of this study were the production of silver nanoparticles using the aqueous extract of green algae *Ulva fasciata* and evaluate the effects of AgNPs on selected pulses (Seed germination assay and seedling growth study).

II. Materials and methods

***Collection and Identification*:**

The green algae *Ulva fasciata* Delile. *(Ulvaceae*) was collected from Kanyakumari Coast, Tamilnadu, South India. The algae was identified and authenticated by Botanical Survey of India, Southern Region, Coimbatore 641013.

***Preparation of seaweed's extract*:**
The samples were washed thoroughly with Milli Q water to remove extraneous materials and washed seaweed was finely cut into small pieces and stirred with 100 mL sterile Milli Q water for 1min and kept in a water bath at 4^0C for 20 minutes. Finally, the extract was filtered with Whatman no .1 filter paper. The filtrate is used as reducing agent and stabilizer.

***Biosynthesis of AgNPs*:**
For the biosynthesis of AgNPs 50ml aqueous seaweed extract was mixed with 50ml of 1mM $AgNO_3$ solution, stirred well for 1 minute, kept in a water bath at 60^0c for 1 hour and then it is incubated in dark at room temperature under static condition. A control set up was also maintained without seaweed extract. The bioreduction of $AgNO_3$ into AgNPs can be confirmed visually by the change in colour from yellow to brown and characterized by UV-Visble spectroscopy, XRD, FESEM, FTIR and EDX (Sowmiya and Ravikumar, 2018).

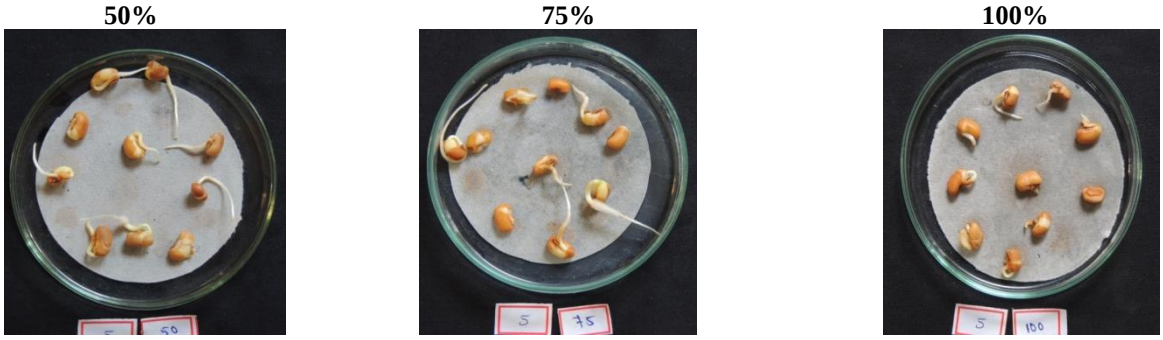
***Seed germination assay (International seed testing Association (ISTA Rules 2014))*:**
Experiment was made to evaluate the effect of AgNPs on the germinability of the selected seeds (*Vigna unguiculata* (L.) walp, *Vigna radiata* (L) R. Wilczek and *Cicer arietinum* (L.)) in a completely randomized design with duplicates as per ISTA rules 2014. The treatments in the experiment were taken in 50%, 75% and 100% concentration of AGNPs. This setup was kept in room temperature of 28°C for 24 hrs. Before the treatment the seeds were pre-soaked in AgNP solution for 12 hrs.

***Seedling growth study*:**
The seedling growth, fresh and dry weight of the embryonic axis and % increase/decrease over the control germinated seeds of *Vigna unguiculata*, *Vigna radiata* and *Cicer arietinum* were calculated in 50%, 75% and 100% concentrated AgNPs solution of *U. fasciata*.

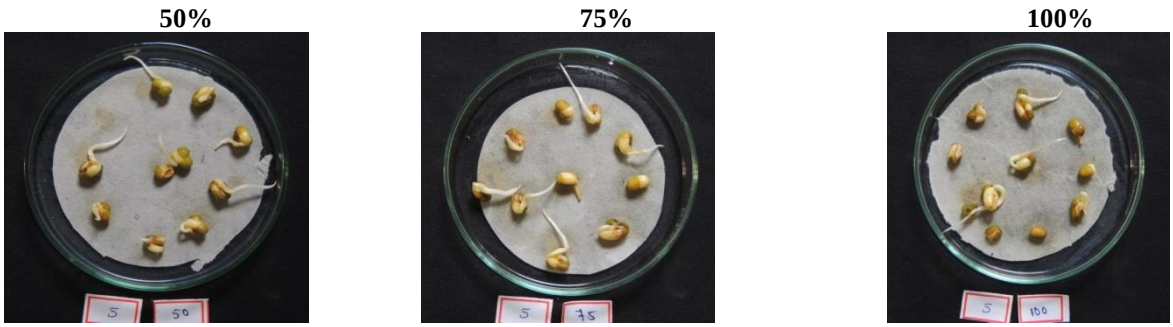
III. Results and discussion

Seed germination assay:
The highest germination percentage and germination rate were observed at different concentrations were depicted in Figure 1 and Table 1. Based on the studies of NPs effects on seed germination mechanism, it is possible that NPs increased the water absorption of the seeds (Zheng *et al*., 2005).
re preferred. Please embed symbol fonts, as well, for math, etc.

Figure 1: Seed Germination Assay *V. unguiculata*



V. radiata



C. arietinum

50% **75%** **100%**



Seedling growth study:

Embryonic axis length observed at concentrations is tabulated in (Table 2). The impact of AgNPs root length and shoot length. Exposure to specific concentrations of AgNPs could enhance plant growth compared with non-exposed plants, whereas higher and lower concentrations could affect growth negatively (Kaveh *et al*., 2013).

Table 1: Seed germination assay

Plant name	Seed name	Concentration	Germination (%)	Increase / Decrease over C
U. fasciata	*V. unguiculata*	Control	60	-
		50%	100	+66.66
		75%	60	0
		100%	90	+50
	V. radiata	Control	80	-
		50%	90	+12.5
		75%	100	+25
		100%	70	-12.5
	C. arietinum	Control	50	-
		50%	50	0
		75%	0	+20
		100%	40	-20

Table 2: Seedling growth study

Seed	Conc. of NPs/ Mean	Seedling growth in cm Mean	% Increase / Decrease of seedling growth over C	FWt g Mean	DWt g Mean	Water g Mean	% Increase / Decrease of DWt over C
V. unguiculata	Control	3.5		0.150	0.062	0.088	-
	50%	3.6	+2.86	0.137	0.055	0.082	-11.29
	75%	2.76	-21.14	0.184	0.056	0.128	-9.68
	100%	1.82	-48	0.165	0.070	0.095	+12.90
V. radiata	Control	2.96		0.175	0.035	0.140	
	50%	2.62	-11.49	0.159	0.045	0.114	+28.57
	75%	2.76	-6.76	0.147	0.040	0.107	+14.29
	100%	2.36	-20.27	0.191	0.058	0.133	+65.71
C. arietinum	Control	1.9		0.585	0.252	0.333	
	50%	2.02	+6.31	0.570	0.264	0.306	+4.76
	75%	2.24	+17.89	0.607	0.276	0.331	+9.52
	100%	2.52	+32.63	0.612	0.268	0.344	+6.35

IV CONCLUSION

Through the study we have confirmed that the aqueous extract of seaweed *U.fasciata* is capable of producing AgNPs by reducing $AgNO_3$ solution and is proved to be an efficient, eco-friendly and simple method. Their abundance and the case of availability also make them good green reagents for the green synthesis of novel AgNPs. Exposure to AgNPs can encourage earlier seed germination and improve plant production. Our results indicated that exposure to AgNPs had significant effects on the seed germination and seedling growth study of *V. unguiculata*, *V. radiata* and *C. arietinum*.

REFERENCE

[1]. A. Nel, T. Xia, L. Madler and N. Li, "Toxic potential of materials at the nanolevel.Science",2006;311, 622-627.
[2]. V. K. Sharma, R. A. Yngard and Y. Lin, Adv. Colloid Sur. Interface, 2009; 145 83.
[3]. A. B. Lansdown, "Silver in health care: antimicrobial effects and safety in use", Curr. Probl. Dermatol. 2006;33:17–34.
[4]. N. Savithramma, S. Ankanna and G. Bhumi, "Effect of nanoparticles on seed germination and seedling growth of Boswellia ovalifoliolata an endemic and endangered medicinal tree" taxon. Nano technology, 2012; 2, 61–68.
[5]. H. M. H. Salama, "Effects of silver nanoparticles in some crop plants, common bean (Phaseolus vulgaris L.) and corn (Zea mays L.)", International Research Journal of Biotechnology,2012; 3, 190–197.
[6]. P. Sharma, A. B. Jha, R. S. Dubey and M. Pessarakli, "Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions" Journal of Botany, 2012; 12, 134–136.
[7]. M. Zaka, B. H. Abbasi, L. Rahman, A. Shah and M. Zia, "Synthesis and characterization of metal nanoparticles and their effects on seed germination and seedling growth in commercially important Eruca sativa", IET Nanobiotechnology 2016; 10, 1–7.
[8]. (https://www.ncbi.nlm.nih.gov/pubmed?term=Marine+alga*)*
[9]. D. Sowmiya and P. Ravikumar, "Green synthesis and characterization of silver nanoparticles from *Ulva fasciata* Delile.", Internat. J. of creative Res. Thoughts (IJCRT), April 2018, Vol. 6, issue 2, pp.20-27.
[10]. F. Zheng, S. Hong and C. Liu, "Effect of nano-Tio_2 on strength of naturally aged seeds and growth of spinach", Biotec. Trace Element, Res., 2005; Vol. 104, no.1.pp 82-93.
[11]. R. Kaveh, Y. S. Li, S. Ranjbar, R. Therani, C. L. Brueck and B. Van Aken, "Changes in Arabidopsi thaliana gene expression in response to silver nanoparticles and silver ions".Environ.Sci.Technol, 2013 ;Vol 47.no.18,pp-10637-44.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Quantitative phytochemical screening of *Filicium decipiens* Wight & Arn. leaves

P. Sathishkumar[1]*, D. Kaviya[2], R. Rakkimuthu[3], A.M. Anandakumar[4], D. Sowmiya[5] and S. Sabirafathima[6]

*Corresponding Author

[1, 2, 3,4,5 and 6] PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi-01 Tamilnadu, India, sathishkumarkutti@gmail.com

Abstract*: The quantitative phytochemical analysis was conducted on the leaves of Filicium decipiens for the content of secondary metabolites like alkaloids, flavonoids, glycosides, saponin, phenol and tannin by gravimetric and spectrophotometric methods. It is revealed that saponin (78 mg/g) and flavonoid (64 mg/g) content was present in maximum amount than the other secondary metabolites in the studied plant sample. The results of the present study support the therapeutic usage of the secondary compounds that can be used in new drugs for the treatment of various diseases. Hence it is confirmed the presence of phytochemicals and their respective quantities in the leaves of F. decipiens and further, the appropriate medicinal value is to be checked with relevant experiments and animal models.*

Keywords*: Filicium decipien; Extracts; Quantitative analysis; Secondary metabolites;therapeutic usage*

I. Introduction

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans and animals against diseases. A number of phytochemical are known, some of which include: alkaloids, flavonoids, glycosides, saponins, tannins, anthraquinones, steroids and terpenoids. They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more [1].

In recent years, secondary plant metabolites (Phytochemical and phytotherapy) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents [2]. Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defence mechanisms against predation by microorganisms, insects and herbivores [3]. Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activities. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings. The evaluation of all the drugs is based on phytochemical and pharmacological approaches which lead to the drug discovery referred as natural products screening [4], [5].

There are large number of medicinal plants whose scientific importance and their phytochemicals has not been explored. All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine, particularly in India. The study plant *Filicium decipiens* White & Arn. belongs to the family sapindaceae were analyzed their phytochemicals quantitatively based on gravimetric and spectrophotometric methods. Hence, the highlight of the present study is there are no reports have been studied previously for quantitative phytochemicals of the species of *F. decipiens* were selected.

II. Methodology

A. Collection of Plant Materials

The healthy and matured leaves of *Filicium decipiens* White & Arn. were collected from our college campus, Pollachi, Coimbatore district and Tamilnadu state in India, during the month of December/January.

B. Preparation of the powder sample

The leaves were washed thoroughly 2 - 3 times with running tap water, leaf material was then air dried under shade. After complete shade drying the plant material was grinded in the mixer, the powder was kept in small plastic bags with proper label.

C. Preparation of Extract - Soxhlet Extraction

Crude plant extract was prepared by Soxhlet extraction method. About 50gm of powdered plant material was packed into a thimble and extracted with 500ml of methanol. The process of extraction was carried out at 55 - 85° C till the solvent become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30 - 40^0 C till all excess solvent got evaporated. Dried extract was kept in refrigerator at 4° C for future use.

D. Quantitative Determination of Phytochemical

i) Determination of alkaloids [6], [7]

Fifty gram of powdered sample was dispersed in 500 ml of 10 % acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 hours before it was filtered. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre - weight filter paper was used to filter off the precipitate and it was washed with 1 % ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60° C for 30 min. and transferred to desiccators to dry and cool and then weighed repeatedly until a constant value was obtained. The weight of the alkaloid was determined by deducting the filter paper weight. Triplicates were maintained.

ii) Determination of total flavonoids [8]

One gram of plant samples was repeatedly extracted with 100 ml of 80 % aqueous methanol at room temperature. The mixture was filtered through a Whatman No. 1 filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into water bath and allowed to evaporate to dryness and weighed.

iii) Determination of total saponins [9]

Fifty gram of powder sample was put into a conical flask and 500 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55° C. The mixture was filtered and the residue was re-extracted with another 500 ml of 20 % ethanol. The combined extracts were reduced to 100 ml by heating over water bath at about 90° C. The concentrate was transferred to a 1000 ml separating funnel and 50 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. To this, 150 ml of n-butanol was added. The combined n-butanol extract were washed twice with 25 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the oven to a constant weight, the saponin content was calculated and expressed in percentage.

iv) Determination of total glycosides [10]

Fifty gram of powdered dry samples of leaves was taken and added to 800 ml of alcohol (20 %) shaken for 6 hours and filtered. The residue was again extracted two times with 800 ml alcohol (20 %) and with chloroform (200 ml x 5) in a separating funnel. All the chloroform extracts (lower layer) were combined and transferred to a conical flask and dried over anhydrous Na_2so_4. The chloroform layer was decanted in the R.B. flask. Na_2So_4 was washed with chloroform (15 ml x 3) and the washings are added to the main chloroform bulk and it was evaporated to dryness. The dried residue is dissolved in a mixture of chloroform and methanol (1: 1) and filtered. The filtered was evaporated to dryness over a water bath on a weighted watch glass and the residue weighted as the glycosides content.

v) Determination of phenol [11]

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2 N of the folin-ciocalteu's reagent and 1.5 ml 20 % of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allow to stand for 2 hours after which the absorbance was taken at 765 nm.

These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

vi) Determination of tannin [12]

The methanolic extract (1 ml) was mixed with folin-ciocalteu's reagent (0.5 ml) followed by the addition of saturated Na_2Co_3 solution (1 ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant was obtained by centrifuge and absorbance was recorded at 725 nm using VU - visible spectrophotometer. Tannins content was calculated as mg tannic acid equivalent from a linear regression equation obtained from a calibration curve.

vii) Detection of Oil (Gravimetric method)

3 g of dry powder and added 10ml of methanol and chloroform (1 : 2) mixture. After centrifuged and collect the supernatant carefully. Add two drops of 0.005 KCL on the supernatant, it was thoroughly shaken and allowed to stand up for 5 min. The bottom lipid layer was collected in a pre - weighted beaker. Then the beaker kept in a water bath to evaporate the methanol and chloroform content. After cooling the beaker were reweighed using an electric balance.

viii) Data analysis

The methodology was repeated for triplicate value. Further the values are converted to mg/g. Results were averaged, and given as mean ± standard deviation, calculated by using the Microsoft excel.

III. Results

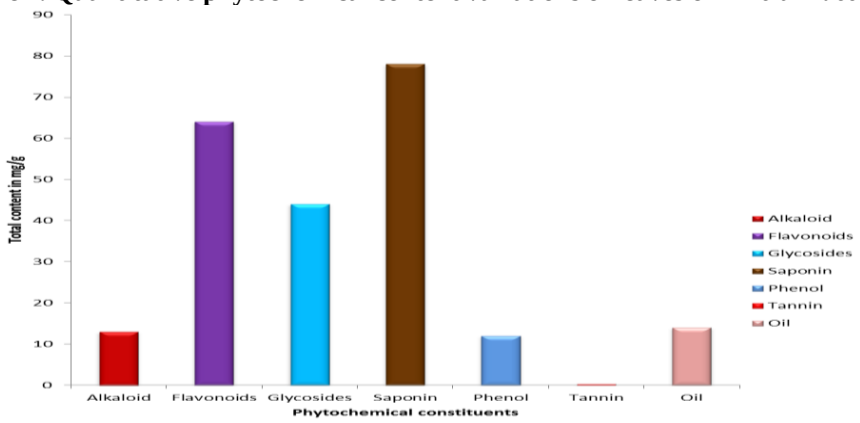
The result of the gravimetric and spectrophotometric studies are revealed that the quantity of major secondary metabolites (alkaloids, flavonoids, glycosides, saponins, phenol, tannins and oil) in the leaves of *Filicium decipiens* White & Arn. are showed in Table 1 and Figure 1. The present study was observed that the content of the following secondary compounds viz. alkaloids 13 mg/g, flavonoids 64 mg/g, glycosides 44 mg/g, saponins 78 mg/g, phenolic content 12 mg/g, tannins 0.3 mg/g and essential oil 14 mg/g of the extract. The overall results revealed that the saponins and flavonoids are present in high amount and the alkaloid and tannin are present in lower amount of the leaves of *F. decipiens* (Table 1).

Table 1. Quantitative analysis of leaves of *Filicium decipiens.*

S.No	Phyto constituents	Total content in mg/g
1	Alkaloid	13 ± 1.53
2	Flavonoids	64 ± 2.52
3	Glycosides	44 ± 1.00
4	Saponin	78 ± 1.53
5	Phenol	12 ± 0.58
6	Tannin	0.3 ± 0.08
7	Oil	14 ± 1.53

N = 3 Values are expressed as mean ± Standard Deviation (SD)

Figure 1. Quantitative phytochemical content variations of leaves of *Filicium decipiens*



IV. Discussion

Many studies have illustrated the differences in composition and concentration of phytochemical among plant extracts of the different species. Our results are agreed with the phytochemical studies of *Cardiospermum halicacabum* revealed that the various plant parts like leaf, seed coat and stem contain a broad spectrum of secondary metabolites such as flavonoids, terpenoids and cardiac glycosides are quantitatively rich in three different solvent extract [13]. Similarly the content of certain secondary metabolites of medicinal importance in leaves of the plant, *Acacia caesia s*howed that the compounds such as alkaloids (3.50 mg/g), flavanoids (11.67 mg/g), glycosides (3.00 mg/g) and saponins (3.00 mg/g) were estimated by gravimetric and spectrophotometric methods [14]. [15] Pragada *et al*. (2011) carried out preliminary phytochemical analysis and quantification of total phenols, and antibacterial activities of the hydro alcoholic (70 % ethanol) extract of *Acalypha indica*.

Alkaloids are the most significant compounds play a metabolic role in the living systems and are involved in the protective function in animals. Steroidal alkaloids are medicinally evolved. Flavonoids have been used against the cancer causing tumors and it inhibits the promotion of growth and progression of tumors [16]. Phenols when mixed with the flavonoids compounds in plants are reported to show multiple activities like antioxidant, anti-carcinogenic, anti-inflammatory etc. [17]. Tannins inhibit the pathogenic fungi and antimicrobial activity of extracts showed better activity by the presence of tannins.

Generally all the sapindaceae members are recorded as saponin rich family similarly the saponins are also rich in the study species *F. decipiens* (78 mg/g). Saponins are steroid and triterpenoid glycosides that display diverse biological activities [18]. Based on various authors saponins have been studied for their wide range of properties, including beneficial and detrimental effects on human health, pesticidal, insecticidal, molluscicidal and fungicidal activity, bitterness and sweetness and other industrial applications such as foaming and surface active agents [19] to [26].

V. Conclusion

The study confirmed the presence of phytochemicals and their respective quantities in the leaves of *F. decipiens* and further, the medicinal value is to be checked with relevant experiments and animal models.

References

[1] A. Doss and S. P. Anand, Purification and characterization of extracellular amylolytic enzyme from Aspergillus species, African Journal of Biotechnology, 2012, Vol. 11, No. 83, pp. 14941-14945.

[2] A.V. Krishnaraju, T. V. Rao and A. Sundararajan, Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Altenaria salania*) lethality assay, Indian Journal of Applied Science Engineering, 2005, No. 2, pp. 125-134.

[3] N. R. Fransworth and R.W. Morris, Higher plants, the sleeping giant of drug Development, American Journal of Pharmaceutical, 1976, No. 148, pp. 46-52.

[4] W.O. Foye, T. L. Lemke and D. A. Williams, Foye's Principles of medicinal chemistry, Philadelphiap, 2008, pp. 44-45.

[5] M. C. Gordon and J. N. David, Natural product drug discovery in the next millennium, Pharmaceutical Biotechnology, 2001, Vol. 39, No. 1, pp. 8-17.

[6] J. B. Harborne, Phytochemical Methods, Chapman and Hall, London, 1973, pp. 11-21.

[7] S. A Adeniyi, C. L. Orjiekwe, J. E. Ehiagbonare, Determination of alkaloids and oxalates in some selected food samples in Nigeria, African Journal of Biotechnology, Jan. 2009; Vol. 8, No. 1, pp. 110-112.

[8] A. B. Boham and A. C. Kocipai, Flavonoid and condensed tannins from leaves of Hawaiian Vaccinium vaticulum and Vicalycinium sp., Pacific science, 1994. No. 48, pp. 458-463.

[9] B. O. Obadoni and P.O. Ochuko, Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, Global Journal Pure Applied Science, 2001, No. 8, pp. 203-208.

[10] A. A. Farooqi and B. S. Sreeramu, Cultivation of medicinal and Aromatic Crops, University press Ltd., India, 2001, pp. 116.

[11] A. Hagerman, I. Muller and H. Makkar, Quantification of tannins in tree foliage, A laboratory manual, Vienna: FAO/IAEA, 2000, pp. 4- 7.

[12] T. N. Fagbemi, A. A. Oshodi and K. O. Ipinmoroti, Processing effects on some anti-nutritional factors and in-vitro multi enzyme protein digestibility (IVPD) of three Tropical seeds: breadnut (*Artocarpus altilis*), cashew nut (*Anacardium occidentale*) and fluted pumpkin (*Telfairia occidentalis*), Pakisthan Journal of Nutrition, 2005, Vol. 4 No. 4, pp. 250-256.

[13] A. Annadurai, V. Elangovan, S. Velmurugan, R. Ravikumar,. Preliminary Phytochemical screening and antibacterial of *Cardiospermum halicacabum* L., Advanced in Applied Science Research, 2013, Vol. 4 No. 5, pp. 302-308.

[14] P. Sathishkumar, S. Paulsamy, A. M. Anandakumar and P. Senthilkumar, Effect of Habitat variation on the content of certain secondary metabolites of medicinal importance in the leaves of the plant, *Acacia caesia* Wild., Advances in plant sciences, 2009, Vol. 22 No. 11, pp. 451-453.

[15] R. R. Pragada, V. Vangepurapu, S. R. Ethadi and V. S. Praneeth, Phytochemical investigation and in - vitro antioxidant, antimicrobial activity of different fractions of *Acalypha indica* L., International Journal pharmacological pharmaceutical science, 2011, Vol.3 No. 4, pp. 314-317.

[16] J. F. Stevens, H. T. Hart, H. Hendriks and T. M. Malingre, Alkaloids of some European *Macaronesian diode* and *Androsace semepervivodeae* (Crassulaceae), Phytochemistry, 1992, No. 31, pp. 3917-3924.

[17] K. Asha, C. T. Rasika, R. D. Nirmala and P. S. Jyoti, Annals of Biological Research, 2011. Vol. 2 No. 1, pp. 176-180.

[18] A. Faizal and D. Geelen, Saponins and their role in biological processes in plants, Phytochemistry Reviews · December 2013, No. 12, pp. 877–893, DOI 10.1007/s11101-013-9322-4.

[19] R. M. Zablotowicz, R. E. Hoagland and S. C. Wagner, Effect of saponins on the growth and activity of rhizosphere bacteria, Advanced Experimental Medical Biology, 1996, pp. No. 405, pp. 83-95.

[20] H. X Sun, Y. Xie, Y. P. Ye Advances in saponin-based adjuvants. Vaccine, 2009, No. 27, pp. 1787–1796.

[21] S. Takahashi, K. Hori, M. Hokari, T. Gotoh, T. Sugiyama, Inhibition of human renin activity by saponins. Biomed Res, 2010, No. 31, pp. 155–159.

[22] S. Takahashi, K. Hori, M. Hokari, T. Gotoh, T. Sugiyama, Inhibition of human renin activity by saponins. Biomed Res, 2010, No. 31, pp. 155–159.

[23] Y. Diab, E. Ioannou, A. Emam, C. Vagias, V. Roussis, Desmettianosides A and B, bisdesmosidic furostanol saponins with molluscicidal activity from *Yucca desmettiana*, Steroids, 2012, No.77, pp. 686–690.

[24] E. De Geyter, G. Smagghe, Y. Rahbe, D. Geelen, Triterpene saponins of Quillaja saponaria show strong aphicidal and deterrent activity against the pea aphid *Acyrthosiphon pisum.* Pest Manag Sci 2012, No. 68, pp. 164–169.

[25] J. Chen, S. Zhang, X. Yang, Control of brown rot on nectarines by tea polyphenol combined with tea saponin. Crop Prot 2013, No. 45, pp. 29–35.

[26] S. Kumar, A. K. Verma, M. Das, S. K. Jain, P. D. Dwivedi, Clinical complications of kidney bean (*Phaseolus vulgarisss* L.) consumption. Nutrition, 2013, No. 29, pp. 821–827.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

SCANNING, IMAGING AND HIGH FREQUENCY PHONONIC ANALYSIS FOR HEALING EFFECT OF CONTROLLING AND MINIMISING THE IMPACT ON BRAIN OF THE STROKE PATIENTS - AN ETHICALITY VIEW

R RANADEVAN[1], A.UMA[2], S JEYAKUMAR[3*],
A. MUNI KUMARI[4], P BIRMANATHAN[5], PRASANTH K[6], K SENTHILKANNAN
[1] Dept. of Biochemistry, Edayathangudy G S Pillay Arts & Science College, Nagapattinam
[2]Dept.of Electronics and communication science, New Prince shri Bavani Arts and Science College, Chennai-100, TN, India
[3]MSAJ group of colleges Chennai, TN India.
[4]Department of Genetics and Genomics, Yogi Vemana University, Kadapa 516003, Andhra Pradesh, India.
[5]Bharathidasan Univ. Model College, Vedaraniyam, Nagapattinam 614810, TN.
[6]Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Coimbatore 641020, TN.
[7]R and D, Edayathangudy G S Pillay Arts & Science College, Nagapattinam 611002, TN
Email: mscgoldmedalist@yahoo.in

Abstract*: Human body as one of the biological specimens having many positive and some hostile cum adverse aspects which are capable or chances of getting diseases or attacked by other parameters [1-8] as the human body is highly perishable in nature. Here, some of the stroke patients are considered for some healing effects to nurture the diseases or control effect [9-16] of stroke in brain by proper investigation by the methods of 1.CT scan, 2.MRI scan, 3. MRA imaging, 4.Doppler ultrasound method … and got permission from ethical committee for performing the heal cum therapy. To magnify the image CTS and for Tissues MRIS and Vascular disease identification MRAI and extra cranial outfit DUS is used*

Keywords*: Stroke patients. CTS. MRIS. MRAI. DUS*

I. Introduction

The World Health Organization (WHO) described stroke as: swiftly developing clinical signs of focal (or global) disturbance of cerebral function with symptoms lasting 24 hours or longer or leading to death [17-25], with no apparent cause other than of vascular origin. Stroke is the abrupt loss of neurological function caused by an interruption of the blood flow to the brain. According to World Health Organization stroke is a neurological deficit of cerebrovascular cause that persists beyond 24 hours or interrupted by death within 24 hours. The prevalence of stroke in India was estimated as 203 per 100,000 population above 20 years, amounting to a total of about 1 million cases. The effect of the stroke may be influenced by the individual's general health and the extent of the damage to the brain, and stroke is the leading cause of long term disability with less than 50% of surviving stroke patients walking in the community [17-25]. Much effort goes in to helping those patients to regain the ability to walk at least in the home. In spite of these effects, approximately 35% of stroke surveyors with initial paralysis of the leg do not regain useful walking function. Many studies have been carried out on gait training of stroke patients with the use of body-weight support during training. Fig.1 and Fig.2 shows the types of strokes and shattering of vessels.

II. AIM

The aim of the study is to find the effectiveness of task oriented healing effect on hemi paretic stroke patients using CTS, MRIS, MRAI and DUS….

CLINICAL MANIFESTATIONS OF SELECTED STROKE SYNDROMES WITH THEIR SYMPTOMS:

VASCULAR TERRITORY	SIGNS AND SYMPTOMS
Anterior cerebral atery	Contralateral:leg>face and arm weakness Frontal signs such as abulia
Middle cerebral artery	Contralateral:face and arm>leg weakness,sensory loss toall modalities,visual field cut,visual-spatial neglect Ipsilateral:gaze preference Dominant hemisphere affected:aphasia,alexia, Agraphia,acalculia
Posterior cerebral artery	Contralateral:homonymous hemianopia With thalamic involvement:Sensory loss to all modalities or pain
Anterior inferior cerebellar artery	Contralateral:hemiparesis and hemisensory loss of pain and temperature Ipsilateral:ataxia
Posterior inferior cerebellar artery	Contralateral: hemibody pain and temperature loss Ipsilateral: facial pain, hemifacial pain and temperature loss, ataxia, nystagmus, nausea/vomiting, vertigo, Horner's syndrome, dysphagia Hiccups
Basilar artery	Bilateral: progressive quadriplegia, facial weakness Lateral gaze weakness with sparing of vertical gaze
Vertebral artery	Contralateral: hemibody weakness, loss of vibration and proprioception Ipsilateral: tongue weakness and/ or atrophy
Vertebral artery	Wallenberg syndrome

III. INVESTIGATION:

Computed Tomography: CTS

Acute bleeding and hemorrhagic transformation are visible on CT scanning. In the sub acute phase, CT scan can delineate the development of cerebral edema (within 3 days), which then fades over the next 2 to 3 weeks. Cerebral infarction (within 3 to 5 days) is visible with the addition of contrast material by showing areas of decreased density.

Magnetic Resonance Imaging: MRIS

MRI is more sensitive in the diagnosis of acute strokes, allowing detection of cerebral ischemia as early as 30 minutes after vascular occlusion and infarction within 2 to 6 hours. It is also able to detail the extent of infarction or hemorrhage and can detect smaller lesions than a CT scan. Use of contrast enhancement allows documentation of changes in an infarct over the first 2 to 3 weeks. MRI scans cannot be performed on individuals with certain implantable devices(e.g., pacemakers) or with patients who are claustrophobic.

Magnetic Resonance Angiography Imaging: MRAI

Magnetic resonance angiography (MRA) is a type of magnetic resonance image that uses special software to create an image of the arteries in the brain. It is used to identify vascular abnormalities (e.g., stenosis), and alterations in blood flow as result of embolus or thrombosis. It provides similar information as classical angiography (x-ray of blood vessels following dye injection) with increased sensitivity of detection and with lowered risks.

Doppler Ultrasound: DUS

Doppler ultrasound imaging is a noninvasive technique that sends sound waves into the body. Echoes bounce off the moving blood and artery and are formed into an image. Diagnostically, transcranial Doppler is used to examine the posterior circulation of the brain (the vertebrobasilar system). Carotid Doppler is used to examine the carotid arteries and typically precedes carotid endarterectomy. It is also used to examine the peripheral arteries in the diagnosis of PAD.

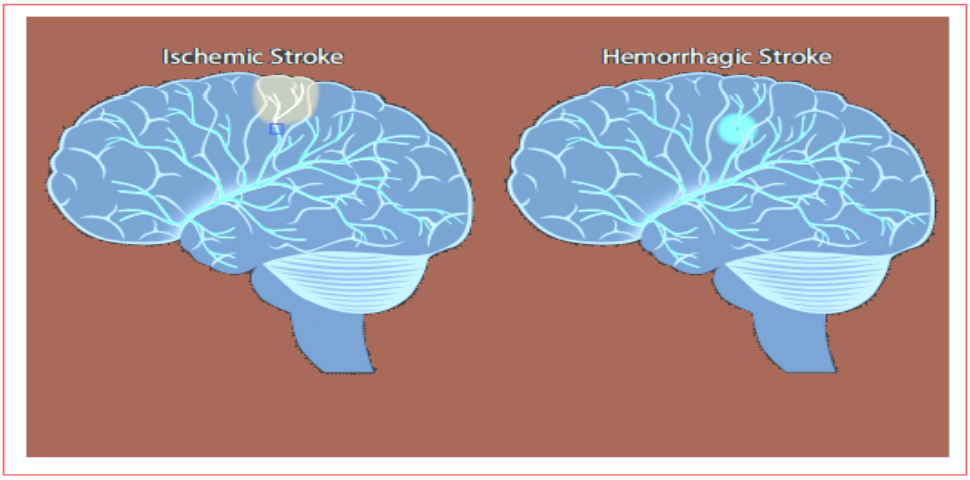


Fig.1 Types of strokes

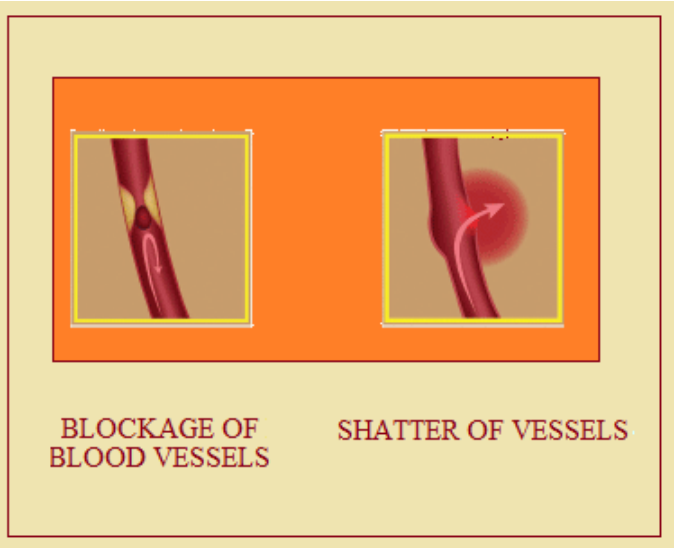


Fig.2 Blockage and shatter of vessels

IV. Conclusion

Stroke is the most common cause of chronic disability, despite the natural spontaneous recovery period, chronic residual impairments in both the contra-lesional upper and lower extremities are present in the majority of individuals. Approximately two thirds of stroke survivors have residual neurological deficits that impair function and approximately 50% are left with disabilities making them dependent on other for activities of daily living. The specificity of impairment is quite heterogeneous and varies with the particular regions of the central nervous system (CNS) that have sustained damage. Ambulatory or walking function is one type of activities of daily living (ADL) that becomes severely impaired following stroke. Here, intracranial investigation is done with the help of CTS, MRIS, MRAI and extra cranial is by DUS and here CTS is for magnifying the image and MRIS is best preferred over CTS for imaging of tissues and MRAI is for vascular variation identification and for high frequency sound by phononic vibrations by DUS and its for outside skull for the analysation and found to be with higher frequencies and delineated that compressibility is high for stroke patients than the normal beings and velocity is low for stroke victims than the counterparts. Data wise the compressibility differs by factor of 2 for the ratio of victims over normal ones and velocity impact by a factor 0.4 - 0.45 depends on the impact of stroke and by persons.

References

1. Ada L, Dean CM, Hall JM, Bampton J, Crompton S (2003). A treadmill and overground walking program improves walking in persons residing in the community after stroke: a placebo-controlled, randomized trial. Arch Phys Med Rehabil. 84(10): 1486-91
2. Ada L, Dean CM, Vargas J, Ennis S (2010). Mechanically assisted walking with body weight support results in more independent walking than assisted overground walking in non-ambulatory patients early after stroke: a systematic review. J. Physiotherapy. 56: 153-161

3. Duncan M, Sean K (2003). Circuit training. In: Porter S. Tidy's Physiotherapy (13th ed), London, Butterworth-Heinemann.), pp. 438-469.
4. English C, Hillier SL (2010). Circuit class therapy for improving mobility after stroke. Cochrane Database of Systematic Reviews. 7: 107120.
5. Enright PL, Sherrill DL (1998). Reference equations for the six-minute walk in healthy adults. American Journal of Respiratory and Critical Care Medicine. 158(5): 1384–1387.
6. Hartona S. experiences from a multicenter stroke register : a preliminary report. BULL WHO 1976;54:541-53
7. Hesse S, Bertelt C, Jahnke MT, Schaffrin A , Baake P, Malezic M, Mauritz KH (1995). Treadmill training with partial body weight support compared with physiotherapy in non-ambulatory hemiparetic patients. Stroke 26; 976-81.
8. Ivey FM, Hafer-Macko CE, Macko RF. Exercise rehabilitation after stroke. Neuro RX. 2006;3(4):439-450
9. Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS. Recovery of walking function in stroke patients: the Copenhagen Stroke Study. Arch Phys Med Rehabil.1995;76:2
10. Lawrence ES, Coshall C, Dundas R, Stewart J, Rudd AG, Howared R. Estiates of acute stroke impairments and disability in a multi-ethnic population. Stroke 2001; 32:1279-1284.
11. Mackay-Lyons MJ ; Makrides L. Exercise capacity early after stroke. Archives of physical medicine and rehabilitation. 2002 ; 83(12): 1697-1702
12. Macko RF, De Souza CA, Tretter LD, Silver KH, Smoth GV, Anderson PA (1997). Treadmill aerobic exercise training reduces the energy expenditure and cardiovascular demands of hemiparetic gait in chronic stroke patients. Stroke. 28(2): 326–30.
13. Macko RF, Katzel LI, Yatacho A, et al., Low velocity graded treadmill stress testing in hemiparetic stroke pts. Stroke 1997; 28(2) : 326-330
14. Mudge S, Barber PA, Stott NS (2009). Circuit based rehabilitation improves gait endurance but not usual walking activity in chronic stroke: a randomized controlled trial. Arch Phys Med Rehabil. 90(12): 1989-96
15. Olney SJ, Richards CL (1996). Hemiparetic gait following stroke. Part I: Characteristics. Gait & Posture. 4: 136–46.
16. Pohl M, Mehrholz J, Ritschel C, Ruckriem S (2002). Speed-dependent treadmill training in ambulatory hemiparetic stroke patients: a randomized control trial. Stroke. 33: 553-558.
17. Richards CL, Olney SJ (1996) Hemiparetic gait following stroke. Part II: Recovery and physical therapy. Gait & posture. 4:149-62.
18. Rose D, Paris T, Crews E, Wu SS, Sun A, Behrman AL, Duncan P (2011). Feasibility and effectiveness of circuit training in acute stroke rehabilitation. Neurorehabil Neural Repair. 25(2); 140-8.
19. Salbach NM, Mayo NE, Wood Dauphinee S et al: a task oriented intervention enhances walking distance and speed in the first year post stroke: a randomized controlled trail. Clin Rehabil,2004,18:509-519
20. Salbach, Mayo et al; a task oriented intervention enhances walking distance and speed in the first year post stroke. RCA clinrehabil 2004:18:509-519
21. Van de Port IGL, Wevers LEG, Lindeman E, Kwakkel G (2012). Effects of circuit training as alternative to usual physiotherapy after stroke: randomised controlled trial. British Medical Journal. 10:344.
22. Verma R, Arya KN, Garg RK, Singh T (2011). Taskoriented circuit class training program with motor imagery for gait rehabilitation in poststroke patients: a randomized contro led trial. Top Stroke Rehabil. 18(1):620-32.l
23. Wade DT, Wood VA, Heller A (1987). Walking after stroke: measurement and recovery over the first three months. Scand J Rehabil Med. 19: 25–30.
24. Wevers L, van de Port I, Vermue M, Mead G, Kwakkel G (2009). Effects of task-oriented circuit class training on walking competency after stroke: a systematic review. Stroke. 40(7): 2450-9.
25. Yocheved L, Dickstein R, Yael C, Marcovitz E (2001). The effect of treadmill training on the ambulation of stroke survivors in the early stages of rehabilitation: A randomized study. Journal of Rehabilitation Research and Development. 38(1): 69–78

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Production of Biodiesel from *Anacardium occidentale* Shell Coat Oil

Nivetha. K, Prethika Murugesan, Suhanika.P, Dr. N. Sivarajasekar*

Department of Biotechnology, Kumaraguru College of Technology

Athipalayam Rd, Chinnavedampatti, Coimbatore, Tamil Nadu 641049, INDIA

nivethakumareson@gmail.com

Abstract: *Biodiesel becomes a popular alternative source of fuel in recent years due to the rise in awareness of the depletion and degradation of fossil fuel resources. In this paper, we have explored the benefits of using non-edible vegetable waste oil (CNSL) for the production of biodiesel. Cashew nut shell liquid oil is a multifaceted by-product of the cashew industry which possesses immense applications in polymer-based industries. Biodiesel was extracted, produced and processed to reduce its viscosity using standard protocols. Transesterification of natural oils with alcohol to alcohol esters is an efficient and technically important reaction in the production of biodiesel from CNSL. Determination of acid value is a prerequisite for the processing of the oil to analyze the free fatty acid content in it. From the results obtained, it was evident that the alkaline treatment of CNSL was required to obtain highly efficient biodiesel. We also conclude that using biodiesel blends are more efficient than using neat biodiesel (100% pure biodiesel obtained from CNSL) since it aids in reducing the vehicle emissions serving us an environmentally friendly option.*

Keywords: *Cashew nut Shell liquid Oil, Biodiesel, acid value, Transesterification, neat biodiesel*

I. Introduction

Developing and populous countries like India are engaged in a lot of research on energy conservation for a better tomorrow. We are obliged to search for an alternative fuel source mainly due to the depletion of fossil fuels and the rise in the crude oil prices. The alternative fuels must be cost-effective, environmentally friendly (low vehicle emission), and easily available. Biodiesel is one of the alternative fuels that are considered highly efficient and can be produced from straight vegetable oil, animal oil/fats, tallow, and waste cooking oil. It is found that biodiesel can significantly reduce vehicle emissions such as CO, HC, and NO_X. It refers to vegetable oil or animal fat-based diesel fuel containing long chain alkyl- esters. Biodiesel should be non-toxic, insoluble in water with a high boiling point and high flash point, producing Sulphur free emissions

Pure oils are not suitable for diesel engines because they can cause problems like engine deposits, injector plugging, or lube oil gelling. So it is necessary to reduce the viscosity of oil which is done using methods like Emulsification, Pyrolysis, Dilution, and Transesterification. Among these, transesterification is the most commonly used method to produce efficient biodiesel.

The cashew nutshell which is unused during cashew nut processing serves as an excellent source. It is a soft honeycomb- like structure which is about 0.3cm thickness. It possesses three layers namely the endocarp, epicarp, and mesocarp. The natural resin present in the mesocarp holds the cashew nut shell oil. Cashew nut shell liquid(CNSL) is a dark reddish brown, viscous liquid which is extracted from the shell coat and the nut. The cashew nut shells are dried to reduce the moisture content and are then milled for obtaining higher content of oil. CNSL consists of anacardic acid, cardanol, and cardol. There are two types of CNSL: Natural CNSL and Technical CNSL. They are distinguished based on their chemical composition. The anacardic acid content is high in Natural CNSL when compared to technical CNSL.

Other than being used as an alternative fuel source, CNSL is also used in other fields. They are food, fodder, medicine, insect repellant manufacturing etc...

Our main objective is to produce biodiesel that helps in reducing the vehicle emissions to a greater extent. It is highly efficient to use biodiesel blended with other fuels since it aids in increased reduction in the vehicle emission of harmful compounds. Hence research is undergone to find more possible solutions in this field to help in pollution control.

Table I shows the physical properties of natural CNSL

S.no	Description	Natural CNSL
1	Molecular formula	C22H303
2	Molecular mass	342
3	Appearance	Dark brown
4	Density g/cm^3	1.009
5	Viscosity, Cp at 30°C	343

II. Experimental Materials and Methods

A. Extraction of Cashew Nut Shell Liquid Oil (CNSL Oil)

There are two types of CNSL, they are
Technical CNSL – rich in cardol
Natural CNSL – rich in anacardic acid
Extraction of CNSL is done using conventional extraction techniques which includes thermal, mechanical and solvent extraction.

1) Thermal Extraction:

Three methods are commonly employed in thermal extraction.

a) Roasting method- the nut shells are to be roasted at a temperature of 180–185ºC. (85-90% recovery)

b) Hot oil bath method- CNSL is obtained in a cylinder in which steam heating is being applied at a temperature of about 200-250°C for 2-3 minutes. (recovery- 7 to 12% by weight)

c) Solar cooker- Oil was extracted by concentrating a solar cooker of 1.4kW capacity. (temperature- 225-300°C)

2) Mechanical Extraction:

For this purpose, an expeller pressing system is needed to extract oil from the raw materials. High pressure is applied to squeeze the raw materials at a single step. The cashew nut shells are put into the expeller pressing system where high pressure is applied to remove CNSL from oil.

3) Solvent Extraction:

This is the most efficient method when compared to all the other methods. It is the extraction method used to separate compounds based on their relative solubilities in two immiscible liquids, water, and an organic solvent. The extraction solvents are of two types,

a) Solvents denser than water- dichloromethane

b) Solvents less dense than water- diethyl ether

B. Free Fatty Acid Determination

Acid value estimates the amount of free fatty acids and mineral acids in the oil sample. It is the amount of KOH needed to neutralize to neutralize the free fatty acids present in one gm of the oil. This should be determined because high fuel acidity owes to corrosion and engine deposits, especially in the fuel injectors. If the acid number is found to increase more than the initial acid number, it can signify intrusion of water or complete fuel degradation. Besides these, the acid number is important for the quality control of feedstocks. Higher acid number reduces the overall ester yield and elevates the NaOH consumption for neutralisation. If the free fatty acid content is found to be high in feedstocks, an acid catalysed transesterification is necessary to process the biodiesel. For biodiesel production, free fatty acid determination is a crucial step to determine the free fatty acid content in the oil (acid value). Determination of pH of the oil is a prerequisite. To produce biodiesel from cashew nut shell liquid, the pH of the liquid is to be found. The pH of the CNSL was found to be 9 and so acid value determination can't be performed.

C. Biodiesel synthesis from Cashew Nut Shell Oil

CNSL cannot be used in its pure form as biodiesel since it has a very high viscosity. The high velocity and the low viscosity of the oil might lead to severe carbon deposits and incomplete combustion, therefore processing of

the oil to obtain biodiesel with all its optimal properties is mandatory. Some of the properties to be considered include,

1) Cold weather properties (cloud point, cold filter plugging point and pour point)

2) Transport and depositing properties (microbial contamination, filterability limit temperature, oxidative and hydrolytic stability, flash point, induction period, etc.)

3) Wear of engine parts (compatibility with materials used to manufacture the fuel system, lubricity, cleaning effect, viscosity, etc.)

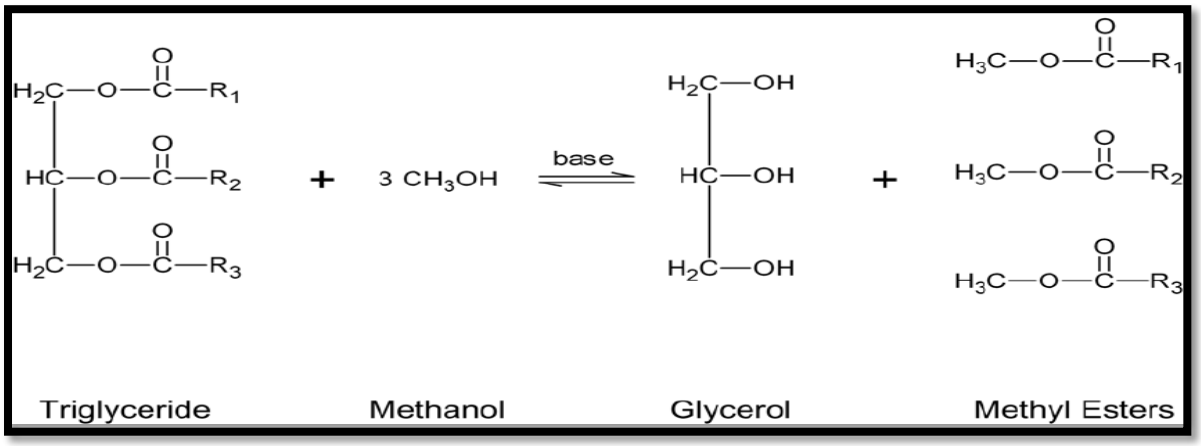
4) Engine properties (exhaust gas formation and quality, ignition qualities, formation and burning of the fuel-air mixture, ease of starting, and the heating value, etc.)

Various methods such as emulsification, pyrolysis, dilution, and transesterification can be employed to reduce the high viscosity of CNSL making it suitable for use as biodiesel.

D. Transesterification

It is the most common method employed to produce biodiesel from oils. Transesterification of natural oils with alcohol to alcohol esters is a technically important reaction in the production of biodiesel from CNSL. There are two types of transesterification, acid catalyzed transesterification and alkali-catalyzed transesterification. Transesterification process is the reaction of a triglyceride with alcohol in the presence of a catalyst which produces esters and glycerol. Most of the biodiesel is produced using a similar chemical process through alkali catalysed transesterification. This method is preferred to acid catalysed transesterification since it is found to be economical and produces 98% conversion with the requirement of low temperature and pressure. A triglyceride has three long-chain fatty acids attached to a glycerin molecule. The nature of the fatty acids attached to the glycerin infers the quality of the fat, which affects the characteristics of the biodiesel.

Figure 1 Transesterification Reaction



For the transesterification process, 17ml of methanol was added to varying concentrations of NaOH (100mg,200mg,300mg,400mg respectively). The methanol oil mixtures were stirred to dissolve NaOH completely and then heated at 60°C for 5 minutes. Simultaneously 50 ml of CNSL was heated to 75-80°C. To this, methanol oil mixture is added in 3 installments (3min interval). The mixture is poured into a separating funnel and left overnight. This was done to obtain separate layers of biodiesel and glycerol which was then washed further to remove dissolved NaOH and other impurities.

The lower phase contains glycerol and the upper phase contains glycerol which was removed and reused after purification in industries such as cosmetic, pharmaceutical, food, and painting industries. Biodiesel was further purified to make it suitable for engine testing. Purification was done to ensure that there is complete removal of glycerol, free fatty acids, alcohol, catalyst, and low Sulphur content.

III. Results and Discussions

The pyrolyzed cashew nut shell oil was subjected to various testing procedures to ensure it is fit for the production of biodiesel. The acid number which is the amount of potassium hydroxide required in milligrams that are required to neutralize one gram of chemical substance. It is a measure of the number of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds.

Biodiesel, when reacted with diethyl ether and phenolphthalein as an indicator and titrated against potassium hydroxide, was around 09.64± 0.01 and the pH at 25 °C is around 7.09 to find out the acid number. This acid number is not fit for biodiesel and hence it is followed with certain other treatment procedures to make it more receptive to biodiesel steps.

The pyrolyzed cashew nut shell oil is subjected to acid – catalyzed transesterification process which involves using a methanolic solution of methyl alcohol and sulfuric acid in the ratio of 1: 16 to the cashew nut shell oil. The mixture is heated and maintained at different temperatures with constant stirring using magnetic stirrer, heating at 60°C for 45 min would be the optimal condition to enable efficient acid- catalyzed esterification process.

The process is further continued with alkaline catalyzed transesterification process. Sodium hydroxide was used to reduce the free fatty acid content present in the cashew nut shell oil which is mixed with sodium hydroxide. Biodiesel would be produced from the reaction between vegetable oil and an alcohol with the aid of strong alkyl catalyst.

Further steps would include the purification of the oil and subjecting it to engine testing before which methanol dissolved in the oil is removed from the cashew nut shell esters to improve the yield of the esters on which we are working on.

IV. Conclusion

The by-products obtained from the cashew processing industries are incorporated for many industrial applications like manufacturing of polymers, friction lining, paints, adhesives. Presence of long carbon linear chain is responsible for its good solubility in diesel. The calorific value of cardanol is high which is closer to diesel. The corrosiveness of it is also very mild due to the nature of phenolic compounds present in it. Higher viscosity is the drawback of every vegetable oil and this viscosity can be reduced while blending with diesel and ethanol. Blending of biodiesel with other fuels is also found to significantly reduce the vehicular emissions of CO, HC, and NO_x thus ensuring and supporting pollution control The pyrolyzed oil was obtained from outside sources and was checked for feasibility for the production of biodiesel, by the following steps such as acid or alkaline treatment which enabled it to lose some of its viscosity and gain the properties of biodiesel. Transesterification is the process which is adapted for the production of the biodiesel, after treating the pyrolyzed oil by acid or alkaline treatment. This is followed by the purification of the biodiesel to undergo engine testing on later stages. This will reduce the dependence of India on the organization of petroleum exporting countries (OPEC) and will lead to the supporting of partial fulfilment of requirements of diesel in India.

References

[1] Kasiraman, G., Nagalingam, B., & Balakrishnan, M. (2012). Performance, emission and combustion improvements in a direct injection diesel engine using cashew nut shell oil as fuel with camphor oil blending. Energy, 47(1), 116-124.

[2] Vedharaj, S., Vallinayagam, R., Yang, W. M., Chou, S. K., Chua, K. J. E., & Lee, P. S. (2014). Experimental and finite element analysis of a coated diesel engine fuelled by cashew nut shell liquid biodiesel. Experimental Thermal and Fluid Science, 53, 259-268.

[3] Velmurugan, A., Loganathan, M., & Gunasekaran, E. J. (2014). Experimental investigations on combustion, performance and emission characteristics of thermal cracked cashew nut shell liquid (TC-CNSL)–diesel blends in a diesel engine. Fuel, 132, 236-245.

[4] Dinesha, P., & Mohanan, P. (2015). Evaluation of combustion, performance and emissions of a diesel engine fuelled with bio-fuel produced from cashew nut shell liquid. Biofuels, 6(1-2), 101-106.

[5] Das, P., Sreelatha, T., & Ganesh, A. (2004). Bio oil from pyrolysis of cashew nut shell-characterisation and related properties. Biomass and bioenergy, 27(3), 265-275.

[6] Devarajan, Y., Nagappan, B. K., & Munuswamy, D. B. (2017). Performance and emissions analysis on diesel engine fuelled with cashew nut shell biodiesel and pentanol blends. Korean Journal of Chemical Engineering, 34(4), 1021-1026.

[7] Ma, F., & Hanna, M. A. (1999). Biodiesel production: a review. Bioresource technology, 70(1), 1-15.

[8] Meher, L. C., Sagar, D. V., & Naik, S. N. (2006). Technical aspects of biodiesel production by transesterification—a review. Renewable and sustainable energy reviews, 10(3), 248-268.

[9] Van Gerpen, J. (2005). Biodiesel processing and production. Fuel processing technology, 86(10), 1097-1107.

[10] Marchetti, J. M., Miguel, V. U., & Errazu, A. F. (2007). Possible methods for biodiesel production. Renewable and sustainable energy reviews, 11(6), 1300-1311.

[11] Das, P., & Ganesh, A. (2003). Bio-oil from pyrolysis of cashew nut shell—a near fuel. Biomass and bioenergy, 25(1), 113-117.

[12] Leung, D. Y., Wu, X., & Leung, M. K. H. (2010). A review on biodiesel production using catalysed transesterification. Applied energy, 87(4), 1083-1095.

[13] Mahanwar, P. A., & Kale, D. D. (1996). Effect of cashew nut shell liquid (CNSL) on properties of phenolic resins. Journal of applied polymer science, 61(12), 2107-2111.

[14] Fukuda, H., Kondo, A., & Noda, H. (2001). Biodiesel fuel production by transesterification of oils. Journal of bioscience and bioengineering, 92(5), 405-416.

[15] Lepage, G., & Roy, C. C. (1986). Direct transesterification of all classes of lipids in a one-step reaction. Journal of lipid research, 27(1), 114-120.

[16] Schuchardt, U., Sercheli, R., & Vargas, R. M. (1998). Transesterification of vegetable oils: a review. Journal of the Brazilian Chemical Society, 9(3), 199-210.

[17] Knothe, G. (2005). Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel processing technology, 86(10), 1059-1070.

[18] Berrios, M., & Skelton, R. L. (2008). Comparison of purification methods for biodiesel. Chemical Engineering Journal, 144(3), 459-465.

[19] Atadashi, I. M., Aroua, M. K., & Aziz, A. A. (2011). Biodiesel separation and purification: a review. Renewable Energy, 36(2), 437-443.
[20] Ramos, M. J., Fernández, C. M., Casas, A., Rodríguez, L., & Pérez, Á. (2009). Influence of fatty acid composition of raw materials on biodiesel properties. Bioresource technology, 100(1), 261-268.
[21] Khalid, A., Tamaldin, N., Jaat, M., Ali, M. F. M., Manshoor, B., & Zaman, I. (2013). Impacts of biodiesel storage duration on fuel properties and emissions. Procedia Engineering, 68, 225-230.
[22] Allen, C. A., Watts, K. C., Ackman, R. G., & Pegg, M. J. (1999). Predicting the viscosity of biodiesel fuels from their fatty acid ester composition. Fuel, 78(11), 1319-1326.
[23] Knothe, G. (2008). "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energy & Fuels, 22(2), 1358-1364.

Acknowledgments

We owe our deepest gratitude to our project guide Dr. N. Sivarajasekar, who took a keen interest on our project work and guided us all along, by providing us all the necessary information and sources to do the project. We express our warm thanks to Dr.N.Saraswathy(Professor and Head, Department of Biotechnology) for her encouragement and support in carrying out the project. We are using this opportunity to express our gratitude to everyone who supported us throughout the project phase with their valuable comments and friendly advice.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

STUDIES ON BIOGAS PRODUCTION FROM DAIRY WASTE BY COGENERATION

Deepamalini. P, Dr. N. Sivarajasekar*
Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu 641049 INDIA, malinipalani98@gmail.com

***Abstract**: In dairy industries after every batch of milk processing the dairy scale formed on the walls of the processing tanks which are rich in carbon sources but these remains are left unused and washed away. It can be utilized in many ways such as feed manufacture for microbial culture, soap manufacture, feed for microalgae to produce biodiesel etc. But these methods are not found efficient and cost effective. So the efficient way to use this waste is to introduce it to biogas production. This dairy waste alone cannot serve as a substrate for the biogas production which could not be digested by the microbes very easily. Cogeneration is the proven technology which takes the industrial/municipal wastes as co-substrates and produce large quantity of biogas. In this case dairy waste can be used as a co-substrate with cow dung. In this study biogas was produced by cogenerating biogas at different compositions of cow dung and dairy waste. The effects of number of days, composition and co- substrate were studied.*

***Keywords:** Dairy waste, Carbon source, Cogeneration.*

I.Introduction

The most important energy sources in our world today are Fossil Fuels. The majority of the huge amount of energy used in the world comes from the burning of three major fossil fuels: coal, petroleum, and natural gas. Fossil fuels are a non-renewable source of energy. the decayed matter of the plants and animals that lived up to 300 million years ago are the fossil fuels on earth today. Deep beneath the earth we can find the fossil fuel. A chemical energy is released when these fuels are burned that is stored within this resource. As we know it energy is essential to modern society. Over 85% of our energy demands are met by the combustion of fossil fuels. It is very important to find an alternate energy because The problem is that the fossil fuel is getting depleted. Searching the energy from renewable sources is a good solution or alternate for this problem, one of such solution is the plant and animal waste that can be converted into biogas.

Biofuel is a fuel that is produced by means of modern technologies such as anaerobic digestion and agriculture, directly biofuels can be derived from plants or indirectly from agricultural, commercial, domestic, and/or industrial wastes.

To treat organic wastes such as cow manure anaerobic digestion is very good, because it produces biogas, a renewable energy source and the remaining digest is an organic fertilizer. Because it is difficult to run a biogas plants with economically profitable results if the process is based only in livestock manure. Co-digestion strategies can be used to enhance the methane production in agricultural biogas plants. Trial and error practice can be used for the addition of co-substrates. There are many advantages of adding fat residues or food waste to their biogas plants. Food waste has a high potential for methane production and can be digested rapidly making it a good source of material for anaerobic co-digestion.

The aim of this work was to study the behavior of co-digestion of cow manure with dairy waste at different composition and optimize the correct ration at which maximum amount of biogas is produced.

Table 1: Composition of Biogas

Component	Concentration (by volume)
Methane (CH_4)	49-53%
Carbon di oxide	30-33%
Nitrogen	5-6%
Hydrogen	5-10%
Water vapor	0.3%

II. Experimental Materials and Methods

A. Experimental Site:

The initial stage of the experiment was carried in laboratory, Kumaraguru College of Technology

B. Climatic condition:

The experiment was carried in normal environmental temperature.

C. Experimental device:

The reactors for the production of biogas was designed in the laboratory itself. Plastic bottles were collected from the stores. The bottle capacity is 2 L and the bottles where checked thoroughly for holes and any other leakages, so that the gas would not escape. Holes were made in the bottle covers to fix the tubes for gas collection. Tubes of length and diameter was connected to the bottle via holes and valves with the same diameter as the tubes were fixed with the tubes to serve as open and close valve. Once the bottle was filled with the feed it was closed and the mouth was wrapped with tape tightly to ensure no leakages.

D. Feed material:

About 5,000 g of cattle waste (cow dung) was collected from the farm and stored at 4°C prior to experiments. Dairy waste was collected from the dairy industry and stored at 4°C. To give a clear explanation about the dairy waste and its origin: In dairy industries large tanks are used to process the milk and to store the processed milk. To maintain the GMP of the production all the tanks were washed after every batch The entire wash water contains contain a high concentration of organic material such as proteins, carbohydrates and lipids, high BOD and COD, and high concentrations of suspended solids and suspended oil-grease.

E. Method:

The substrate and co-substrates was mixed in 6 different combinations and filled in the reactor for the biogas production. The substrates are mixed in different combination and introduced into the reactor. The combinations are shown in Figure 1. It is ensured that the cover was tightly closed after the feed introduction and the valve opening was closed.

Table 2: Combination of the substrates at different ratio

Combination	1	2	3	4	5	6
Cow dung(g)	1000	900	800	700	600	500
Dairy waste(g)	0	100	200	300	400	500

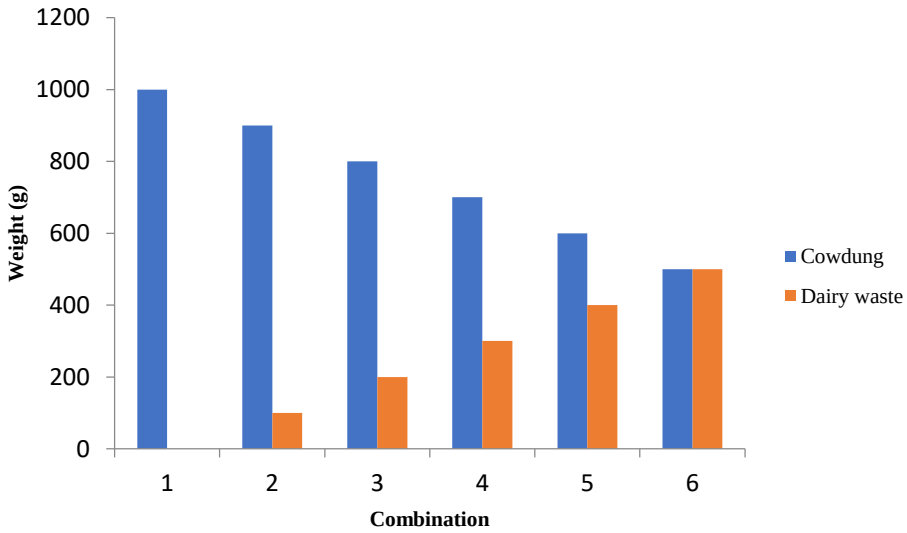
Figure 1: **The bioreactors after the introduction of feed.**



Figure 2: Buried bioreactors with the tubes extended out for gas collection



Figure 3: The ratio of the substrate and co-substrate.



F. Measurement of the gas by weighing method:

The reactors with the mixed substrates were carefully buried underground and the pipes were stretched outwards for the gas collection. The gas was collected using balloons and the amount of gas produced was measured by subtracting the weight of the empty balloon from the weight of the balloon with gas.

Weight of the empty balloon = 1.73 g.

The balloon was fixed to the valve and the gas produced was filled in the balloon, after that the weight of the balloon with the gas was weighed, then the amount of gas actually produced was measured by using the formula,

Amount of gas collected (g) = Weight of the balloon with gas (g) – Weight of the empty balloon (g)

III. Result and discussion

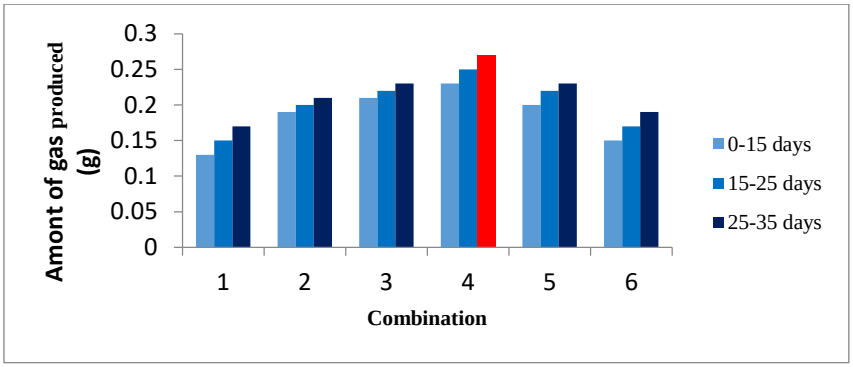
Amount of gas produced in batch studies:

Table 3: Amount of gas produced from different composition of substrate and at regular time intervals.

Proportion(cow dung : dairy waste)	Number of days		
	0-15	15-25	25-35
	Amount of gas produced in g		
10:0	0.13	0.15	0.17
9:1	0.19	0.20	0.21
8:2	0.21	0.22	0.23
7:3	0.23	0.25	0.27
6:4	0.20	0.22	0.23
5:5	0.15	0.17	0.19

Combination at which the maximum amount of gas is produced:

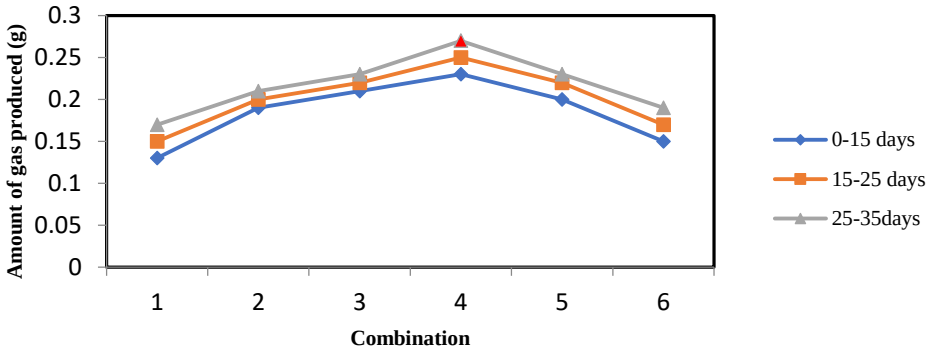
Figure 4: The combination for which the maximum amount of the gas is produced.



From the graph- Figure 4 it is clear that the maximum amount of gas is produced in 4th combination that is the mixture of 700 g cow dung and 300 g dairy waste.

Time interval at which the maximum amount of gas is produced:

Figure 5: The time interval at which the maximum amount of gas is produced.



From the graph- Figure 5 it is clear that the maximum amount of gas is produced at the time interval of 25-35 days.

IV. Conclusion

The results of this study show that the production of methane by co-digestion of dairy waste and cattle slurry is possible. By using this strategy, the dairy waste from many such industries can be utilized efficiently. The main users of the gas produced from this co-digestion will be the population around the industries who already have a biogas plant so that the dairy waste can be added additionally in the above optimized combination to get reasonable amount of bio gas. The composition of the biogas is not much important here because the main usage of the biogas produced is for heating (burning fuel). Further studies can be carried out by optimizing various parameters such as temperature, pH etc., and quantify the amount of biogas produced.

References

[1] E. Marañón , L. Castrillón , G. Quiroga , Y. Fernández-Nava , L. Gómez , M.M. García .Co-digestion of cattle manure with food waste and sludge to increase biogas production,(2012).

[2] Kondusamy, D., & Kalamdhad, A. S. (2014). Pre-treatment and anaerobic digestion of food waste for high rate methane production – A review. *Journal of Environmental Chemical Engineering*, *2*(3), 1821–1830. https://doi.org/10.1016/j.jece.2014.07.024

[3] L. Neves, R. Oliveira, M.M. Alves. Co-digestion of cow manure, food waste and intermittent input of fat. (28 November 2008).

[4] M.R. Al-Masri. Changes in biogas production due to dierent ratios of some animal and agricultural wastes (2000).

[5] Maritza Macias-Corral , Zohrab Samani a, Adrian Hanson , Geoffrey Smith , Paul Funk , Hui Yu , John Longworth (2008).

[6] Martin, M., & Parsapour, A. (2012). Upcycling wastes with biogas production: An exergy and economic analysis. *Venice*.

[7] Njogu, P., Kinyua, R., Muthoni, P., & Nemoto, Y. (2015). Biogas Production Using Water Hyacinth (Eicchornia crassipes) for Electricity Generation in Kenya. *Energy and Power Engineering*, (May), 209–216. https://doi.org/10.4236/epe.2015.75021

[8] Patinvoh, R. J., Osadolor, O. A., Chandolias, K., Sárvári Horváth, I., & Taherzadeh, M. J. (2017). Innovative pretreatment strategies for biogas production. *Bioresource Technology*, https://doi.org/10.1016/j.biortech.2016.11.083

[9] Ren, Y., Yu, M., Wu, C., Wang, Q., Gao, M., Huang, Q., & Liu, Y. (2018). A comprehensive review on food waste anaerobic digestion: Research updates and tendencies. *Bioresource Technology*, *247*(July 2017), 1069–1076. https://doi.org/10.1016/j.biortech.2017.09.109

[10] Zamanzadeh, M., Hagen, L. H., Svensson, K., Linjordet, R., & Horn, S. J. (2017). Biogas production from food waste via co-digestion and digestioneffects on performance and microbial ecology. *Scientific Reports*, *7*(1), 1–12. https://doi.org/10.1038/s41598-017-15784-w

[11] Zhang, R., El-Mashad, H. M., Hartman, K., Wang, F., Liu, G., Choate, C., & Gamble, P. (2007). Characterization of food waste as feedstock for anaerobic digestion. *Bioresource Technology*, *98*(4), 929–935. https://doi.org/10.1016/j.biortech.2006.02.039

Acknowledgments

We owe our deepest gratitude to our project guide Dr. N. Sivarajasekar, who took a keen interest on our project work and guided us all along, by providing us all the necessary information and sources to do the project. We express our warm thanks to Dr. N.Saraswathy (Professor and Head, Department of Biotechnology) for her encouragement and support in carrying out the project. We are using this opportunity to express our gratitude to everyone who supported us throughout the project phase with their valuable comments and friendly advice.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Thermal death kinetics analysis for effective sterilization of microorganisms and shelf life improvement of milk

Elakya .M.V.[1] Suvetha. R[2] and G. Bharath[3]

Department of Food technology, K.S. Rangasamy College of Technology

elakyamv@gmail.com

Abstract*: India is the world's largest producer of milk and is a leading exporter of skimmed milk powder, which is a perishable food which needs preservation. The composition of cow's milk is 87.7% water, 4.9% lactose, 3.4% fat, 3.3% protein and 0.7% minerals, milk is highly nutritious, high moisture content, possesses a neutral or slightly acidic and therefore allows the growth of a wide range of microorganisms. The purpose of the paper is to evaluate the growth rate and identify the activation energy efficacy mathematical model that describes microbial growth in milk. The microorganisms Listeria monocytogenes, Yesinia enterocolitica, Shigella flexneri, Bacillus cereus are the most common milk spoilage organisms, the analysis of Log C value on various time periods to sterilization temperature was studied. The results showed that the yield coefficient and Monod kinetics was linear and the individual growth rates were analysed for the effective determination of thermal death time. The activation energy required along with various sterilization temperature was analysed.*

Keywords: Yield coefficient, Monod kinetics, Activation energy.

I. Introduction

Mathematical modelling is translating problems from an application area into tractable mathematical formulations [1]. A model may help to explain a system and to study the effects of different components, and to make predictions about behaviour India is the world's largest producer of milk [3] and is a leading exporter of skimmed milk powder, which is a perishable food which needs preservation [9]. The composition of cow's milk is 87.7% water, 4.9% lactose, 3.4% fat, 3.3% protein and 0.7% minerals, milk is highly nutritious, possesses a neutral or slightly acidic and high moisture content, and therefore allows the growth of a wide range of microorganisms[11]. The purpose of the paper is to evaluate the growth rate and identify the activation energy efficacy of mathematical model that describes microbial growth in milk [7]. The main objective of the proposed work was to evaluate a mathematical model that describes microbial growth in milk [8]. In order to test the ability of the model to deal with experimental data, four different datasets of microbial growth in dairy products were selected from Combase (www.combase.cc), which is the most important database in the area [2]. The parameters obtained were used to estimate the duration of the exponential and lag phase of the microorganisms studied.

II. Materials and Methods

A. Experimental data

The experimental data for the analysis was collected from combase(www.combase.cc). The data consists the growth of four different microorganism found in milk. The datasets used in this analysis can be obtained using the following Id codes

- *Listeria monocytogenes:* Buchanan_91a
- *Yesinia enterocolitica:* FSA-CCFRA
- *Shigella Flexner:*Zaika_98
- *Bacillus cereus.*STU-BA

Table I shows value from combase

MICROORGANISM							
Listeria monocytogens		***Yersinia enterocolitia***		***Shigella Flexneri***		***bacillus cereus***	
Time	Logc	Time	Logc	Time	Logc	Tim	Logc
0	2.9	0	3.66	0	2.99	0	2.18
8	3.1	24	5.04	19.5	3.67	28	2.45
24	4.7	48	6.49	27	4.12	52	2.73
36	5.4	72	7.46	45	5.01	76	3.69
48	6.5	96	8.46	52.5	5.43	100	4.23
55	6.7	168	8.81	70.5	6.69	148	5.88
72	7	192	8.85	76.5	7.05	172	6.46

B. Computation of graphical model

The computation of graphical model was carried out using OriginPro 8 software. The fitted parameters were used to determine the specific growth rate (μ), maximum growth rate (μ_{max}) using the monod equation and is given by

$$\mu = \mu_{max}\frac{[S]}{k_S + [S]} \quad (1)$$

Another parameter which may be useful in the food industry is the growth of microorganism at specific time and concentration of the substrate. Cell mass and product formation by microorganisms can be described quantitatively by yield coefficient. It is expressed as the mass of cells or product formed per unit mass of substrate consumed.

$$\text{Yield coefficient(Y)} = \frac{mass\ of\ new\ cells}{mass\ of\ substrate\ consumed} \quad (2)$$

Another important parameter to consider is the cell mass production per unit of time. Specific growth rate is the rate of increase in cell mass per unit of time.

$$\text{Specificgrowthrate} = \frac{\text{mass of cells produced}}{\text{original mass of cells X time}} \text{X } \frac{1}{\text{time}} \quad (3)$$

The beginning of the log phase,logc, is the point of inflation of the growth curve which can be explained as the point of maximum acceleration on growth.the time corresponding to this point can be found from the graph. The growth kinetics for the microorganism were found inorder to predict the death kinetics of each microorganism. The following procedure was used in this study.

The calculations comprises the following steps:

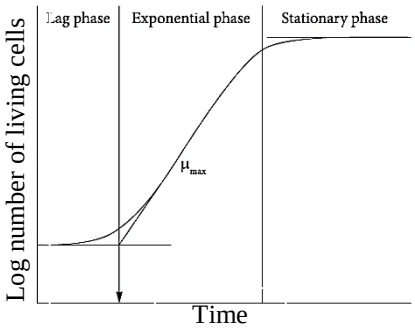
1) Fit of experimental data selected from Combase using the curve-fitting software Origin 8.0.

2) Use of the fitted parameters to calculate μ using Equation 1.

3) Use of the fitted parameters to calculate Y for different values of substrate using Equation 2.

4) Use of the fitted parameters to plot the specific growth rate of each microorganism using Equation 3.

5) Determination of the parameter Ea defined as $Ea = \frac{2.303\ RTminTmax}{z}$, which corresponds to the activation energy.

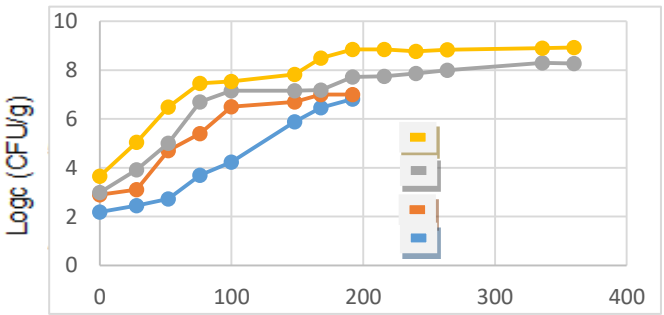


III. RESULTS AND DISCUSSIONS:

C. Analysis of data

The initial values for the analysis were collected from combase for four different microorganisms and were plotted in a graph as shown in figure 1

Figure 1 represents the time Vs logc value for the microorganism.



Time

The curve fitting for four organisms were plotted in origin, the fittings are represented in the following figures.

Figure 2 represents *bacillus cereus*

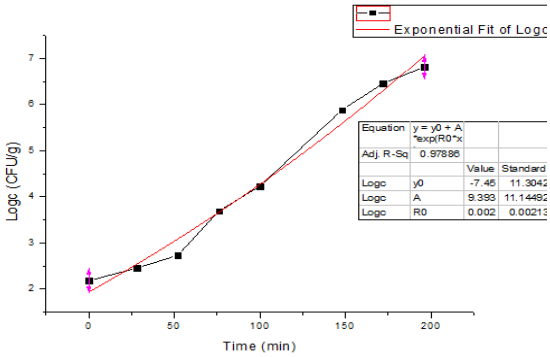


Figure 3 represents *listeria monocytogenes*

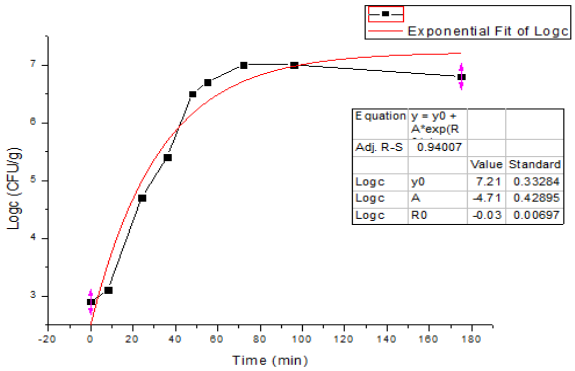


Figure 4 represents *shigella flexneri*

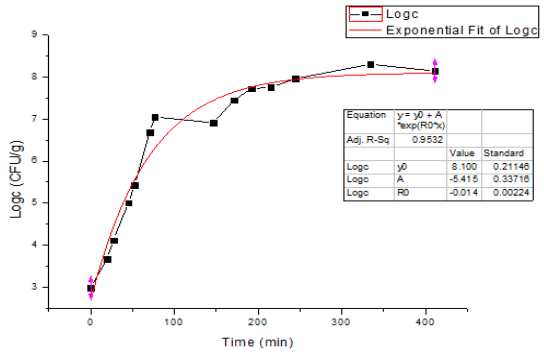
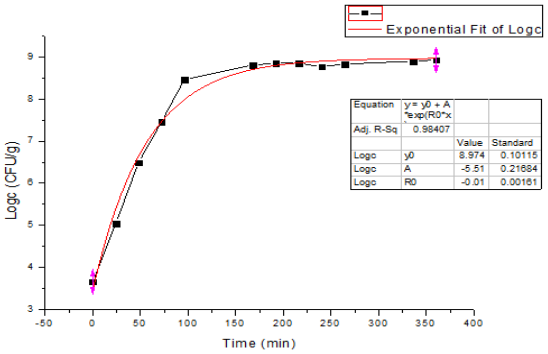


Figure 5 represents *Yersinia enterocolitica*.



D. Growth kinetics

i. Monard equation

Figure 6 shows the fitted curve curves for the five microorganisms used in this paper. As can be seen, the determination coefficient was higher than 0.99, which indicates a good fit.

ii. Yield coefficient

The results obtained for the yield coefficient is summarized in Table II. It represents fitted parameters using Equation 2 for each microorganism.

Figure 6 Represents the graph for Monod equation

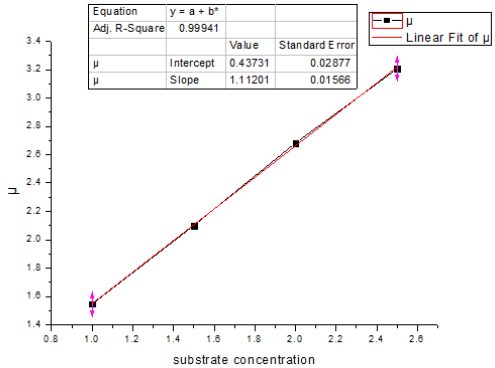


Table II shows yield coefficient value

Mass of new cells	Mass of substrate consumed	Yield coefficient (y)
4.63	1	4.63
5.9	1.5	3.933333333
6.63	2	3.315
8.12	2.5	3.248

iii. Specific growth rate

The specific growth rate for the microorganisms obtained from the equation 1 are represented in the following figures.

Figure 7 Represents *bacillus cereus*

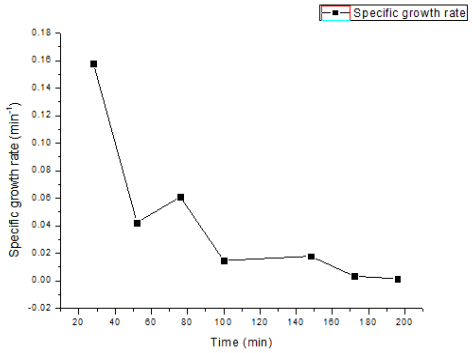


Figure 8 Represents *listeria monocytogenes*

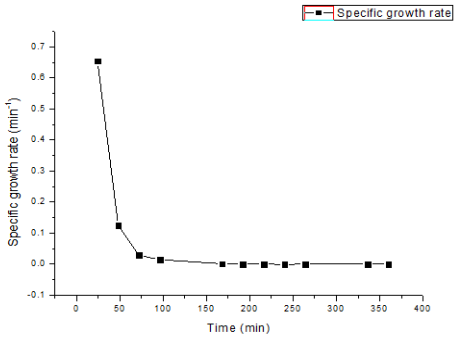


Figure 9 Represents *shigella flexneri.*

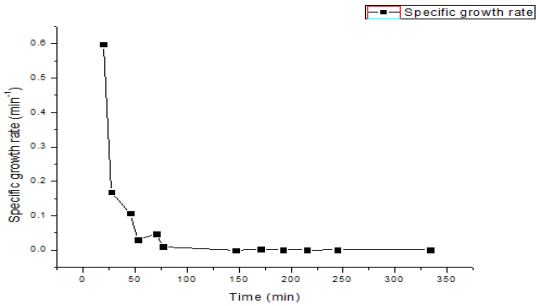
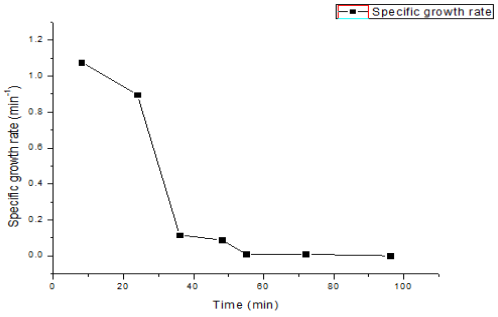


Figure 10 Represents *yersinia enterocolitica*



iv. Activation energy

The activation energy for the organism were calculated by the following equation.

$$Ea = \frac{2.303\ RTminTmax}{Z}$$

Where,

R = gas constant = 8.314

T_{min} = minimum time required for its growth

T_{max} = maximum time required for its growth

Z = sterilization temperature.

Table III shows for *bacillus cereus*

T_{min}	T_{max}	Z	Ea
1.1	196	100	41.28123815
1	197	102	36.98026445
1.3	195	101	48.05743066

Table IV shows for *shigella flexneri*

T_{min}	T_{max}	Z	Ea
4	360	42	656.47344
4.3	361	44	675.5024665
4.6	360.5	47	675.5682038

Table V shows for *Yersinia enterocolitica*

T_{min}	T_{max}	Z	Ea
2.9	411	41	556.621428
2.5	412	42.5	464.0366179
2.7	413.5	46	464.7136236

Table VI shows for *listeria monocytogenes*

T_{min}	T_{max}	Z	Ea
3	175	79.4	126.6026392
3.5	174	74	157.5758038
2.9	176	75.6	129.2685354

The coefficient of determina

tion for the **fig: 2,3,4,5** curve fitting is 0.97886, 0.94007, 0.9532, 0.98407 which represents a good fit and helped to determine the saturation point which was used to find the beginning of stationary phase. The equation for the graph was determined and values were noted from graph.

The specific growth rate helps to find the growth of microorganism at specific interval of time. The growth rate of *bacillus cereus* and *listeria monocytogenes* decreases over time and reaches its death phase at around 3 hours and 2 hours. The growth rate of *shigella flexneri* and *yersinia enterocolitica* decreases over time and reaches its death phase at around 6 hours and 6 and half hours.

VI. Conclusion

The growth kinetics of the microorganism was predicted for four different microorganisms present in milk. The data from combase shows the growth of four microorganisms in dairy products were fitted with curve and the results showed good agreement between the theory and experiment. The curve fitting was used to determine the saturation points which helps to find the end of log phase. By using this, the growth kinetics of the microbes was studied using Monod equation. The results showed that the yield coefficient and Monod kinetics was linear and the individual growth rates were analysed for the effective determination of thermal death time. The activation energy required along with various sterilization temperature was analysed. In order to test the ability of the model to deal with new situations that were not tested in the derivation of the model, such as time-varying temperature conditions, further studies are necessary. Currently the model predicts the optimal temperature for pasteurisation for killing the major spore forming bacteria.

References

[1] BARANYI, J.; ROBERTS, T. A. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, v. 23, p. 277-294, 1994. http://dx.doi. org/10.1016/0168-1605(94)90157-0.

[2] BARANYI, J.; ROBERTS, T. A.; MCCLURE, P. A non-autonomous differential equation to model bacterial growth. Food Microbiology, v. 10, p. 43-59, 1993. http://dx.doi.org/10.1006/fmic.1993.1005.

[3] ZWIETERING, M. H. et al. Modeling of the Bacterial Growth Curve. Applied and Environmental Microbiology, v. 56, n. 6, p. 1875-1881, 1990. PMid:16348228. PMCid:184525.

[4] BUCHANAN, R. L.; CYGNAROWICZ, M. L. A mathematical approach toward defining and calculating the duration of the lag phase. Food Microbiology7, p. 237-240, 1990. http://dx.doi. org/10.1016/0740-0020(90)90029-H.

[5] BUCHANAN, R. L.; SOLBERG, M. Interaction of sodium nitrite, oxygen and pH on growth of Staphylococcus aureus. Journal of Food Science, v. 37, p. 81-85, 1972. http://dx.doi. org/10.1111/j.1365-2621.1972.tb03391.x.

[6] CHEN, H. Use of linear, Weibull, and log-logistic functions to model pressure inactivation of seven foodborne pathogens in milk. Food Microbiology, v. 24, p197-204, 2007. PMid:17188197. http:// dx.doi.org/10.1016/j.fm.2006.06.004.

[7] CHEN, H.; HOOVER, D. G. Modeling the combined effect of high hydrostatic pressure and mild heat on the inactivation kinetics of Listeria monocytogenes Scott A in whole milk. Innovative Food Science & Emerging Technologies, v. 4, p. 25-34, 2003. http:// dx.doi.org/10.1016/S1466-8564(02)00083-8.

[8] CONTE, A. et al. Active Packaging Systems to Prolong the Shelf Life of Mozzarella Cheese. Journal of Dairy Science, v. 90, p. 2126-2131, 2007. PMid:17430909. http://dx.doi.org/10.3168/jds.2006-709.

[9] DUYVESTEIN, W. S.; SHIMONI, E.; LABUZA, T. P. Determination of the End of Shelf-life for Milk using Weibull Hazard Method. Lebensmittel-Wissenschaft und-Technologie - Food Science and Technology, v. 34, p. 143-148, 2001.

[10] FRANCO, B. D. G. M.; LANDGRAF, M. Microbiologia dos alimentos. São Paulo: Atheneu, 2007. 182 p.

[11] FUJIKAWA, H.; MOROZUMI, S. Modeling Staphylococcus aureus growth and enteroxin production in milk. Food Microbiology, v. 23, p. 260-267, 2006. PMid:16943012. http://dx.doi.org/10.1016/j. fm.2005.04.005.

[12] GIBSON, A. M.; BRATCHELL, N.; ROBERTS, T. A. The effect of NaCl and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry. Journal of Applied Bacteriology, v. 62, p. 479-490, 1987. http://dx.doi. org/10.1111/j.1365-2672.1987.tb02680.x.

[13] GOUGOULI, M.; ANGELIDIS, A. S.; KOUTSOUMANIS, K. A Study on the Kinetic Behavior of Listeria monocytogenes in Ice Cream Stored Under Static and Dynamic Chilling and Freezing Conditions. Journal of Dairy Science, v. 91, p. 523-530, 2008. PMid:18218738. http://dx.doi.org/10.3168/jds.2007-0255.

[14] GUAN, D.; CHEN, H.; HOOVER, D. G. Inactivation of Salmonella typhimurium DT 104 in UHT whole milk by high hydrostatic pressure. International Journal of Food Microbiology, v. 164, p. 145-153, 2005. PMid:16099523. http://dx.doi.org/10.1016/j. ijfoodmicro.2005.01.014.

[15] LE MARC, Y.; VALÍK, L.; MEDVED'OVÁ, A. Modelling the effect of the starter culture on the growth of Staphylococcus aureus in milk. International Journal of Food Microbiology, v. 129, p. 306-311, 2009. PMid:19157613. http://dx.doi.org/10.1016/j. ijfoodmicro.2008.12.015.

[16] NAKASHIMA, S. M. K.; ANDRÉ, C. D. S.; FRANCO, B. D. G. M. Revisão: Aspectos Básicos da Microbiologia Preditiva. Brazilian Journal of Food Technology, v. 3, p. 41-51, 2000.

[17] NDAGIJIMANA, M. et al. Growth and metabolites production by Penicillium brevicompactum in yoghurt. International Journal of Food Microbiology.http://dx.doi.org/10.1016/j.ijfoodmicro.2008.07.019.

[18] RATKOWSKY, D. A. et al. Relationship Between Temperature and Growth Rate of Bacterial Cultures. Journal of Bacteriology, v. 154, n. 1, p. 1222-1226, 1982.

[19] RATKOWSKY, D. A. et al. Model for Bacterial Culture Growth Rate Throughout the Entire Biokinetic Temperature Range. Journal of Bacteriology, v. 154, n. 3, p. 1222-1226, 1983. PMid:6853443. PMCid:217594.

[20] RICHARDS, F. J. A flexible growth functions for empirical use. Journal of Experimental Botany, v. 10, p. 290-300, 1959. http://dx.doi. org/10.1093/jxb/10.2.290.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

EXTRACTION OF PHYTOCONSTITUENTS FROM *Rocella montagnei* AND EVALUATION OF ITS ANTI APOPTOTIC POTENTIAL

R.Niroshini[1], V.Evangelin Shaloom[1], B.Lakshmi Priya[1], R.Shyam Kumar[1], D. Mathews LurthRaj[1], A.Ronaldo Anuf[1]*

[1]Department of Biotechnology, Kamaraj College of Engineering and Technology, K.Vellakulam, Near Virudhunagar, Madurai, India, *Mail id: ronaldoanuf@gmail.com*

***Abstract**: Lichens are abundant in secondary metabolites that have been identified to contain anti-cancer and many other therapeutically significant properties. Rocella montagnei , an epiphytic lichen which remains to be one of the least exploited lichen species is expected to be a potential drug source for cancer. Extraction of phytoconstituents was performed using Microwave assisted extraction from Rocella montagnei is crucial. The solvent system and process parameters such as time, pH, microwave power and liquid to solid ratio were optimized using one variable at a time (OVAT) method. Maximum yield of 31.6 mg GAE/g.dw was obtained when the sample was treated at a microwave power of 630 W for 60 seconds with a liquid to solid ratio of 10 mL/g .The crude extract was then subjected to Gas Chromatography- Mass Spectroscopy analysis for the identification of potential phytoconstituents. The compounds elucidated from Gas Chromatography- Mass Spectroscopy were then docked with anti-apoptotic protein BCL2 to evaluate the anti-apoptotic activity of phytoconstituents. Molecular docking of the phytoconstituents against BCL2 (PDB ID 2W3L) was performed using AutoDockVina. The docking score was found to be the highest for 6-Methoxyflavanone with a binding energy of -7.4kCal/mol. Further in vitro and in vivo analyses are to be performed to prove the efficacy of the drug.*

***Keywords**: Lichen;Rocella montagnei;Phytoconstituents;6-methoxyflavanone; Bcl2; Apoptosis, Cancer.*

I. Introduction

The increase in the number of multiresistant organisms has created a demand for novel drugs. Lichens, a symbiotic association of algae and fungi [1], contain a wide range of secondary metabolites. They can withstand extreme conditions like drought, high salinity and high concentration of air pollutants [2]. Substances present in lichen are classified as primary and secondary metabolites. The primary metabolites perform the structural functions and cellular metabolism. These substances are similar to those found in plants [3]. The secondary metabolites can be found as extracellular crystals on the cell wall of the mycobiont. These metabolites defend the lichens from abiotic and biotic stress. They possess anti-cancer, anti-bacterial and many other therapeutically important properties. It was evident from the chemical structure that these components were mostly phenols and their derivatives [1].

Rocella montagnei, a fruticose lichen, belongs to the family Rocellaceae. This epiphytic lichen is commonly found along the Coromandel coast of India. They are found in abundance in the mangrove forests of Pichavaram. This species of lichen is rich in secondary metabolites such as montagnetol, -sitosterol, orcinol, -carotene, roccellic acid, methyl orsellinate, lecanoric acid and erythritol[4]. Researches have proved that *R.montagnei* possesses anti-inflammatory [5], antimicrobial [1] and insecticidal activity[6].

Cancer is one of the deadliest diseases in the world [7]. It has been identified as the second leading cause of death in developing countries [8].According to the World Health Organization , cancer is responsible for around 9.6 million deaths worldwide and the number is predicted to increase in the following years. The present treatment methods include chemotherapy, radiation, immunotherapy and surgery. Due to poor oral bioavailability , low therapeutic indices, high toxicity , low solubility , non-specific bio distribution , specific delivery of anticancer drugs and inconsistency in circulation make the treatment of this disease highly challenging [9, 10]. Drugs with high efficiency should be developed in order to combat cancer. Polyphenols have been identified as potential drug candidates for the treatment of cancer. They contain one or more hydroxyl groups attached to at least one aromatic ring. They are divided into phenolic acids, stilbenes, lignans, flavonoids and other polyphenols depending on their chemical structure. The most common classes are the natural polyphenols and flavonoids making up to 30% and 60% respectively. Polyphenols control pathways associated with proliferation, migration, cell survival, angiogenesis, detoxification enzymes, differentiation, immune response, hormonal activity, etc., [14]. Their ability to modulate the signaling pathways and molecular targets in

addition to their antioxidant and anti-inflammatory activities makes it an excellent drug for the treatment of cancer.

The treatment methods for cancer target proteins involved in the apoptotic activity of cells. One such protein has been used as the target in this study.BCL-2 is a protein belonging to the family of BCL-2 that controls the intrinsic pathway of apoptosis. They work by inhibiting the release of cytochrome C in the mitochondria. The anti-apoptotic class of BCL-2 proteins binds to the pro-apoptotic class of BCL-2 proteins, thus preventing the cells from undergoing apoptosis.

R.montagnei is rich in polyphenols. In this study, polyphenol extraction was done using microwave extraction which is comparatively a novel method. Both the consumption of solvent and the time of extraction is reduced in this method. However, optimization of the process is necessary for high yield and cost efficiency [12].OVAT, a traditional method of optimization was used in this experiment[13].

II. Materials and methods

Collection Of Sample :

The lichen sample was collected from Karaikal district in the district of the union territory of Puducherry. It was found on the surface of palm trees present in the coastal regions of the Coromandel Coast. The lichen was sun-dried and powdered using a blender to increase the surface area. The powdered sample was stored in an airtight container.

Optimization:

The univariate, conventional technique for optimization is One Variable At a Time (OVAT) approach. It gives an easier interpretation of the whole process [15]. The condition for extraction of polyphenols from lichen was optimized using the OVAT approach. The process was found to be dependent on the following factors: liquid to solid ratio, time and microwave power. The effect of the parameters on the sample was studied using this approach. The factors were varied as LSR 10-50 mL/g, microwave power 450-810 W, time 30-120 s and solvent (methanol, ethanol, hexane, water, and acetone).

Solvent Selection:

The solvent was varied as methanol, ethanol, hexane, water, and acetone based on their polarity. Phytoconstituents were found to be insoluble in water and mostly extracted in organic solvents. Hence it was expected to be extracted by the polar solvents. Methanol was found to be used as a commonly used solvent for the extraction of the bioactive compounds with antioxidant activity from the lichen sample[16].

Extraction :

1 g of air-dried, the powdered sample was added to 10 mL of methanol and the sample was exposed to different microwave conditions (Microwave power 450-810, time 30-120 s) by the varying irradiation time, microwave power and the solid/liquid ratio. The extract was filtered using a filter paper. The methanol extract was stored and refrigerated for further analysis.

Assay For Total Phenolics:

The total phenol content of the methanol extract was determined using the Foiln-Ciocalteu reagent. 1:10 ratio of reagent to distilled water was prepared. 100µL of the diluted reagent was added to the well of ELISA reader. Briefly,100µL of 7.5% sodium carbonate was added to it. 10µL of the extract was added. Reading was taken in 750 nm respectively. The standard graph was constructed for Gallic acid using the same procedure.

Gas Chromatography-Mass Spectroscopy :

The lichen extract was analyzed using the Gas Chromatography-Mass Spectrum analyzer. With the help of a microinjector, 1 µL of lichen extract was injected into the equipment and scanned for 45 minutes. Gas flow rate, oven temperature, and the electron gun were set accordingly before the initiation of the process. The eluted compounds entered the detector which was then processed by the computer. The intensity of the signal was found to increase with an increase in the concentration of the sample. The mass spectrum graph gives the fingerprint of the molecules. Comparing the spectra with NIST 11.L library helped in the identification of the compounds.

Ligand And Protein Screening:

The protein target was selected based on literature review. The 3D structure of the selected protein molecule Bcl2 (PDB ID: 2W3L) was retrieved from the Protein Data Bank (https://www.rcsb.org/) in .pdb format. The 3D structures of the Ligand compounds present in the lichen *Rocella montagnei*, that are elucidated from GCMS were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). They were downloaded in .sdf format and converted to .pdb (for docking) and .mol(for ADME analysis) using Open Babel tool.

ADME analysis :

To enhance the activity of the drug and neglect any crisis in drug delivery, the molecular properties of the drug should be analyzed. The absorption, distribution, metabolism, and excretion (ADME) of the molecule should be performed[17]. The ligand molecules were subjected to ADME analysis using PreADMET software. The parameters considered include CaCO2, MDCK, BBB, PLASMA binding capacity.

Lipinski's Rule:

The Lipinski's rule of five should be passed in order to use a molecule as a drug. SwissADME software was used to obtain the molecular weight, number of H bond acceptors, number of H bond donors and MLOGP of the molecules. The rule shows that the molecule has less permeation and absorption when the following conditions are present. a) greater than 5 hydrogen bond acceptors, b) mLogP is greater than4.15, c) greater than 10 hydrogen bond acceptors and d) molecular weight greater than 500[18]. Only those that pass this analysis were taken to further studies.

Molecular Docking :

The molecular docking has the accuracy in substantial degree and the prediction of the ligand or small molecule conformation in the binding site pocket of the target molecule. It became an essential tool to predict drug binding efficiency[19].

The crystal structure of the protein, BCL2 was retrieved from the protein data bank (PDB) with PDB id: 2W3L. The small molecules were removed and hydrogens were added using Pymol software. The protein was prepared for docking. The three-dimensional structures of ligand and the protein were loaded into Auto Dock software, where the molecular docking was performed. Each ligand was docked with the protein target in quest of finding the best fit. The exhaustiveness was set to 5. The output of the docking was stored in .pdbqt format. The files were opened in pymol viewer to find out the hydrogen bond interactions between the protein and the ligand. The interacting residues, bond length and the number of bonds were all viewed from this software.

III. Results and discussion

A.Screening of solvent system:

The polyphenols from the lichen sample were extracted using a different solvent such as Methanol, Ethanol, Acetone, Hexane and Water by microwave analysis. The phenols in the extract were tested using Folin-Ciocalteu reagent test. They were evaluated using Gallic Acid as standard. The methanol extract was found to contain higher phenolic content when compared with other solvents. Methanol extract contained about 30.2mgGAE/g.dw Total phenolic content (TPC).

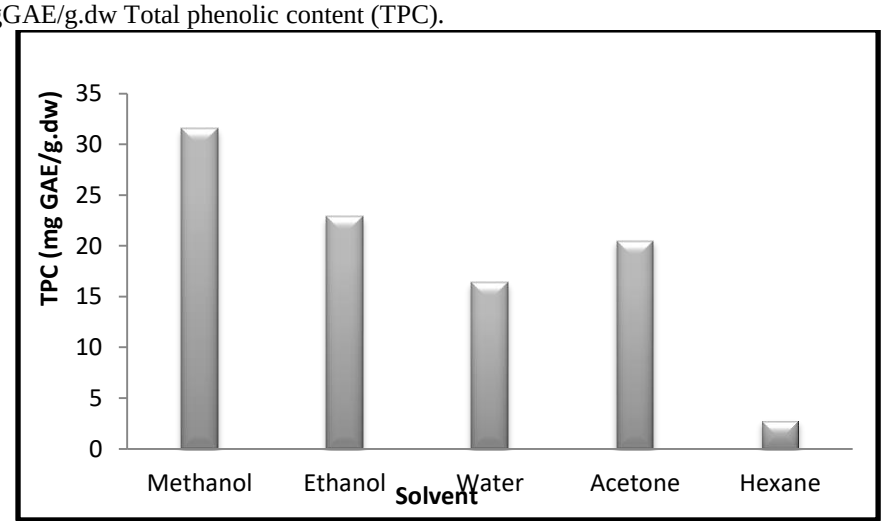


Fig.1. Effect of different solvents on total phenolic content

B.Effect of process variables:

Using one variable at a time approach the optimum conditions for maximum extraction of polyphenols was evaluated. Three different variables were evaluated namely, Liquid to Solid ratio(10 to 50 mL/g),

Microwave power (450 to 810 W)and Time (30 to 120 s). Maximum yield of Phenol was obtained at a Power of 630 W, Time of 60 Sec, and LSR of 10 ml/gm. About 28 mg GAE/g.dw of phenols were obtained while extracted using 100% methanol solvent. This solvent concentration was used for the optimization of other process parameters. When the liquid to solid ratio increased, the extraction of phenols decreased. From 0.5g of sample 3, mgGAE/g.dw of phenols were extracted. On exposing 0.5g of the sample for 120s in Microwave extracted 32 mgGAE/g.dw of phenols. 630W of microwave power extracted 33 mgGAE/g.dw of phenols.

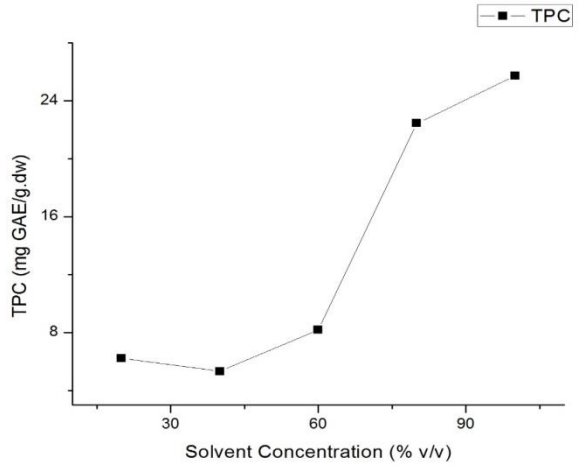


Fig2 (a): Effect of solvent concentration

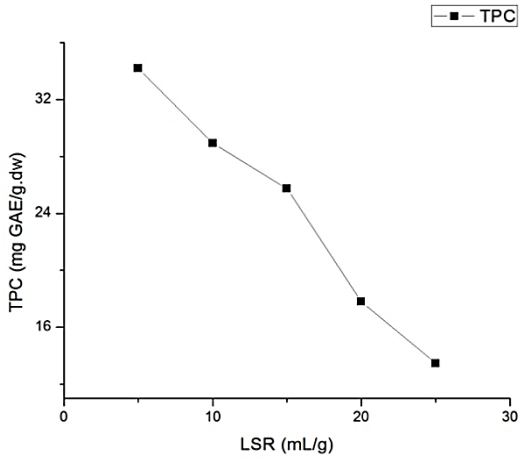


Fig2 (b): Effect of LSR

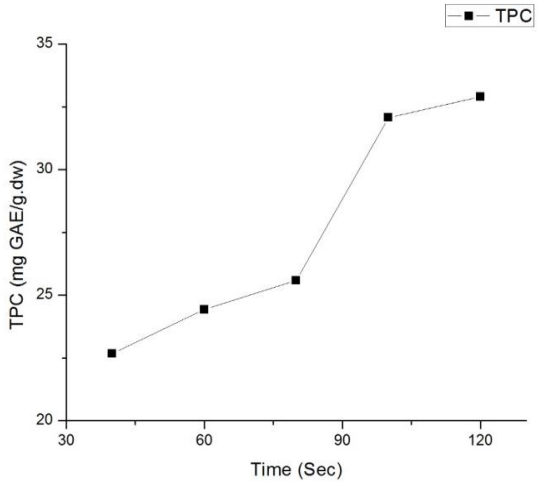


Fig2 (c): Effect of Time

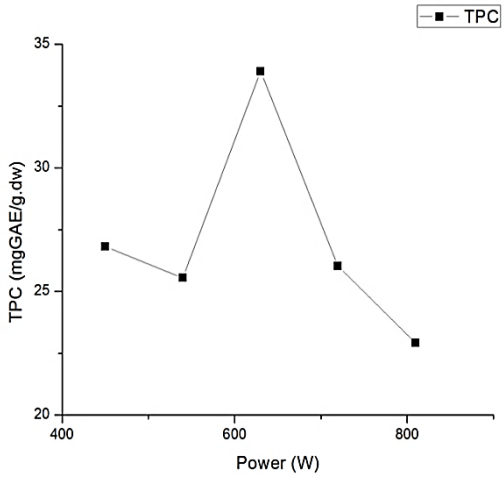


Fig2 (d): Effect of Microwave power

C. Profiling of constituents using GC-MS

The Extract was taken for GC-MS analysis in order to identify the Ligands. Totally, 30 compounds were identified and listed in Table1.

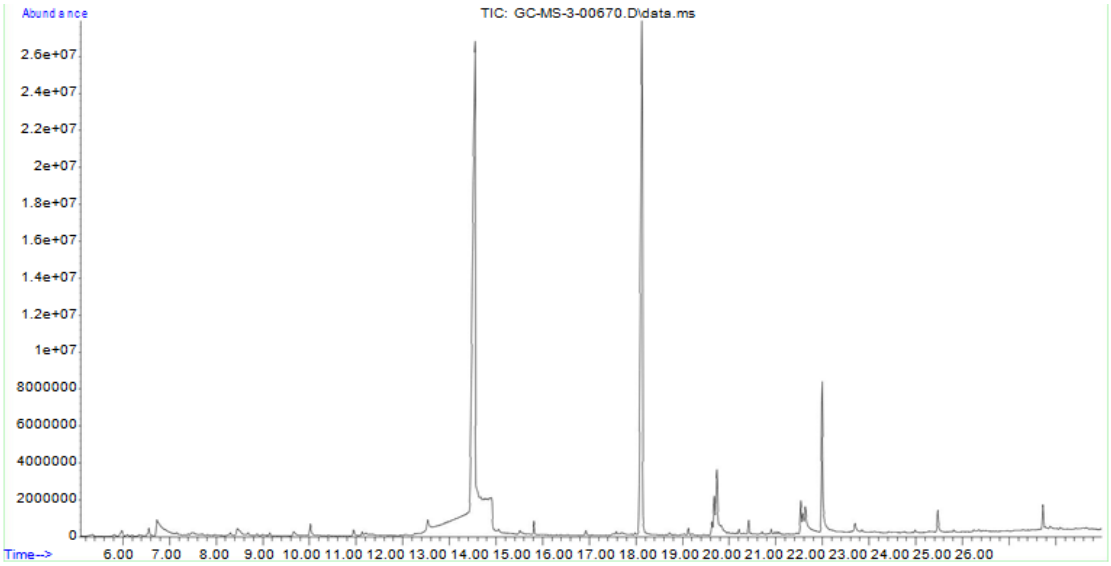


Figure 2 The result obtained from GCMS analysis.

D. ADME Analysis

The drug-likeness and pharmacokinetic properties of our ligand are been screened using two different methods: 1. ADME analysis 2. Rule of Five or Lipinski's rule. Total of 20 ligands was chosen and they were screened for drug-likeness using PreADMET tool (https://preadmet.bmdrc.kr/adme/).

TABLE I: Result of ADME analysis using PreADMET tool

NAME	CaCo2	MDCK	BBB	PLASMA BINDING CAPACITY	Molecular weight	Num. H-bond acceptors	Num. H-bond donors	mollogP
1,2,3,4-Butanetetrol	0.564717	0.654577	0.0966139	85.238551	122.12 g/mol	4	4	-1.91
1,2,3-trimethyl-benzene	23.4334	14.8514*	4.16516	100	120.19 g/mol	0	0	4.17
1,4-Benzenediol	18.7106	73.5844	2.33875	100	124.14 g/mol	2	2	1.15
1-cyclododecyl-Ethanone	47.7798	118.466	6.08617	100	210.36 g/mol	1	0	3.41
1-pentenyl-benzene	23.2515*	215.276	5.72152	89.905645	146.23 g/mol	0	0	4.68
3-Methoxy-5-methylphenol	33.9767	65.2492	1.38511	100	138.16 g/mol	2	1	1.48
6-Methoxyflavanone	50.8432	27.7124	0.0477329	96.546883	254.28 g/mol	3	0	2.1
8-Heptadecene	23.1934*	68.0057*	19.4535	100	238.45 g/mol	0	0	6.54
9,12-Octadecadienoic acid (Z,Z)	28.0819	72.0045*	7.31647	100	280.45 g/mol	2	1	4.47
9,12-Octadecadienoic acid	28.0819	72.0045*	7.31647	100	280.45 g/mol	2	1	4.47
9-Octadecenoic acid(E)	28.1906	70.3359*	7.48966	100	282.46 g/mol	2	1	4.57
Benzene, 1-ethyl-3-methyl-	23.4578	13.9408*	4.06466	100	120.19 g/mol	0	0	4.17
Cyclohexyldimethylisopropoxysilane	26.7994	101.382	11.1907	100	200.39 g/mol	1	0	2.74
Erythritol	0.564717	0.654577	0.0966139	85.238551	122.12 g/mol	4	4	-1.91
n-Hexadecanoic acid	26.0735	70.5083*	8.21885	100	256.42 g/mol	2	1	4.19
Oleic Acid	28.1906	70.3359*	7.48966	100	282.46 g/mol	2	1	4.57
Orcinol	18.7119	65.4637	2.33802	100	124.14 g/mol	2	2	1.15

Palmitoleic acid	25.9744	72.4761	4.59242	100	254.41 g/mol	2	1	4.09
Squalene	23.4049 *	68.0119 *	27.4639*	100	410.72 g/mol	0	0	7.93
tetrahydro-, trans3,4-Furandiol	3.48798	290.543	0.249514	81.43045	108.13 g/mol	3	2	-1.45

On performing the Lipinski's rule, 10 of the molecules got neglected. Since they had at least 1 violation .10 molecules were found eligible as a drug molecule.

Table II Lipinski's rule of five analysis

NAME	Bioavailability Score	Lipinski
1,2,3,4-Butanetetrol	0.55	Yes; 0 violation
1,2,3-trimethyl-benzene	0.55	Yes; 1 violation: MLOGP>4.15
1,4-Benzenediol	0.55	Yes; 0 violation
1-cyclododecyl-Ethanone	0.55	Yes; 0 violation
1-pentenyl-benzene	0.55	Yes; 1 violation: MLOGP>4.15
3-Methoxy-5-methylphenol	0.55	Yes; 0 violation
6-Methoxyflavanone	0.55	Yes; 0 violation
8-Heptadecene	0.55	Yes; 1 violation: MLOGP>4.15
9,12-Octadecadienoic acid (Z,Z)	0.56	Yes; 1 violation: MLOGP>4.15
9,12-Octadecadienoic acid	0.56	Yes; 1 violation: MLOGP>4.15
9-Octadecenoic acid(E)	0.56	Yes; 1 violation: MLOGP>4.15
Benzene, 1-ethyl-3-methyl-	0.55	Yes; 1 violation: MLOGP>4.15
Cyclohexyldimethylisopropoxysilane	0.55	Yes; 0 violation
Erythritol	0.55	Yes; 0 violation
n-Hexadecanoic acid	0.56	Yes; 1 violation: MLOGP>4.15
Oleic Acid	0.56	Yes; 1 violation: MLOGP>4.15
Orcinol	0.55	Yes; 0 violation
Palmitoleic acid	0.56	Yes; 0 violation
Squalene	0.55	Yes; 1 violation: MLOGP>4.15
tetrahydro-, trans3,4-Furandiol	0.55	Yes; 0 violation

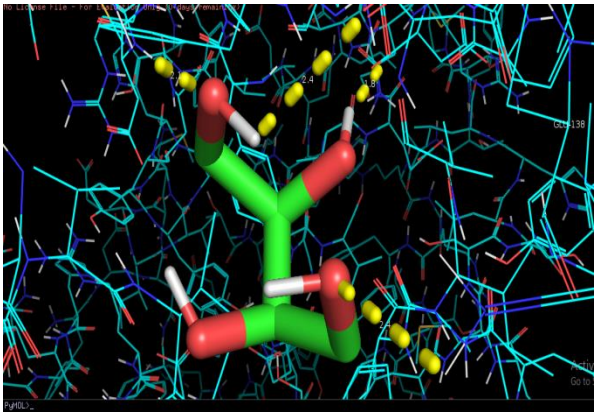
E. Molecular Docking:

The effects of Ligands over the cancer cells were analyzed using Molecular docking. Docking study was carried out using AutoDock 4.2. The Ligands were selected based on the Docking Scores and Hydrogen interaction. The bcl2 molecule was docked with the 10 Ligands that were screened from the Lipinski test namely,

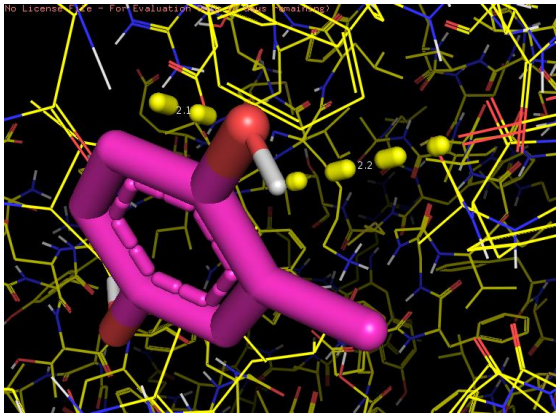
- 1,2,3,4-Butanetetrol
- 1,4-Benzenediol
- 1-cyclododecyl-Ethanone
- 3-Methoxy-5-methylphenol
- 6-Methoxyflavanone
- Erythritol
- Orcinol
- Palmitoleic acid

- tetrahydro-, trans3,4-Furandiol

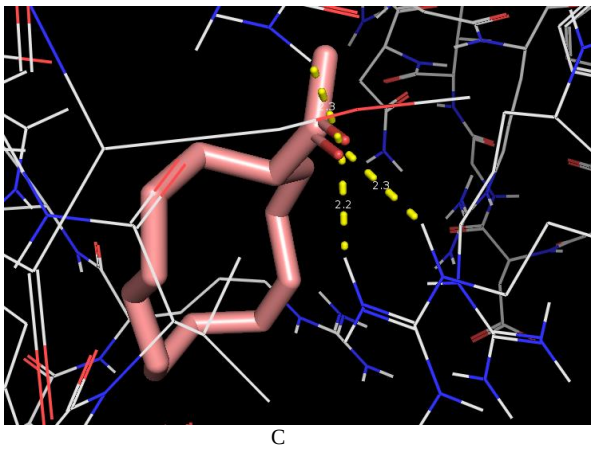
Out of these 10 molecules 6-Methoxyflavanone gave best hit of -7.4Kcal/mol and bound to the residue, LYS 22, with the bond length of 2.6. Erythritol had most number of hits with least docking score -3.7Kcal/mol. Erythritol has 6 interactions, interacting with the residues ARG 65, SER 64, LYS 22, ARG 66 and 2 with ARG 68. 1-cyclododecyl-Ethanone and protein interaction gave binding affinity of -6.2Kcal/mol by binding in 3 positions, namely 2 with ARG 26 of bond lengths 2.2 and 2.3and another interaction with ASN 122 of bond length 2.3. 3-Methoxy-5-methylphenol formed interaction with 3 of the residues of binding site namely one with GLU 138 and two with ARG 86. The bond length with GLU 138 is 2.2, with ARG 86 are 2.6 and 1.0 with bindinf affinity -5.5Kcal/mol.



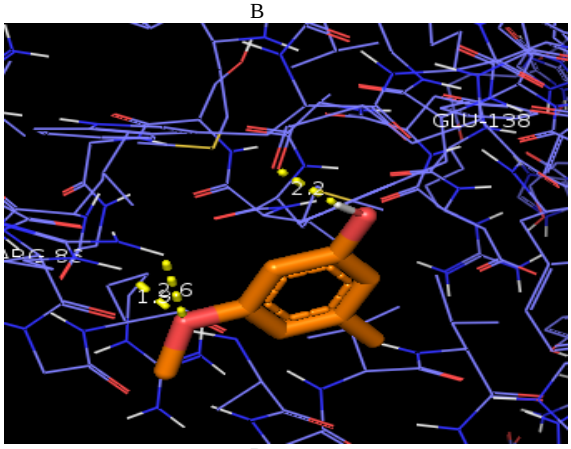
A



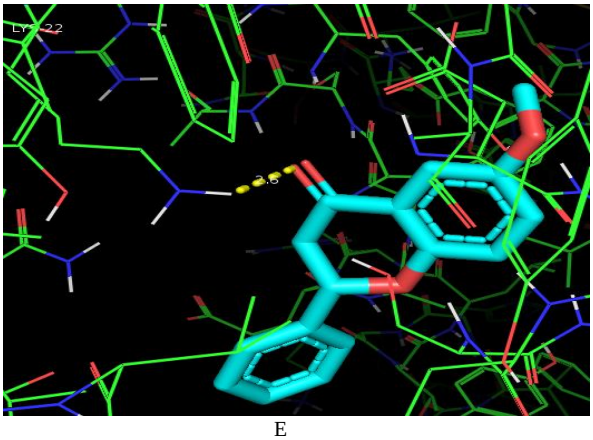
B



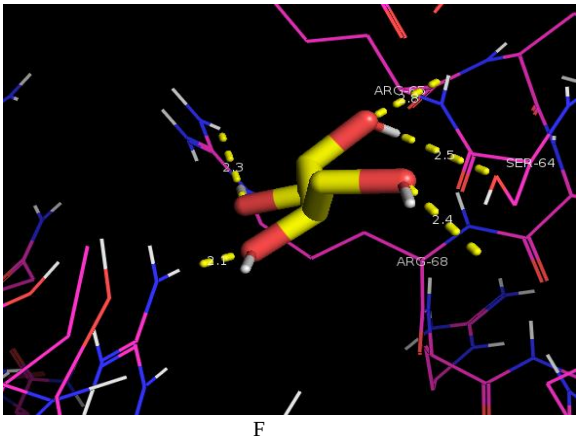
C



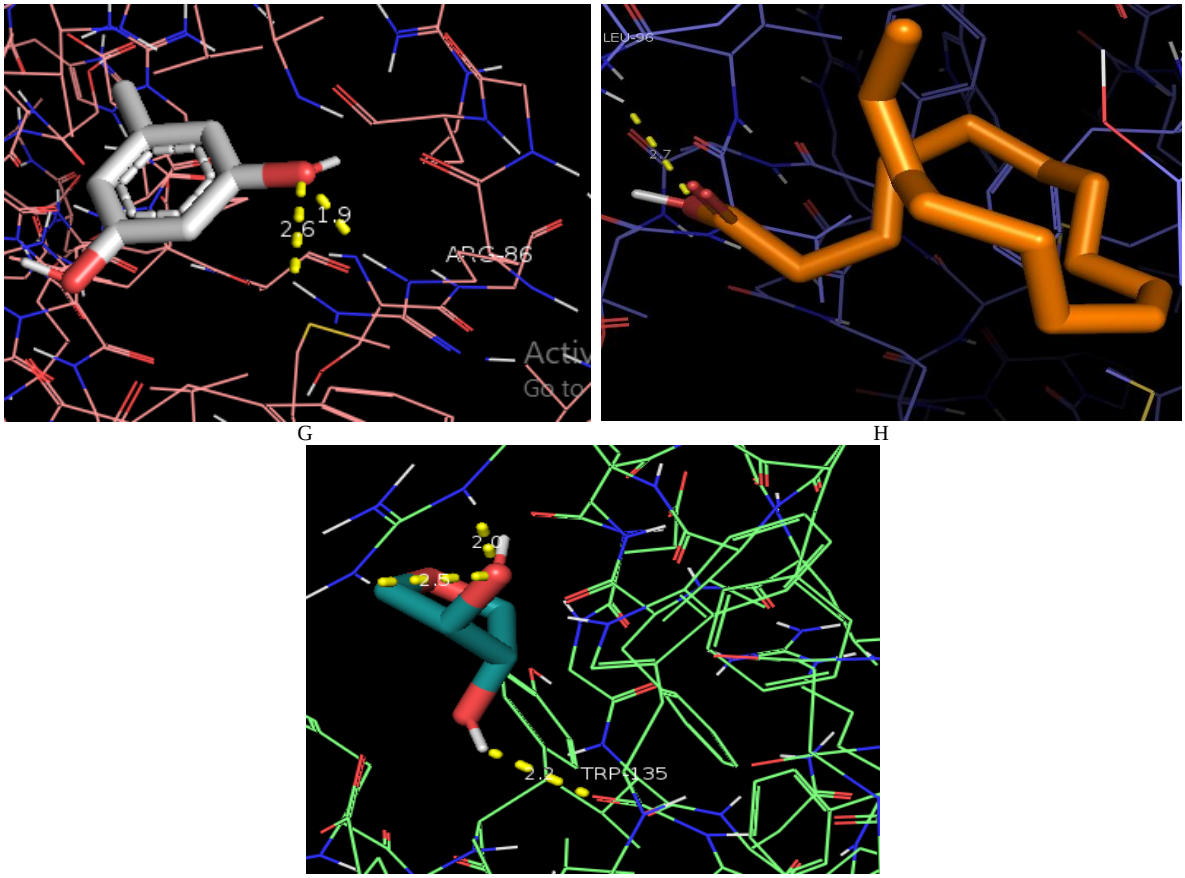
D



E



F



G H I

Figure 3 Respective Ligands docked with Bcl2 exhibiting their Interacting residues and chain length (A) 1,2,3,4-Butanetetrol (B)1,4-Benzenediol (C)1-cyclododecyl-Ethanone (D)3-Methoxy-5-methylphenol (E) 6-Methoxyflavanone (F) Erythritol (G) Orcinol (H)Palmitoleic acid (I)tetrahydro-, trans3,4-Furandiol

Table III Docking scores of Polyphenols with Bcl2

Poly phenols	Binding affinity	No. of interaction	interacting residues	bond length
1,2,3,4-Butanetetrol	-3.6	4	ARG 142, GLU 138, TRP 135, ARG 86	2.4, 1.8, 2.4, 2.1
1,4-Benzenediol	-5	2	GLU 138, ARG 86	2.2,2.1
1-cyclododecyl-Ethanone	-6.2	3	ARG 26, ARG 26, ASN 122	2.2, 2.3, 2.3
3-Methoxy-5-methylphenol	-5.5	3	GLU 138, ARG 86, ARG 86	2.2,2.6,1.0
6-Methoxyflavanone	-7.4	1	LYS 22	2.6
Erythritol	-3.7	6	ARG 68, ARG 68, ARG 65, SER 64, LYS 22, ARG 66	2.2, 2.3, 2.8, 2.5, 2.4, 2.1
Orcinol	-5.4	2	ARG 86, ARG 86	2.6, 1.9
Palmitoleic acid	-5.3	1	LEU 96	2.7
tetrahydro-, trans3,4-Furandiol	-3.7	3	ARG 86, ARG 86, TRP 135	2.0, 2.5, 2.2

IV. Conclusion

Numerous synthetic drugs are available against cancer. These synthetic drugs are said to have various limitations and side effects in usage. In spite of that, isolation of drug molecule from a natural source has driven a keen interest among the research community. This study is focused on identifying a drug molecule from Lichen species *Rocella montagnei* against cancer cells. The molecule which serves as a promising drug over cancer is been identified using In-Silico analysis. Further In-Vivo analysis and animal testing are to be done in order to find the efficiency of the drug molecule.

References

[1] BaÅŸ, D. and I. H. BoyacÄ± (2007). "Modeling and optimization I: Usability of response surface methodology." Journal of food engineering **78**(3): 836-845.

[2] Balaji, P., P. Bharath, et al. (2006). "In vitro antimicrobial activity of Roccella montagnei thallus extracts." Journal of Tropical Medicinal Plants **7**(2): 169.

[3] Cetin, H., O. Tufan-Cetin, et al. (2008). "Insecticidal activity of major lichen compounds,(âˆ’)-and (+)-usnic acid, against the larvae of house mosquito, Culex pipiens L." Parasitology research **102**(6): 1277-1279.

[4] Cho, K., X. U. Wang, et al. (2008). "Therapeutic nanoparticles for drug delivery in cancer." Clinical cancer research **14**(5): 1310-1316.

[5] Dahmoune, F., B. Nayak, et al. "Optimization of microwave-assisted extraction of polyphenols from Myrtus communis L. leaves." Food chemistry **166**: 585-595.

[6] Ferlay, J., I. Soerjomataram, et al. "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012." International journal of cancer **136**(5): E359-E386.

[7] Ferreira, L., R. dos Santos, et al. "Molecular docking and structure-based drug design strategies." Molecules **20**(7): 13384-13421.

[8] Khajeh, M. (2009). "Optimization of microwave-assisted extraction procedure for zinc and copper determination in food samples by Box-Behnken design." Journal of Food Composition and Analysis **22**(4): 343-346.

[9] Mishra, T., S. Shukla, et al. "Isolation and identification of cytotoxic compounds from a fruticose lichen Roccella montagnei, and it's in silico docking study against CDK-10." Revista Brasileira de Farmacognosia **27**(6): 724-728.

[10] Nanayakkara, C., K. Bombuwala, et al. "Effect of some lichen extracts from Sri Lanka on larvae Ofaedes aegypti and the fungus Cladosporium cladosporioides." Journal of the National Science Foundation of Sri Lanka **33**(2).

[11] Nash Iii, T. H. (2008). "Lichen sensitivity to air pollution." Lichen biology **2**.

[12] Podterob, A. P. (2008). "Chemical composition of lichens and their medical applications." Pharmaceutical Chemistry Journal **42**(10): 582-588.

[13] Sankari, S., K. Ramaraj, et al. "In Silico analysis and molecular interactions studies of selected phytoconstituents from Andrographis paniculata as potential inhibitors of monoamine oxidase B." Asian J Pharm Clin Res **9**(2): 239-242.

[14] Siegel, R., D. Naishadham, et al. "Cancer statistics, 2013." CA: a cancer journal for clinicians **63**(1): 11-30.

[15] Soni, G. and K. S. Yadav "Applications of nanoparticles in treatment and diagnosis of leukemia." Materials Science and Engineering: C **47**: 156-164.

[16] Tsilidis, K. K., J. C. Kasimis, et al. "Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies." Bmj **350**: g7607.

[17] Wang, N.-N., J. Dong, et al. "ADME properties evaluation in drug discovery: prediction of Caco-2 cell permeability using a combination of NSGA-II and boosting." Journal of chemical information and modeling **56**(4): 763-773.

[18] Zambare, V. P. and L. P. Christopher "Biopharmaceutical potential of lichens." Pharmaceutical Biology **50**(6): 778-798.

[19] Zhou, Y., J. Zheng, et al. "Natural polyphenols for prevention and treatment of cancer." Nutrients **8**(8): 515.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Molecular Docking Study of Apigenin-7-O-β-D-Glucuronide with Viral Oncoproteins

C.Nirmala[1], Sambhasan Banerjee[2], M.Sridevi[3,] R.Rajeshwari[4]

[1, 3] Department of Biotechnology, Vinayaka Mission's Kirupananda Variyar Engineering College

[2] Department of Computational Biology

[4]Department of Biochemistry, Vinayaka Missions Medical College

[1, 3] Vinayaka Missions Research Foundation (Deemed to be University), Salem-636 308, Tamilnadu, India

[2]Anna University, Chennai, nirmalabt@gmail.com

Abstract*: The cancer has invaded the mankind in an exponential way. Major cases of cancer is reported to be caused by certain viral infection such as Human Papilloma Virus, Epstein Barr Virus, Kaposi's sarcoma associated Herpesvirus. Cervical cancer and the Oral cancer are the two main cancers detected among the Indian population. The virus infects the cells of the human body and leads to the alteration of signal transduction pathways which in return cause the proliferation of the cell. Apigenin-7-O-β-D-glucuronide, a flavone derivative extracted from Cardiospermum halicacabum is found to have many pharmacological properties. This study is focused on the molecular docking of Apigenin-7-O-β-D-glucuronide with the viral oncoproteins such as HPV E6, HPV E7, EBNA1, EBNA2 and KSHV. The docking study signified effective binding of the ligand with the selected viral oncoproteins by forming hydrogen bonds with minimum global energy, that may prevent the binding of tumor suppressor proteins, restricts the transcriptional regulation of the viral episomes and inhibits the degradation of the apoptotic proteins which suggests the compound might be implemented as an anticancer therapeutic agent.*

Keywords*: Cancer, Viral Oncoproteins, Apigenin-7-O-β-D-glucuronide, Molecular Docking*

I. Introduction

Cancer includes the conditions associated with malignant tumors which grow by somatic mutation from the simple body cell or group of cells. There are various types of cancer, like oral cancer, breast cancer, lung cancer, stomach cancer, Leukemia, cervical cancer, etc. Each of these cancers occurs by the activation of specific incorporation or mutated tumor suppressor protein and sometimes also spread due to metastasis. Long term exposure to certain mutagens like smoking, tobacco chewing, asbestos, chemical exposure, nuclear exposure, etc. gives way to any of these cancers. Various viral proteins are found to be involved to derive malignancy in cells.. The fourth most frequent cancer in women is cervical cancer, with an estimated 570,000 new cases in 2018 representing 6.6% of all female cancers [30].

An approximate of 99.7% cervical cancer cases are directly linked to previous infection with the oncogenic types of Human Papilloma Virus (HPV) [11]. In the last 20 years the HPV infected Oral squamous cell carcinoma has seen a growth from less than 20% to more than 70% in United States of America [20]. The active proliferation of the cell is brought about by the two major oncogenes E6, E7 present in the HPV genome [9], [20]. These oncogenes (E6, E7) are associated with the tumor suppressor proteins p53 and pRB respectively. The E6 protein forms a trimeric complex with p53 and cellular ubiquitination enzymes (E6-AP) which promote the proliferation of the cells. The E7 binds at the pocket domains of pRb proteins disrupting the interaction of retinoblastoma protein Rb and E2F factors leading to the reverse of tumor suppression [7]. More than 90 % of the adults become carriers of Epstein–Barr virus (EBV) [2] that leads to malignancies like Burkitt lymphoma, nasopharyngeal carcinoma, Hodgkin Lymphoma, T cell lymphoma, smooth muscle tumors, gastric cancers [16], cervical cancer [17] and hepatobillary system cancer [27]. Other Other virus like Kaposi's sarcoma Herpes virus (KSHV) infects malignant and progenitor cells of kaposi's sarcoma, multicentric Castleman's disease and lymphoma [3], [11].

To resist the invasion of the viral agents in the infliction of cellular malignancy, the activity of viral oncoproteins can be inhibited by phytochemicals. Medicinal plants are good sources of bioactive components that have different pharmacological activities such as antioxidant, antiinflammatory, anticancerous, hepatoprotective activities etc., Apigenin is a flavanoid present abundantly in fruits and vegetables such as grapefruits, parsley, onions, oranges, tea, chamomile, wheat sprouts, plant-derived beverages, some seasonings [8] and medicinal plants. Many *in vitro* studies have shown apigenin has antiproliferative, antiinflammatory, antioxidant,

antispasmodic, antibacterial, and antiphlogistic effects. It is described as non-mutagenic, non-toxic and has low intrinsic toxicity and striking effects on cancer. It inhibits skin papillomas and showed the tendency to decrease conversion of papillomas to carcinomas [28]. Thus it has many beneficial pharmacological effects in different stages of cancer.

Cardiospermum halicacabum (*C. halicacabum*), an herbaceous climber, belongs to the Sapindaceae family, commonly found in Asia and Africa, is rich in pharmacological properties due to the presence of flavonoids, tannins, terpenoids, protein, saponin, glycosides, carbohydrates, volatile esters and variety of fatty acids. The Apigenin-7-O-β-D-glucuronide one of the flavone isolated from *C. halicacabum* [23] has been proved to have antiinflammatory activity [27], Antiproliferative activity, antioxidant activity [14], [15] and hepatoprotective activity [22]. The present study aims in predicting the anticancerous properties of Apigenin-7-O-β-D-glucuronide against selected viral oncoproteins.

II. Methodology

Preparation of Ligand structure

The canonical smile string of the ligand Apigenin-7-O-β-D-glucuronide was retrieved from PubChem Compound Database [31], from the Computed Descriptors to analyze its molecular Structure. In chimera software, Molecular graphics and analyses were performed with the UCSF Chimera package which is a highly interactive program for visualization and analysis of structures and related data, including density maps, supramolecular assemblies of molecules. In Chimera Software [32], the structure was created through Tools>Structure Editing>Build Structure. In the window, the canonical smiles string is pasted to build the 3D structure. The structure is then exported to pdb file format for the docking process.

Selection of Target Oncoproteins

The three-dimensional structure of selected oncoproteins such as HPV E6, HPV E7, EBNA1, EBNA2 and KSHV was downloaded from the Protein Data Bank (PDB), a crystallographic repositary that accesses the three dimensional structural data of various biological molecules especially proteins and nucleic acids [33].

Molecular Docking

Molecular docking studies of the ligand, Apigenin-7-O-β-D-glucuronide with selected oncoproteins, HPV E6, HPV E7, EBNA1, EBNA2 and KSHV was performed using PatchDock to determine the binding efficiency of the ligand in the active site region of the oncoprteins. PatchDock is an algorithm for molecular docking inspired by object recognition and image segmentation techniques used in Computer Vision. The algorithm has three major stages, Molecular Shape Representation, Surface Patch Matching, Filtering and Scoring [4], [6]. The Ligand, Apigenin-7-O-β-D-glucuronide and selected target oncoproteins (HPV E6, HPV E7, EBNA1, EBNA2 and KSHV) are uploaded in '.pdb' format in PatchDock to predict the predominat binding models. Binding interactions were analysed based on Atomic Contact Energy (ACE) and docking scores were obtained for each docked conformations. The obtained docked complexes were then refined through Firedock (Fast Interaction Refinement in Molecular Docking) and rescoring of best rigid body protein-ligand solutions were obtained.

Analysis of the Best docked conformations

The best refined docked protein-ligand complex from Fire dock are then analyzed using PDB sum Generate, a database [34] that provides an overview of the contents of each 3D macromolecular structure deposited in the Protein Data Bank and anlyse the interactions between the ligand and target proteins.

III. Results and Discussion

Preparation of Ligand structure

The molecular structure of Apigenin-7-O-β-D-glucuronide was retrieved from PubChem Compound Database with CID 5319484 (Figure 1). The molecular formula is $C_{21}H_{18}O_{11}$ and the molecular weight of the compound is 446.364 g/mol. The Canonical SMILES of Apigenin-7-O-β-D-glucuronide is : C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O obtained from the Computed Descriptors of PubChem Compound Database.

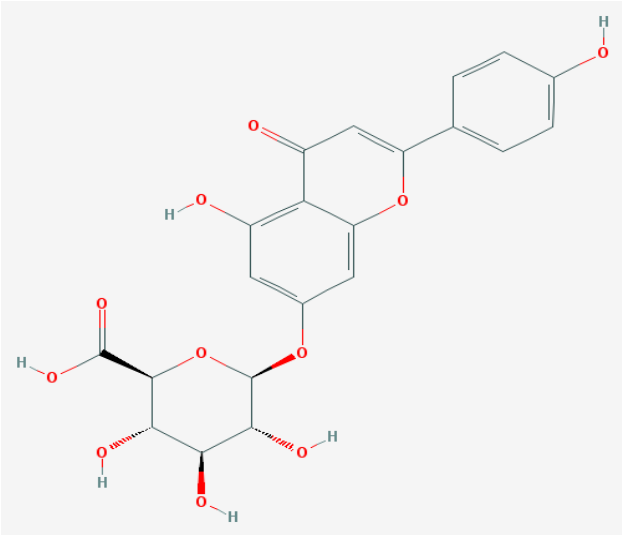


Figure 1: Molecular structure of Apigenin-7-O-β-D-glucuronide

The 3D structure of Apigenin-7-O-β-D-glucuronide was prepared using the Chimera software from the Canonical SMILES of PubChem Compound Database and downloaded in '.pdb format' (Figure 2).

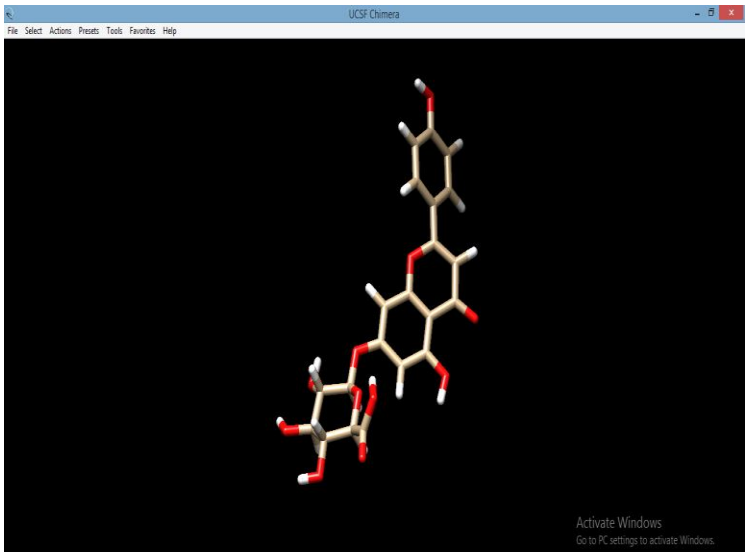
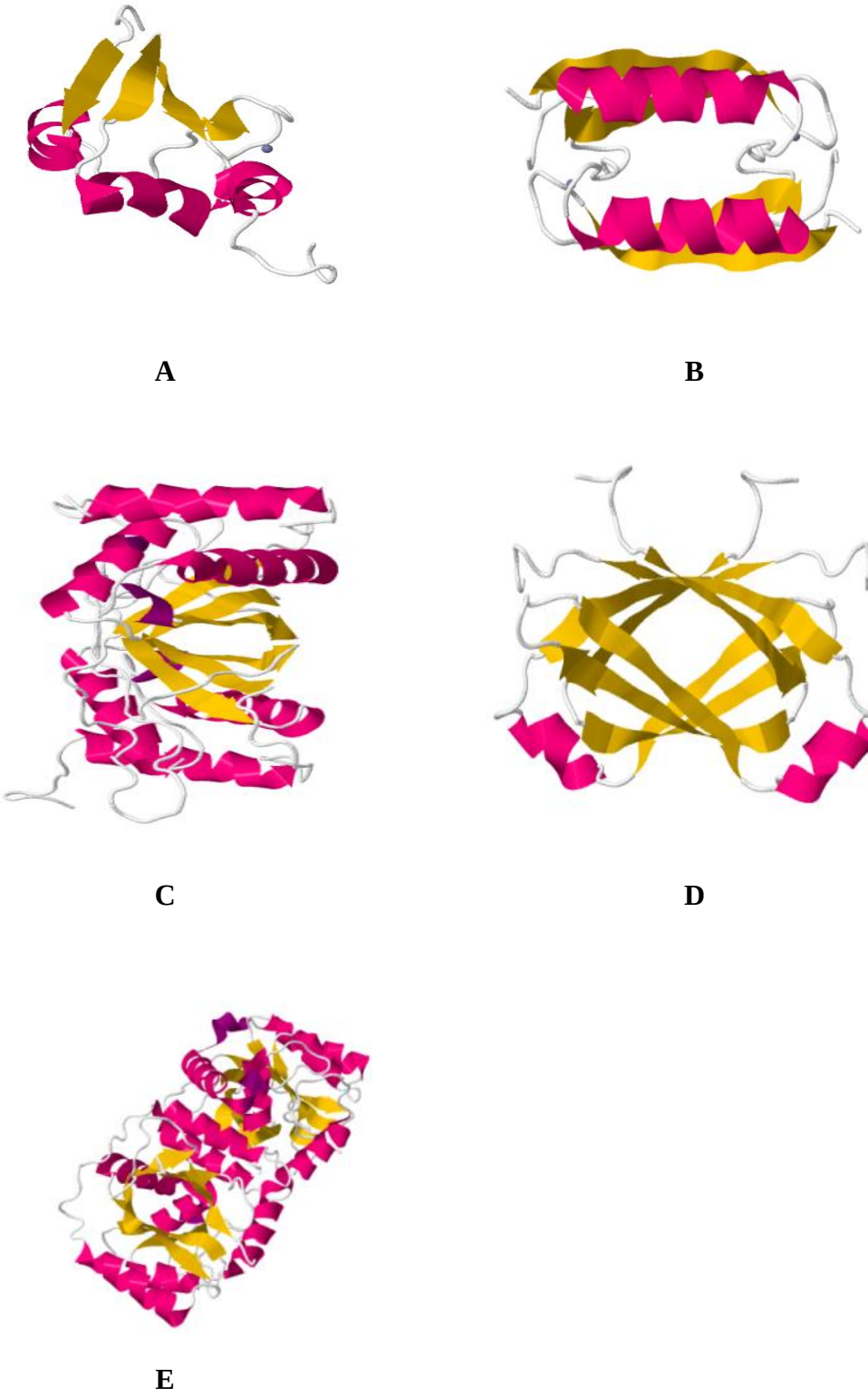


Figure 2: 3D structure of Apigenin-7-O-β-D-glucuronide

Selection of Target Oncoproteins

The 3 Dimensional structure of selected oncoproteins as target molecules were retrieved from RCSB Protein Data Bank (PDB). They are HPV E6 (PDB ID : 2M3L), HPV E7 protein (PDB ID: 2F8B), EBV EBNA1 (PDB ID: 1VHI), EBV EBNA2 (PDB ID: 2N2J), KHSV protease (PDB ID: 1FL1) and downloaded in PDB format (Figure 3).



A **B**

C **D**

E

Figure 3: 3 D structure of oncoproteins A. HPV E6, B. HPV E7, C. EBV EBNA1, D. EBV EBNA2, E. KHSV

Molecular Docking and analysis

Molecular docking studies of the ligand Apigenin-7-O-β-D-glucuronide with selected oncoproteins HPV E6, HPV E7, EBNA1, EBNA2 and KSHV was performed using PatchDock. The docked complex of Apigenin-7-O-

β-D-glucuronide with the selected oncoproteins with lowest global energy from Firedock is uploaded to the PDB sum analysis. The PDB sum generate the docked molecule with analysis of the hydrogen bonds and other interactions formed with the 3D structure of receptor-ligand complex. The results show that 4 hydrogen bonds are formed between glycine 111 glutamic acid 113, 114, cysteine 136 of HPV E6 with and 7th, 9th and 6th oxygen atom of the ligand. The interactions are shown in Figure 4 and number of hydrogen bondings was listed in Table 1.

Table 1: List of Protein – Ligand Interactions for HPV E6

Hydrogen Bonds											
Atom 1						Atom2					
Atom No.	Atom Name	Res Name	Res No.	Chain		Atom No.	Atom Name	Res Name	Res No	Chain	Distance
410	N	GLY	111	A		7	O7	UNK	900		2.85
422	N	GLU	113	A		9	O9	UNK	900		2.59
432	N	GLU	114	A		9	O9	UNK	900		2.59
693	SG	CYS	136	A		6	O6	UNK	900		3.05

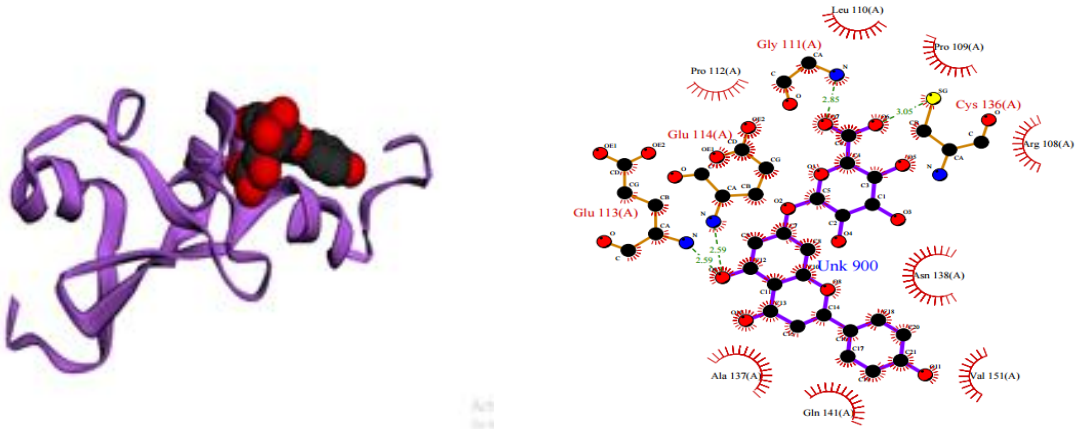


Figure 4: Interaction between Apigenin-7-O-β-D-glucuronide and HPV E6

In case of HPV16 E6 protein, amino acid residues 113–122 (CQKPLCPEEK) were associated with p53 (tumor suppressor protein) binding [20]. Molecular interaction of HPV16 E6 proteins with natural inhibitors around these p53 binding site help in regaining the normal function of p53 [7]. The study of E6 protein being targeted by flavonoids early [10] also suggests that the ligands binding at the alpha helix motif inhibits the activity of the protein molecule [24], [25]. In the docked complex of Apigenin-7-O-β-D-glucuronide with HPV E6, the best docking conformations were obtained with lowest global energy as -30.90. 4 hydrogen bonds were formed at residues 111, 113,114 and 136 that prevents the binding of p53 with E6 and evokes the anticancer effects.

In the docked complex of Apigenin-7-O-β-D-glucuronide with HPV E7, the PDB sum analysis showed hydrogen bond between asparagine 82 of the target protein and 4th oxygen atom of the ligand. The interactions are shown in Figure 5 and listed in Table 2.

Table 2: List of Protein – Ligand Interactions for HPV E7

Hydrogen Bonds											
	Atom 1							Atom 2			
Atom No.	Atom Name	Res Name	Res No.	Chain		Atom No.	Atom Name	Res Name	Res No	Chain	Distance
940	ND2	ASN	82	B		4	O4	UNK	900		2.34

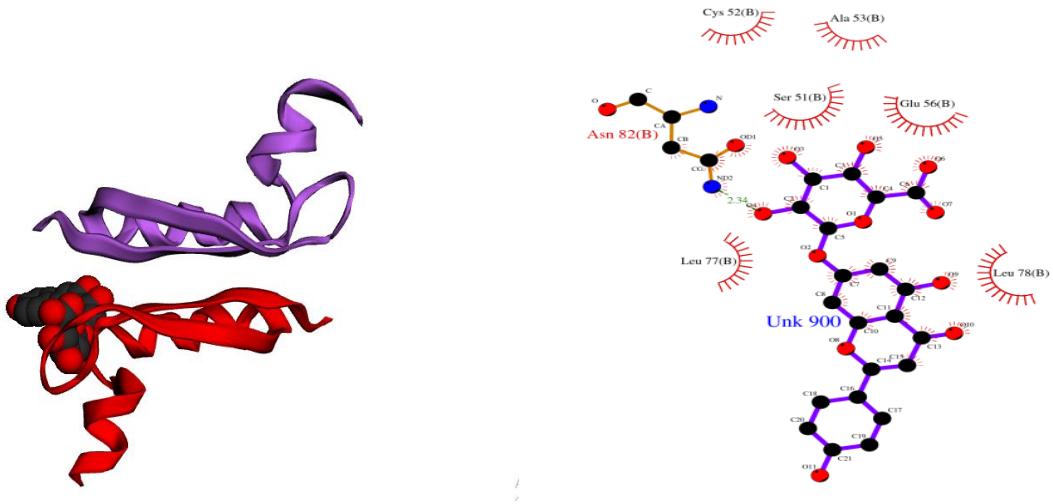


Figure 5: Interaction between Apigenin-7-O-β-D-glucuronide and HPV E7

It is reported that in case of HPV16 E7 protein [26], the CR3 domain (aa 55-106) in the C terminal region contains two CXXC zinc binding domain to form the dimerization and interaction with large number of cellular proteins involved in cell cycle regulation like pRb and p21. Molecular interaction of HPV16 E7 proteins with natural inhibitors around these CR3 binding site might help in regaining the normal function of pRb. In the docking analysis of Apigenin-7-O-β-D-glucuronide with HPV E7, one hydrogen bond was formed at residue asparagine 82 of the oncoprtein with 4th oxygen atom of the ligand that may prevent the binding of pRb with E7 and enhances the anticancer effects. Similar result was shown while identifing antagonists of pRb inactivation by viral oncoproteins [5], [21]. The best docked conformations was obtained with lowest global energy as -22.62.

The binding of Apigenin-7-O-β-D-glucuronide and EBV EBNA1 showed two hydrogen bonds between arginine 291, threonine 585 of EBNA 1 oncoprotein and oxygen atom of 3rd and 7th position in ligand as shown in Table 3 and Figure 6.

Table 3: List of Protein – Ligand Interactions for EBNA1

Hydrogen Bonds											
	Atom 1							Atom 2			
Atom No.	Atom Name	Res Name	Res No.	Chain		Atom No.	Atom Name	Res Name	Res No	Chain	Distance
225	NH2	ARG	491	A	>	7	O7	UNK	900		2.73
110	OG1	THR	585	A	>	3	O3	UNK	900		2.48

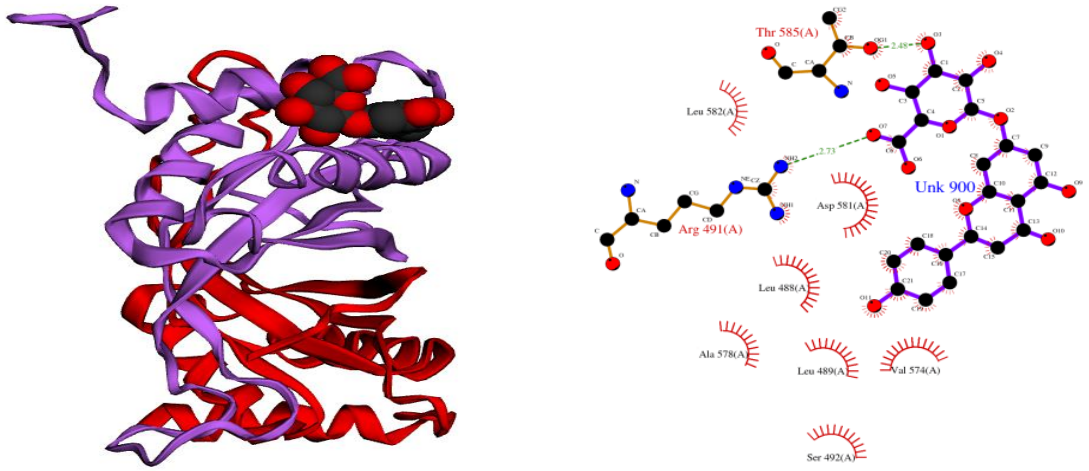


Figure 6: Interaction between Apigenin-7-O-β-D-glucuronide and EBV EBNA1

It is reported that SC 7 binding with the EBNA1 at the Arginine 469 and Tyrosine 518 on one hand while the SC19 binds with EBNA1 at lysine 514, Tyrosine 518, Arginine 522, Leucine 554, Arginine 469 and Tyrosine 518 on the other hand and shows inhibitory effect *in vitro* [19]. The docked molecule of EBNA1 with Apigenin-7-O-

β-D-glucuronide provides the lowest binding energy at -22.82 and binding residues at Arginine 491 and Threonine585 which in proximity to the cited binding region. It can therefore be considered to have inhibitory effect on EBNA-1 molecule which further restricts the replication and maintenance of episomal EBV genome [16].

In the docked complex of Apigenin-7-O-β-D-glucuronide and EBV EBNA2, 3 hydrogen bonds are formed between histidine 13, serine 45, leucine 49 residues of EBNA2 and 8th and 11th oxygen atom of ligand molecule. The interactions are shown in table 4 and Figure 7.

Table 4: List of Protein – Ligand Interactions for EBNA2

List of Protein Ligand Interactions PDB Id h941											
Hydrogen Bonds											
Atom 1						Atom 2					
Atom No.	Atom Name	Res Name	Res No.	Cha in		Atom No.	Atom Name	Res Name	Res No	Cha in	Distance
117	NE2	HIS	13	A	>	11	O11	UNK	900		2.71
422	OG	SER	45	A	>	11	O11	UNK	900		2.81
449	N	LEU	49	A	>	8	O8	UNK	900		3.21

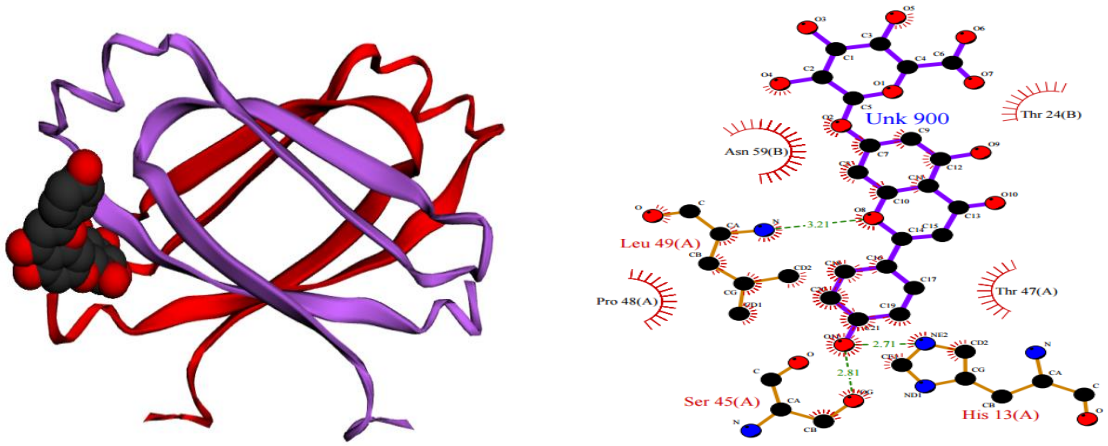


Figure 7: Interaction between Apigenin-7-O-β-D-glucuronide and EBV EBNA2

N –terminal domain of EBNA2 (END) comprises 58 residues which mediate multiple molecular functions including self association, transactivation and functional cooperation with EBNA-LP, which is important for EBV induced immortalisation of B Lymphocytes [1]. The docked molecule of EBNA2 with Apigenin-7-O-β-D-glucuronide provided the lowest binding energy at -46.43 and binding residues at Histidine13, Serine 45 and Leucine 49 that forms hydrogen bonds with oxygen atom in 8th and 11th position of the ligand. The N-terminal fragment of the EBNA2 is a novel END domains exhibit a therapeutic target around the HIS15. In this study Apigenin-7-O-β-D-glucuronide has bound to the END domain of the EBNA2, which infers the anti proliferative activity of the ligand by restricting the transactivation of cellular and viral genes.

In the docked complex of Apigenin-7-O-β-D-glucuronide and KSHV protein, the list of protein- ligand Interactions is shown in Table 5. Hydrogen bond is formed between serine 117 of KSHV oncoprotein and 4th oxygen atom of ligand (Figure 8).

Table 5: List of Protein – Ligand Interactions for KSHV

List of Protein – Ligand Interactions PDB code: h967											
Hydrogen Bonds											
Atom 1						Atom 2					
Atom No.	Atom Name	Res Name	Res No.	Cha in		Atom No.	Atom Name	Res Name	Res No	Cha in	Distance
2836	OG	SER	117	B	>	4	O4	UNK	900		2.3

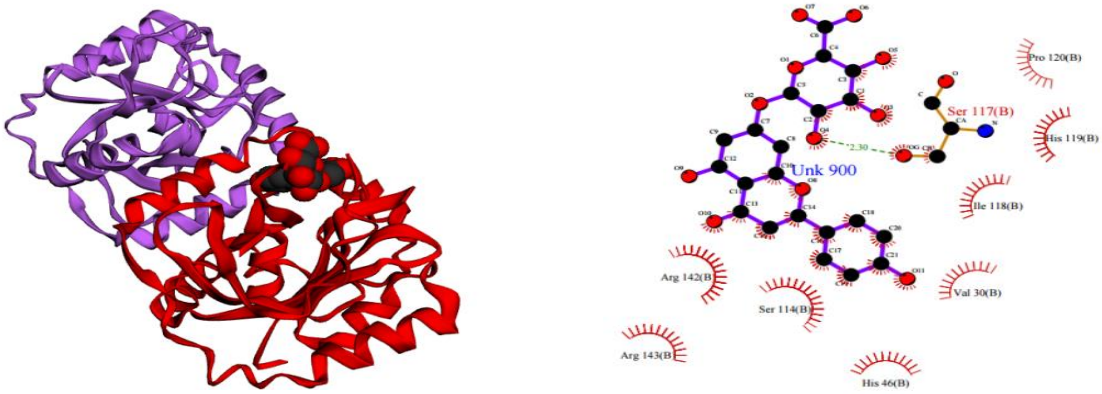


Figure 8: Interaction between Apigenin-7-O-β-D-glucuronide and KSHV protease

KSHV protease has two antiviral drug target sites, the catalytic triad of KSHV Protease (Ser114, His46, His157) and transition-state stabilization site [10], [12], [13]. The docking result with apigenin-7-O-β-D-glucuronide shows the lowest energy at -41.30 with hydrogen bond formed between serine117 of the KSHV protease with the ligand at O4. Thus the ligand Apigenin-7-O-β-D-glucuronide binds in the tumor suppressor protein interaction regions of the selected oncoproteins and prevents their degradation. Hence they can be used as drug candidate molecule against cancer property.

Conclusion

The phytochemical compounds extracted from plants can be used as antiviral agents to prevent various viral infections, for they bind either to the onco proteins of the virus or to the binding molecules of the virus. The study predicts the use of Apigenin-7-O-β-D-glucuronide, a flavonoid from *Cardiopsermum halicacabum* as a ligand against the main types of oncoprotein HPV E6, HPV E7, EBNA1, EBNA2 and KSHV from Human Papilloma Virus, Epstein Barr Virus, Kaposis sarcoma associated Herpesvirus which affects almost 15 % of the total cancer cases in the world population. The ligand binds with the virulence proteins HPV E6, HPV E7 and forms hydrogen bonds with minimum global energy of -30.90, -22.62 respectively that prevents the binding of tumor suppressor proteins such as p53 and p21. The ligand binds with EBNA1, EBNA2 the virulence proteins by forming hydrogen binds with minimum global energy of -46.43, -28.82 respectively and may prevent proliferation by restricting the transcriptional regulation of the viral episomes and also the transformation of the B-cells. KSHV protease bound by the ligand with minimum global energy of -41.30 may restricts the protease activity and hence inhibits the degradation of the apoptotic and tumor suppressor proteins.

References

[1] A. Friberg, S. Thumann, J. Hennig, P. Zou, E. Nössner, P. D. Ling, M. Sattler, and B. Kempkes, "The EBNA-2 N-Terminal Transactivation Domain Folds into a Dimeric Structure Required for Target Gene Activation", PLoS pathogens, vol. 11, no. 5, pp.e1004910, 2015.

[2] A. Kaneda, K. Matsusaka, H. Aburatani, and M. Fukayama, "Epstein–Barr virus infection as an epigenetic driver of tumorigenesis", Cancer research, vol. 72, no. 14, pp. 3445-3450, 2012.

[3] C.Bais, B. Santomasso, O. Coso, L. Arvanitakis, E. G. Raaka, J. S. Gutkind, A. S. Asch, E. Cesarman, M. C. Gerhengorn, and E. A. Mesri, "G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator", Nature, vol. 391, no.6662, pp.86, 1998.

[4] D. Duhovny, R. Nussinov, and H. J. Wolfson, H. J, "Efficient unbound docking of rigid molecules", In International workshop on algorithms in bioinformatics, pp. 185-200, Springer, Berlin, Heidelberg. 2002.

[5] D. Fera, D. C. Schultz, S. Hodawadekar, M. Reichman, P. S. Donover, J. Melvin, S. Troutman, J. L. Kissil, D. M. Huryn, and R. Marmorstein, "Identification and characterization of small molecule antagonists of pRb inactivation by viral oncoproteins", Chemistry & biology, vol. 19, no. 4, pp. 518-528, 2012.

[6] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, and H.J. Wolfson, "PatchDock and SymmDock: servers for rigid and symmetric docking", Nucleic acids research, 33(suppl_2), pp. W363-W367, 2005.

[7] E. K. Yim, and J. S. Park, "The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis", Cancer research and treatment: official journal of Korean Cancer Association, vol. 37, no. 6, pp. 319, 2005.

[8] Hoensch, H., Richling, E., Kruis, W., & Kirch, W. (2010). Colorectal cancer prevention by flavonoids. Medizinische Klinik (Munich, Germany: 1983), 105(8), 554-559.

[9] J. Doorbar, "The papillomavirus life cycle", Journal of clinical virology, vol. 32, pp. 7-15, 2005.

[10] J. J. Cherry, A. Rietz, A. Malinkevich, Y. Liu, M. Xie, M. Bartolowits, V. J. Davisson, J. D. Baleja, and E. J. Androphy, "Structure based identification and characterization of flavonoids that disrupt human papillomavirus-16 E6 function". PloS one, vol. 8, no. 12, pp.e84506, 2013.

[11] J. M. Walboomers, M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K.V. Shah, and N. Muñoz, "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide", The Journal of pathology, vol. 189, no. 1, Dec. 1999, pp. 12-19.

[12] J. Soulier, L. Grollet, E. Oksenhendler, P. Cacoub, D. Cazals-Hatem, P. Babinet, M. F. d'Agay, J. P. Clauvel, M. Raphael, L. Degos, and F. Sigaux, "Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease", Blood, vol. 86, no. 4, pp. 1276-1280, 1995
[13] K. K. Reiling, T. R. Pray, C. S. Craik, and R. M. Stroud, "Functional consequences of the Kaposi's sarcoma-associated herpesvirus protease structure: regulation of activity and dimerization by conserved structural elements", Biochemistry, vol. 39. no. 42, pp. 12796-12803, 2000.
[14] K. Kamalakararao, D. G. Rao, M. Muthulingam, V. K. Gopalakrishnan, Z. Hagos, P. J. Dogulas, and K. K. Chaithanya, "Antiproliferative activity of isolated bioactive flavonoid apigenin-7-O-β-D-glucuronide methyl ester from ethyl acetate leaf extract of *Manilkara zapota*", Drug Invention Today, vol.10, 2018.
[15] K. Kamalakararao, V. K. Gopalakrishnan, Z. Hagos, Y. Satyaprasad, and K. C. Karri, "In vitro antioxidant activities of isolated bioactive flavonoid apigenin-7-O-β-D-glucuronide methyl ester from ethyl acetate leaf extract of *Manilkara zapota*", Drug Invention Today, vol. 10, no. 7, 2018.
[16] L. Jiang, C. Xie, H. L. Lung, K. W. Lo, G. L. Law, N. K. Mak, and K. L. Wong, K.L, "EBNA1-targeted inhibitors: Novel approaches for the treatment of Epstein-Barr virus-associated cancers", Theranostics, vol. 8, no. 19, pp. 5307, 2018.
[17] L. S. Young, and A. B. Rickinson, "Epstein–Barr virus: 40 years on.", Nature Reviews Cancer, vol. 4, no. 10, pp. 757, 2004.
[18] M. A. de Lima, P. J. Neto, L. P. Lima, J. G. Júnior, A. G. Junior, I. P. Teodoro, H. T. Facundo, C. G. da Silva, M. V. Lima, "Association between Epstein-Barr virus (EBV) and cervical carcinoma: A meta-analysis", Gynecologic Oncology, vol. 148, no. 2, pp. 317-328, 2018.
[19] N. Li, S. Thompson, D. C. Schultz, W. Zhu, H. Jiang, C. Luo, and P. M. Lieberman, "Discovery of selective inhibitors against EBNA1 via high throughput in silico virtual screening", PloS one, vol. 5, no. 4, pp. e10126, 2010.
[20] N. Sathish, X. Wang, and Y. Yuan, "Human papillomavirus (HPV)-associated oral cancers and treatment strategies", Journal of dental research, vol. 93, no.7_suppl, pp.29S-36S, 2014.
[21] O. Ohlenschläger, T. Seiboth, H. Zengerling, L. Briese, A. Marchanka, R. Ramachandran, M. Baum, M. Korbas, W. Meyer-Klaucke, M. Dürst, and M. Görlach, "Solution structure of the partially folded high-risk human papilloma virus 45 oncoprotein E7", Oncogene, vol. 25. No. 44, p.5953, 2006.
[22] Q. S. Zheng, X. L. Sun, B. Xu, G. Li, and M. Song, "Mechanisms of apigenin-7-glucoside as a hepatoprotective agent", Biomedical and Environmental Sciences, vol. 18, no. 1, pp. 65-70, 2005.
[23] R. Rajeswari, and M. Sridevi, "Isolation of active compounds from hydro alcoholic extract of *Cardiospermum halicacabum* Linn." International Journal of Pharmaceutical Sciences and Research, vol. 5, no. 11, pp. 4832-4837, 2014.
[24] S. Kumar, L. Jena, M. Sahoo, M. Kakde, S. Daf, and A. K. Varma, "In Silico Docking to Explicate Interface between Plant-Originated Inhibitors and E6 Oncogenic Protein of Highly Threatening Human Papillomavirus 18", Genomics & informatics, vol. 13, no. (2), pp. 60, 2015.
[25] S. Kumar, L. Jena, S. Galande, S. Daf, K. Mohod, and A. K. Varma, "Elucidating molecular interactions of natural inhibitors with HPV-16 E6 oncoprotein through docking analysis", Genomics & informatics, vol. 12, no. 2, pp. 64, 2014.
[26] V. Tomaić, "Functional roles of E6 and E7 oncoproteins in HPV-induced malignancies at diverse anatomical sites", Cancers, vol. 8, no. 10, pp. 95, 2016.
[27] W. Hu, X. Wang, L. Wu, T. Shen, L. Ji, X. Zhao, C. L. Si, Y. Jiang, and G. Wang, "Apigenin-7-O-β-D-glucuronide inhibits LPS-induced inflammation through the inactivation of AP-1 and MAPK signaling pathways in RAW 264.7 macrophages and protects mice against endotoxin shock", Food & function, vol. 7, no. 2, pp.1002-1013, 2016.
[28] H. Wei, L. Tye, E. Bresnick, and D. F. Birt, "Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice", Cancer research, vol. 50, no. 3), pp. 499-502, 1990.
[29] Z. X. Chen, X. T. Peng, L. Tan, G. Q. Zhai, G. Chen, T. Q. Gan, and J. J. Li, "EBV as a potential risk factor for hepatobiliary system cancer: A meta-analysis with 918 cases", Pathology-Research and Practice, vol. 215, no. 2, pp. 278-285, 2018.
[30] https://www.who.int/cancer
[31] https://pubchem.ncbi.nlm.nih.gov/
[32] https://www.cgl.ucsf.edu/chimera/
[33] http://www.rcsb.org/pdb/home/home.do
[34] www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

ADVANCED CONTROLLERS IN BIOPROCESS

Dhivya Priya.K.S, Kabila.N, Hemalatha.G, Vyshali.P, Geeta.A.M, Livya.M, Sivarajasekar. N

Department of Biotechnology, Kumaraguru college of technology

Coimbatore-641049, India, kabila.16bt@kct.ac.in

Abstract*: Optimal environmental condition is required for cell growth and metabolic production in a bioreactor. Maintenance of sterile environment for growth, development and multiplication of organism for desired product is done with the help of controllers. Proportional Integral Controller is commonly used in a bioreactor. This Proportional Integral Controller has limiting function hence advanced controllers were developed. The advanced controllers include Fuzzy logic Controller, Adaptive Controller, Non linear Model Predictive Controller, Decoupled Input-Output Linearized Controller and Feed forward Artificial Neural Network Controller. The Adaptive Controller was developed to overcome the difficulty of finding the specific reaction rate, hence it is the most convenient controller to use with the known parameters. Among the advanced controllers the most efficient controller was found to be Model Predictive Controller in which the product quality is increased while the production cost is reduced. This Model Predictive Controller is Non linearised to increase efficiency.*

Keywords*: Fuzzylogic, Adaptive, NMPC, DIOLC, FANN.*

I. Introduction

A bioreactor is an apparatus for growing organisms to produce products like vaccines, pharmaceuticals and also raw materials into byproducts. These devices provide a biologically optimum environment for the reactions to be carried out. Bioprocess control establishes a program to manage the environment, which is near optimum for microbes to grow and produce desired products. However, there are special challenges due to significant process variability and the complexity of biological systems. Bioprocess is used for producing chemical compound synthesized by microbes; for utilization and extraction of metabolites of biomass. It has provision for continuous monitoring and control of gasses, agitation, pH, temperature, etc. These are the most widely controlled parameters. In large scale production, only critical parameters can be measured to ensure proper control of the reactor, but suitable controllers and sensors have not made the idea implementable.

Advanced controllers are being applied efficiently nowadays. There are various advanced control techniques and they include adaptive, cascade, fuzzy logic, feed forward, internal model, model predictive and neural network control. The hybrid form of more than one type of model has shown to be more efficient in controlling. The many number of models can be categorized into various ways including deterministic, non-deterministic, linguistic and knowledge driven.

In this paper, we have discussed the recent developments and improvements in advanced controller in bioreactors. The attributes and advantages of controller in bioprocesses are described. The delicate problems that arise in bioprocess controllers are mostly due to the rough approximation available in the biological models.

I. Fuzzy logic control

Fuzzy logic controllers are intended for stabilization. Proportional Integral (PI) controllers have simple control system, design and low cost of operation. The only limitation is poor function. It is a multi value logic controller value ranges from 0 to 1.In traditional system, output depends on set points. But, in fuzzy the output doesn't need any predefined set points. In bioprocess, the need of control system is due to non-linearity and variance of time. The non-linearity is dealt with fuzzy logic controllers. The main element of fuzzy controller is inference engine where rules are formulated. The fuzzy control helps to overcome the problem of PI controllers. At transient phase, its shows high performance. This controller converts the experience given by the human into computational algorithms. Some tools like SIMULINK, MATLAB, BIOSIM or fuzzy toolbox are used. It is of two types as direct and indirect type. Mainly, biomass and substrate concentration are determined. This controller provides quick and fast output than conventional controllers. The output of fuzzy not only depends on the input but also the environmental conditions. Recent advances are made to combine PI controller with fuzzy controllers

II. Adaptive controller

In bioprocess, product concentration and biomass concentration are obtained at equilibrium and it is based on substrate concentration. The adaptive control law is performed with known parameters to reach the targeted set point. The adaptive controller is designed based on the following:

1) The specific reaction rates are not known
2) The state variables are not accessed.
3) The value of $Q_p = C_p P$ were estimated but not used to design the controller.
4) The on-line measurement of initial substrate concentration is available and other measurements are calculated.
5) CO_2 is not practically used.
6) Other kinetics and coefficients are given.

Example:

The adaptive controller is used in waste water treatment in CSTR.

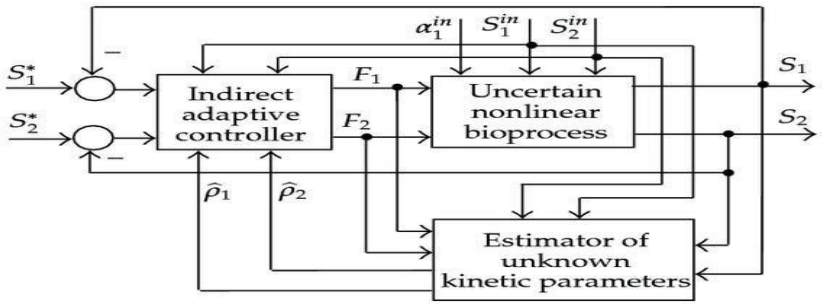


Fig. 1. Structure of adaptive controller in bioprocess. [11]

III. Non linear model predictive controller (NMPC):

This controller was designed to increase the product quality, decrease the production cost and provide batch to batch product reproducibility. This controller is used in Chinese Hamster Ovary (CHO) mammalian cell fed batch process. The non linear bioprocess model was developed in CHO mammalian cells fed batch bioprocess reactor. The tuning of non linear model predictive controller was done through off-line simulation. This was then applied to control glucose concentration in a 15 L Pilot-Plant bioreactor. The control of glucose concentration is not so easy due to long measurement intervals, measurement noise and high process variability. In the presence of bioprocess attributes NMPC was successful in controlling and fixing closed loop set point.

IV. Decoupled input-output linearized controller (DIOLC):

This is a feedback controller. This is used to control ethanol and biomass production in *Saccharomyces cerevisiea.* Based on the linear schedule the performance of decoupled input-output linearized controller was compared with Proportional Integrative-Derivative (PID). The performance of DIOLC was better when compared to PIC in various experiments including accurate response to simultaneous changes in dissolved oxygen concentration and trajectories of the substrate. Further the DIOLC was found more significant in exhibiting good performance in perturbation experiments of the most important parameters, this was determined through statistical design of experiments which involved 730 simulations. Adding to this the DIOLC was able to constrain the implementation process in metabolic regimes in demand of low/high oxygen during maximization of ethanol concentration.

V. Feed forward artificial neural network:

Though so many Artificial Neural Network (ANN) architectures were proposed, the 'feed forward' artificial neural network (FANN) is applied most widely. This is because FANN that has double layers of non-linearity is adequate to imprecise or approximates any continuous non-linear function. Using neural network is more appealing than any other form of model and this is due to its ability to represent complex nonlinear systems effectively. Certainly this attribute is used in the growth of advanced controllers. Standard Proportional Integral Derivative (PID) control improves a model's performance accurately. This case of controller is applied to significant dead-time or severe non-linearity systems. Modern control theory benefits are significant and are industrially accepted over non standard controller structure.

An industrially appropriate alternative approach for designing standard controllers like PID is utilizing the model. Non-linearity, dead-time and constraints can be lodged by variation in controller parameter. Along with the advantages of a common controller's structural conformity, additional industrial advantage is that evaluations can be done of the suggested controller settings' validity.

VI. Conclusion

The ultimate objective of this review article is to compare the advancements in the controller of bioreactors. The advancements are made mainly on the basis of reduction of cyclic time. The controllers are aimed to maintain safety and quality. Thus advancements are required to increase efficiency of the controllers. The most efficient controller as of now was found to be Non linearized Model Predictive Controller for its increased productivity and cost efficiency..

References

[1] Craven, S., Whelan, J., & Glennon, B. (2014). Glucose concentration control of a fed-batch mammalian cell bioprocess using a nonlinear model predictive controller. *Journal of Process Control*, *24*(4), 344-357.

[2] Persad, A., Chopda, V. R., Rathore, A. S., & Gomes, J. (2013). Comparative performance of decoupled input–output linearizing controller and linear interpolation PID controller: enhancing biomass and ethanol production in Saccharomyces cerevisiae. *Applied biochemistry and biotechnology*, *169*(4), 1219-1240.

[3] Kuprijanov, A., Gnoth, S., Simutis, R., & Lübbert, A. (2009). Advanced control of dissolved oxygen concentration in fed batch cultures during recombinant protein production. *Applied microbiology and biotechnology*, *82*(2), 221-229.

[4] Gnoth, S., Simutis, R., & Lübbert, A. (2011). Fermentation process supervision and strategies for fail-safe operation: A practical approach. *Engineering in Life Sciences*, *11*(1), 94-106.

[5] Mulchandani, A., & Bassi, A. S. (1995). Principles and applications of biosensors for bioprocess monitoring and control. *Critical reviews in biotechnology*, *15*(2), 105-124.

[6] Doran, P. M. (1995). *Bioprocess engineering principles*. Elsevier.

[7] Scheper, T., Hitzmann, B., Stärk, E., Ulber, R., Faurie, R., Sosnitza, P., & Reardon, K. F. (1999). Bioanalytics: detailed insight into bioprocesses. *Analytica Chimica Acta*, *400*(1-3), 121-134.

[8] Pérez-Correa, R., & Agosin, E. (2009). Instrumentation and control of bioprocesses. *BIOTECHNOLOGY-Volume II: Fundamentals in Biotechnology*, *2*, 23.

[9] King, J. P. (2014). Instrumentation and Control Systems. In *Fermentation and Biochemical Engineering Handbook (Third Edition)* (pp. 401-414).

[10] Turner, A. P. F., & White, S. F. (2002). Process monitoring. *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*.

[11] Petre, E., Selişteanu, D., & Şendrescu, D. (2013). Adaptive and robust-adaptive control strategies for anaerobic wastewater treatment bioprocesses. *Chemical engineering journal*, *217*, 363-378.

[12] Reed, R., & MarksII, R. J. (1999). *Neural smithing: supervised learning in feedforward artificial neural networks*. Mit Press.

[13] Karaboga, D., Akay, B., & Ozturk, C. (2007, August). Artificial bee colony (ABC) optimization algorithm for training feed-forward neural networks. In *International conference on modeling decisions for artificial intelligence* (pp. 318-329). Springer, Berlin, Heidelberg.

[14] Lippmann, R. (1987). An introduction to computing with neural nets. *IEEE Assp magazine*, *4*(2), 4-22.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Nanobiotechnology and clinical diagnosis of Diabetes mellitus

Swathi. G, Kanish Siddarth. R, Manopriya. M, Sivarajasekar. N

[2]Department of biotechnology, Kumaraguru college of technology, Coimbatore, Tamil Nadu 641049, India

swathi.peegee@gmail.com

Abstract*: Diabetes, a metabolic disorder which was rare earlier and affected only elderly people. Now, due to lifestyle changes and varied diet, it has become more common even in younger people too. There are different types of diagnosis and treatment for diabetes. Diagnosis and treatment of diabetes in a non-invasive manner has been approached using nanotechnology. It includes both detection and treatment. Several methods have been approached for diagnosis and treatment, but each method has its own advantages and disadvantages. Nano sensors for detecting blood glucose levels, Nanomedicine for effective intake of drug and Nanocarriers for delivering drug on the target site are some approaches by nanotechnology towards diabetes. 'Artificial Pancreas' is also based on nanotechnology which can cure diabetes on permanent basis. The purpose of this review is to understand the importance of nanotechnology in treatment of diabetes and discuss about potent applications.*

Keywords*: Diabetes: Nanotechnology; Nano sensors; Nanomedicine; Nanocarriers.*

I. Introduction

Diabetes, a metabolic disorder which lasts lifelong has invaded billions of people's lives. It has raised from a rare disorder to the most common disorder affection younger people too. It occurs usually due to a combination of heredity and environmental causes, which results in high blood sugar levels (hyperglycaemia)[1]. It is mainly characterised by elevated blood glucose level which can be detected by various clinical tests. Type 1 is a state where there is deficiency in the production of insulin and Type 2 is where the cells become insulin resistant. Type 2 DM is more common than Type 1 DM. If untreated it may lead to nerve problems, blindness, heart and kidney disease etc. The number of affected individuals is estimated to be around 400 million adults by 2030 [2].

Insulin, a hormone with 51 amino acids, helps to lower blood sugar level by increasing the intake of glucose in liver and muscles. It is produced by the β-cells of pancreas. Insulin increases the glucose transport to target cells. The main goal of type 1 and type 2 diabetes is to achieve blood glucose levels within normal glycaemic range (70–140 mg per dl or 4–8 mM). The first and main treatment for diabetes is to supply insulin by artificial means (Insulin therapy).It is done mainly through injection and tablet. Sometimes insulin overtreatment may lead to hypoglycaemia, a condition of very low blood sugar level which causes seizures, unconsciousness or death [2]. Other treatments excluding insulin supply is maintaining a controlled diet, regular exercise, lifestyle changes etc. Alternative treatment includes vitamins, minerals, herbal remedies etc. These existing treatments are only useful for controlling existing deficiency and preventing further complications. They don't help in complete cure of the disease. Now, various treatment and methods have been developed to cure as well as prevent the occurrence of the disease.

Nanotechnology has improved in various medical fields over the past years. The physical, chemical and biological properties of nanoparticles have attracted great interest in biomedical applications. Nanoparticles are used in drug delivery, disease detection, killing tumour cells etc [3]. In diabetes, the arrival of improved glucose sensor technology using nanotechnology has had an immediate impact on the health of patients, as improved sensing of blood glucose level helps in accurate insulin dosing and management. Nanotechnology based sensors are capable of convenient blood glucose measurements. It has enabled more accurate insulin delivery systems to maintain normal levels. Nano based sensors can measure even minute amounts of insulin and blood glucose which helps to detect even a small change in the levels. Insulin couldn't be provided orally because the intestinal epithelium lies a major barrier. Hydrophilic drugs don't diffuse through the lipid-bilayer cell membranes to the blood stream. Due to this problem, insulin was provided through injection. A Nano based carrier system can help in the diffusion of insulin from the intestine to the blood stream. This can help in administration of insulin orally which leads to painless treatment of diabetes [1]. Nanotechnology has overall lead to effective control and treatment of diabetes.

II. Prevailing technologies for diagnosis of diabetes mellitus

The clinical diagnosis criteria for diabetes mellitus varies according to the type of diabetes which is based on one of the four abnormalities like glycated haemoglobin, fasting plasma glucose, random high glucose with symptoms or abnormal oral glucose tolerance. Diabetes patients are found to have more glycated proteins than non diabetic subjects. The measurement of the glycated haemoglobin (HbA1c) as a diagosnitic tool was proposed by ADA and WHO**[4]**. The main advantage of this method is that it does not require any kind of special preparation and it can be done at anytime. But the assay requires whole blood which can be affected by anaemic patients, hemoglobinop athies and hepatic cirrhosis. HbA1c as a molecular marker under such conditions may be imprecise and inappropriate. [5], [6]

Compared with HbA1c, a newly available assay using the amount of glucose in serum or plasma attached to albumin is accurate for assessing glycemic control in subjects with anaemia ,hemoglobinopathesis and hemodialysis. GA requires only a serum sample which can be freeze stored for long term. So it is considered as an advantage for detecting undiagnosed diabetes. [7]

However measuring blood glucose at frequent levels causes unnecessary discomfort and mental trauma to patients, and hence a much simpler and non-invasive technique for the diagnosis and monitoring of diabetes is enviable. To overcome saliva was used as a diagnostic tool for diabetes as it is obtained easily and a non invasive method[8]- [10]. As like serum saliva has some antibodies, enzymes, growth factors, hormones and microbes and their products and it has several biomarkers which make it useful for assay. Proteins present in blood are similarly present in saliva from fluid leakage at the gum line. It is a safer and easier method and saliva has played a significant role in early detection and prognosis. The use of salivary diagnostics in a clinical setting is becoming a reality based on the enhanced efficiency of genomic and proteomic technologies. [11]

Population based study is being done in many parts of the country for diagnosing and risk factors of diabetes based on heredity of diabetes, clinical symptoms, levels of C peptide, genetic analyses and detection of auto antibodies. Obesity is also said to increase the risk of type 2 diabetes which is associated with impaired insulin uptake or insulin resistance. Biomarkers measurement that reflect underlying biological mechanism for incresased risk is also an alternative for diabetes diagnosis. [12]

III. Nanobiotechnology in clinical diagnosis

The various types of nanoparticles that are currently studied for their use as drug delivery systems are as follows:

- Polymeric biodegradable nanoparticles that include nanospheres and Nano capsules.
- Ceramic nanoparticles
- Inhalable polymeric nanoparticles
- BioMEMS
- Polymeric micelles
- Dendrimer
- Liposomes
- Niosomes based drug delivery system
- Micelle based drug delivery system for anti- diabetic drug delivery system
- Poly(amidoamine) dendrimer-based drug delivery system

POLYMERIC BIODEGRADABE NANOPARTICLES:

Polymeric nanoparticles are solid, colloidal that vary in size from 10 nm to 1000 nm. These particles consisting of macromolecular substances, are categorised into two types: nanospheres and Nano capsules.

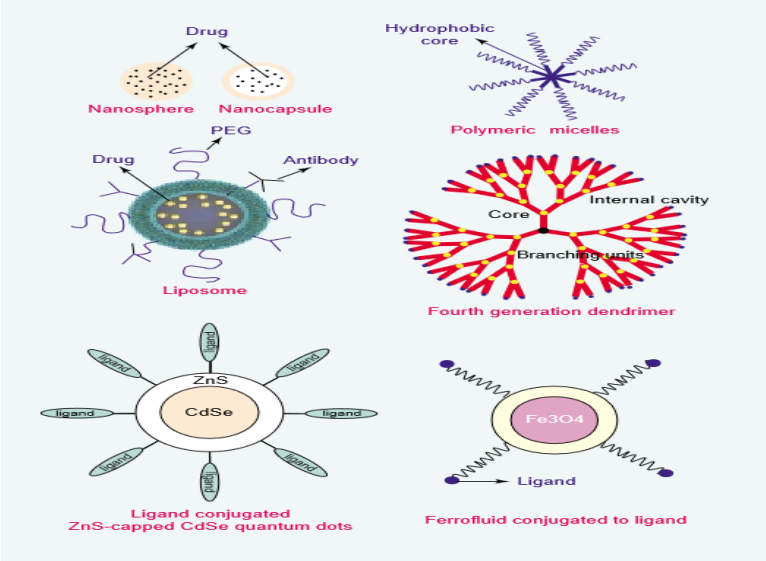
Nanospheres are matrix system that disperses the drug physically and uniformly whereas Nano capsules are vesicular system that is bound in a polymer membrane. These nanostructures are biodegradable and deliver the medication in the targeted tissue perfectly.

Cytotoxicity test showed that the drug from nanostructures at certain drug release and do not cause cell damage. Also, they are engineered to deliver higher concentration at specific spots.[3]

Nanoparticles are small polymeric colloidal particles with a therapeutic agent either dispersed in polymer matrix or encapsulated in polymer. Polymeric micelles are self-assembled block co-polymers, which in aqueous solution arrange to form an outer hydrophilic layer and an inner hydrophobic core. The miceller core can be loaded with a water insoluble therapeutic agent. Liposomes are lipid structures that can be made 'stealth' by PEGylation and further conjugated to antibodies for targeting. Dendrimers are monodispersed symmetric macromolecules built around a small molecule with an internal cavity surrounded by a large number of reactive end groups. Quantum dots are fluorescent

nanocrystals that can be conjugated to a ligand and thus can be used for imaging purposes. Ferrofluids are colloidal solutions of iron oxide magnetic nanoparticles surrounded by a polymeric layer, which can be further coated with affinity molecules such as antibodies **[15]**

Fig. 1. Schematics of different nanotechnology-based drug delivery systems. [15]



CERAMIC NANOPARTICLES:

Ceramic nanoparticles are much preferred for their ultra-low size that is about 50nm and dimensional stability. They also have high biocompatibility. These are used in photodynamic therapy in cancer treatment as they show excellent results in entrapping Water-insoluble photosensitizing anticancer drugs. Also, it does not cause swelling or porosity changes with the change in surrounding environment.[3]

INHALABLE POLYMERIC NANOPARTICLES:

Initially, it was tried in tuberculosis,but this can be applied in diabetes effectively. Insulin molecules are encapsulated within the nanoparticles and this can be administered into the lungs via inhalation of the dry powder formulation of insulin. These nanoparticles showed good insulin loading capacity. It also provides the release of 75–80% insulin upon administration. [3]

BioMEMS:

BioMEMS are Implantable Bio Micro Electro Mechanical Systems (BioMEMS). It can be used as insulin pumps for controlled release of insulin. If there is an increase in blood glucose level, BioMEMS may come into application. BioMEMS device has a drug reservoir which is a compartment filled with insulin molecules. Biosensors and nonporous membranes with pores of 6 nm in diameter are used. These are in the exterior to detect the changes in blood glucose level and for insulin release. A review about the fabrication of a glucose-sensitive microvalve MEMS device has been performed. It discusses extensively about the research attempts on insulin delivery done in the past few decades.[3]

GOLD NANOPARTICLES:

Gold nanoparticles have long term stability in terms of aggregation and good insulin loading. They also have higher uptake of insulin across oral and nasal mucosa; improved pharmacodynamic activity of insulin. Their limitations are widespread distribution in organs like liver, lung, spleen, kidney, brain and heart.[3]

LIPOSOMES:

The advantages of liposomes are Biodegradable, non-toxic and non-immunogenic. Their limitations are Drug loading capacity remains inconclusive; it is captured by the human body's defence system (reticuloendothelial system (RES)); post-treatment accumulation in skin and eye.[3]

NIOSOME BASED DRUG DELIVERY SYSTEM:

Niosomes are synthetic microscopic vesicles formed by non-surfactants incorporated with cholesterol as excipient. They are of nanomolecular size. Their property of acting as drug reservoirs for maximum entrapment in sustained and prolonged drug release makes it suitable for drug delivery systems. They are also being tested for vaginal delivery system of insulin.[3]

MICELLE BASED DRUG DELIVERY SYSTEM FOR ANTI- DIABETIC DRUG DELIVERY SYSTEM:

Polymeric micelles are formed my amphiphilic co polymers. It formed by self-assembly upon reaching critical micellar concentration. These polymeric micelles aggregates into core shell micellar structure. They have enhanced pharmacokinetics and biodistribution. The major drawback is aggregation of insulin causes formulation instability which makes it difficult for drug delivery. But, to stabilise insulin, DDT-denatured insulin is used which has high stability. Also, conjugation with multifunctional PEG is performed with biodegradable polymers. Using PEG makes it stable against aggregation.
Also, micelles with stimuli have cargo-release behaviour approach. Another effective method is using phenyl boronic acid (PBA). It has potential application in drug delivery. PBA based complex micelles have high stability and biodegradability[13]

POLY(AMIDOAMINE) DENDRIMER BASED DRUG DELIVERY SYSTEM:

Poly(amidoamine) dendrimer is Nano sized, polymeric, highly branched macromolecule. It has a 3D nanostructure with tree-like morphology. It consists of central core and branched monomers with different end groups. With their chemical and physical properties, it aids them in being effective in drug delivery system. It is widely used in pharmaceutical and biological application. PAMAM G4 mimics hypoglycaemic action through reduction of higher plasma glucose level. PAMAM increases absorption ability of entrapped components. It is concentration dependent and it causes no increase in protein production or LDH activities that shows indication of no lung tissue by dendrimers. Dendrimers have become important component in gene delivery.[13]

IV. Conclusion

This paper briefly discusses the various prevailing technologies in the clinical diagnosis of diabetes mellitus, their disadvantages and advantages and how nanotechnology can gradually replace them. The ability of nanotechnology to enhance numerous regenerative therapies specifically would benefit diabetic patients with poor vascularization. However, the application of nanotechnology in the field of clinical diagnosis has certain limitations like larger surface area than their volume, friction and clumping of the nanobioparticles into a larger structure may affect their function in drug delivery. Nanobiotechnology is a revolutionary approach that can be adopted in the management of diagnosis if their limitations and technological hurdles are met.

References

[1] Arya, A. K., Kumar, L., Pokharia, D., & Tripathi, K. (2008). Applications of nanotechnology in diabetes. *Digest journal of Nanomaterials and Biostructures*, *3*(4), 221-25.

[2] Veiseh, O., Tang, B. C., Whitehead, K. A., Anderson, D. G., & Langer, R. (2015). Managing diabetes with nanomedicine: challenges and opportunities. *Nature Reviews Drug Discovery*, *14*(1), 45.

[3] Subramani, K., Pathak, S., & Hosseinkhani, H. (2012). RECENT TRENDS IN DIABETES TREATMENT USING NANOTECHNOLOGY. *Digest Journal of Nanomaterials & Biostructures (DJNB)*, *7*(1).

[4] World Health Organization. (2011). Report of a World Health Organization Consultation: Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. *Diabetes Res Clin Pract*, *93*, 299-309.

[5] Kim, C., Bullard, K. M., Herman, W. H., & Beckles, G. L. (2010). Association between iron deficiency and HbA1c levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999–2006. *Diabetes care*.

[6] Ford, E. S., Cowie, C. C., Li, C., Handelsman, Y., & Bloomgarden, Z. T. (2011). Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US. *Journal of diabetes*, *3*(1), 67-73.

[7] Ikezaki, H., Furusyo, N., Ihara, T., Hayashi, T., Ura, K., Hiramine, S., ... & Ai, M. (2015). Glycated albumin as a diagnostic tool for diabetes in a general Japanese population. *Metabolism*, *64*(6), 698-705.

[8] Ravindran, R., GopINATHAN, D. M., & SuKuMARAN, S. (2015). Estimation of salivary glucose and glycogen content in exfoliated buccal mucosal cells of patients with type II diabetes mellitus. *Journal of clinical and diagnostic research: JCDR*, *9*(5), ZC89.

[9] Abikshyeet, P., Ramesh, V., & Oza, N. (2012). Glucose estimation in the salivary secretion of diabetes mellitus patients. *Diabetes, metabolic syndrome and obesity: targets and therapy*, *5*, 149.

[10] Kadashetti, V., Baad, R., Malik, N., Shivakumar, K. M., Vibhute, N., Belgaumi, U., ... & Pramod, R. C. (2015). Glucose level estimation in diabetes mellitus by saliva: a bloodless revolution. *Romanian Journal Of Internal Medicine*, *53*(3), 248-252.

[11] Viswanath, B., Choi, C. S., Lee, K., & Kim, S. (2017). Recent trends in the development of diagnostic tools for diabetes mellitus using patient saliva. *TrAC Trends in Analytical Chemistry*, *89*, 60-67.

[12] Nimptsch, K., Konigorski, S., & Pischon, T. (2018). Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine. *Metabolism*.

[13] Kesharwani, P., Gorain, B., Low, S. Y., Tan, S. A., Ling, E. C. S., Lim, Y. K., ... & Choudhury, H. (2018). Nanotechnology based approaches for anti-diabetic drugs delivery. *Diabetes research and clinical practice*, *136*, 52-77.

[14] Sahoo, S. K., & Labhasetwar, V. (2003). Nanotech approaches to drug delivery and imaging. *Drug discovery today*, *8*(24), 1112-1120

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

NANOTECHNOLOGY IN CANCER DIAGNOSTICS AND TREATMENT

Senthilrani.N[1], Shruthi.A[2],Chrislyne Antonette Fernandez.J[2], Srividya Priyadarshini[2], Niveda[2], Aishwarya[2], Sanjana[2], Sivarajasekar. N[2]

[1]Department of Medicine, Government Medical College and ESI Hospital, Coimbatore – 641015, India

[2]Kumaraguru College of Technology, Coimbatore-641049, India

shruthi.16bt@kct.ac.in

Abstract*: Cancer is a disease caused by the uncontrolled cell division of abnormal cells in a part of the body. They grow continuously to form a mass of cells called tumor. There are different kinds of cancer namely Prostrate, Leukemia, Pancreatic, Breast, Liver, Lung etc. The nanotechnology advancements can be used to treat many types of cancer.In prostrate cancer, carbon nanotubes are used with laser irradiation to enhance treatment. Leukemia detection can be done easier with nanopore which includes fluorescent Anisotropic method. The incorporation of gold nanoparticles and iron oxide nanoparticles can help in potential imaging of pancreatic cancer. Gold nanocrystals are conjugated into antibodies to detect breast tumor cells. Super magnetic iron oxide nanoparticles are used as contrast agent in CT imaging to enhance the imaging of liver cancer. Hybrid nanoparticles are able to deliver multiple drugs used to target tumor cells for lung cancer.This review article cites the nanoparticles usage in various types of cancer and their progress among the other treatments of cancer.*

Keywords: Nanoparticles, Nanodiagnostics, Imaging, Drug delivery, cancer therapy, hyperthermia

I. Introduction

Cancer is a disease caused by the uncontrolled cell division of abnormal cells in a part of the body. It begins when genetic changes interfere with the orderly process.When these cells start to grow uncontrollably, they form a mass called a tumor. Cancer forms a subset of neoplasm which is an abnormal growth of tissue in some part of the body.All solid tumors metastasize. Liquid cancers like leukemia does not metastasize because there is no primary tumor.There are different kinds of cancer treatment and the treatment depends on the type of cancer,risk factors and many parameters.The commonly used treatments include chemotherapy,immunotherapy,surgery and radiation.The usage of nanoparticles stands as a pinnacle in cancer treatment.It outcasts the other treatments due to the minimal damage of healthy tissue and hyperthermia.

Nanotechnology holds great potential for overcoming many of the problems associated with conventional methods which faces some difficulties in the detection, treatment, and diagnosis of cancer. By using nanotechnology, nanomaterials have been evolved for cancer diagnostics. Thus, nanodiagnostics, defined as the use of nanotechnology for clinical diagnostic purposes were developed to meet the demands of clinical diagnostics for increased sensitivity and earlier detection of disease.This paves the way to the enhancement of cancer treatment using nanoparticles.

Nanoparticles in cancer therapy and diagnosis can reduce the side effects of chemotherapy.It can be used for diagnosing cancer by molecular imaging.With the use of nanoparticles in molecular imaging, the acquired images will be of high precision and accuracy. In the treatment of cancer, nanoparticles can be used as delivery agents to the targeted affected areas. The most common delivery agents are liposomes. With these delivery agents, each and every type of cancer can be detected and treated with less damage to healthy issues compared to the traditional methods.

The usage of nanoparticles has its own risks. With the usage of nanoparticles as delivery agents,the targeted areas might develop drug resistance leading to the failure of the therapy. To overcome these risks,the nanoparticles are combined with traditional methods of treatment and make his therapy a huge success.So in each and every type of cancer,the specific nano particles are combined with traditional treatments like chemotherapy, surgery and radiation. This review article gives a deeper understanding on the different tuypes of cancers and its treatment and diagnostics.[1]

II. Prostate cancer:

The survival rate of prostrate cancer in males depends on the cancer stage and the patient's age.It is mainly associated with precursor lesions. Prostrate cancer is prone to patients whose diet is composed of high quantities of red meat and low quantities of vegetables[2].The traditional methods of treatment includes cryotherapy, radiation therapy and radial prostatectomy. The risks in this method includes urinary incontinence and impotency.To avoid these risks and hyperthermia associated with traditional methods of treatment, usage of nanoparticles are considered.

Carbon Nanotubes(CNT) are used with laser irradiation to enhance treatments. Gold nanoparticles are considered for their biocompatibility and bioinertness. Initially, nanoshells were considered. In this type of treatment, solutions containing gold nanoshells were infused into the patients and accumulates in the targeted areas. Secondly, laser light is applied to the lesion and when the laser is fired, the gold nanoshells absorb the laser light and become hot and raises the temperature of the lesion to a point where cell death occurs. After this, the body reabsorbs the dead tissue and the lesion heals . Magnetic nanoparticles such as iron oxide are used for magnetically mediated hyperthermia. Nanorods are also considered for prostrate cancer treatment due to its small size.[3]

III.Leukemia:

Leukemia is a fatal cancer which is common in children. It occurs when the DNA of immature blood cells becomes damaged which results in blood cell proliferation. As proliferation occurs, they stop the growth of healthy WBC by crowding out space in blood. The suspected risk factors are-artificial ionising radiation,hair dyes ,smoking, petrochemicals, Down's syndrome. The nanoparticle advancements helps to deliver the anti-cancer agent to the target area and maintaining the viability of non-malignant cells.[4]

Liposomes are used as drug delivery systems in leukemia. Anti-cancer drugs such as Abraxane and Doxil are delivered to the targeted area by protecting the encapsulated anti-cancer agent with its sealed core. Nanodiamonds coupled with anti-cancer drugs are used as drug delivery system especially in leukemia since these cancer cells pump out the chemotherapy drugs limiting its effects.Due to its limited effectiveness,the nanodiamonds are used which makes the cancer cells hard to pump out the nanodiamonds thereby increasing the effectiveness of this drug.[5]

Leukemia detection can be done easier with nanoparticles. The detection method includes Fluoroscence Anisotropy Method in white blood cells which is more easier than separation method. Nanoparticle modified glassy carbon electrodes are used in diagnostics to distinguish between drug resistant and drug sensitive leukemia cells.[6]

IV. Brain tumor:

Brain tumors are one of the most fatal tumors. It can lead to severe side effects like paralysis, amnesia,etc. Nanoparticles are promising "therapeutic agents in the treatment of brain tumors. They can be used for both the diagnostics,treatment and imaging methods.The traditional methods such as removal of tumor may lead to death or sometimes long term effects.

The usage of nanoparticles for treatment makes the anti-tumor drugs to pass easily through the blood-brain barrier which stands as an obstacle in traditional methods of treatment. Mostly lung and breast cancer can metastasize to brain tumor.The vessels in the brain is of complicated architecture.[7]

Polymeric nanoparticles are the most powerful nanotechnological method. They have been known as variable carriers for therapeutic cancer drugs. Polymeric nanoparticles carry not only small drug molecules but also macromolecules like genes and proteins. Thus nanoparticles are used as great drug delivery methods for anti-cancer drugs[8]

Nanoparticles are used for imaging because of high precision and quality.The traditional methods of imaging are Computed tomography (CT), and magnetic resonance imaging (MRI). But the nanoparticles such as magnetic nanoparticles can be used as contrasting agents to increase accuracy.The most promising contrasting agent which can be injected directed into the tumor to enhance imaging process are gadolinium conjugated with nanoparticles.[9]

V. Pancreatic cancer:

Pancreatic cancer occur when there are mutations in the pancreas cell's DNA. It occurs in the tissue of pancreas. It spreads rapidly to the nearby organs thus making its early detection seldom.Their most common risk factors include smoking,diabetes, weight loss. Pancreatic cancer mostly leads to liver metastasis.[10]

Nanoparticles in pancreatic cancer are used for drug delivery, detection and imaging. Nanoparticles encapsulated with anti-cancer drugs such as Doxil,Abraxane are most common delivery methods.Nanoparticle formulation of Gemcitabine, the chemotherapeutic agent is found to have more impact on unresectable tumors.This is a novel treatment strategy since it reduces its metastasis to a certain level.[11]

The Nano-Diagnostics of pancreatic cancer is used for its accuracy and cost effectiveness. To increase the sensitivity of tumor biomarkers,various electrochemical immunosensors with nanomaterials such as magnetic particles, gold nanoparticles, quantum dots, and carbon nanotubes are used.The incorporation of contrast agents in nanoparticles such as gold nanoparticles and iron oxide nanoparticles helps in the potential imaging of pancreatic cancer.[12]

VI.Breast cancer:

Breast cancer is the most frequent carcinoma in females and the second most common cause of cancer-related mortality in women worldwide.For the diagnosis and treatment of breast cancer using Nanobiotechnology, three crucial biomarkers are detected and quantified in each part of the tumor sections by employing nanoparticles conjugated to antibodies. Breast cancer express protein protein biomarkers such as oestrogen receptor , progesterone receptor and ERBB2(Erb-B2 Receptor Tyrosine Kinase 2). These are the sites where clinical diagnosis are made.[13]

Quantum dots, the semiconductor fluorescent nanocrystals are conjugated into antibodies and gold containing nanoparticles allows accurate detection and quantification of these markers in breast tumor sections. The most commonly used Nanocarriers maybe either organic or inorganic. The organic carriers include Liposome, dendrimers, carbon nanotubes, polymers etc. Liposomes are used in drug delivery in breast cancer diagnostics. Doxorubicin is formulated with Liposome delivery system to maintain efficiency of drug and it decreases cardiac toxic effects. Tumor- selective delivery of anticancer agents is preferred as it is important to protect healthy tissue from exposure to a cytotoxic agent and to reduce toxic effects , nanoparticles are used for this benefit. Nanoparticles conjugated to antibodies is used to detect multiple molecular targets on small tumor samples on which the decision for the treatment can be made. [14]

VII. Liver cancer:

It is one of the malignant tumors with low survival rate.The common risk factors include diabetes, alcohol consumption and hepatitis. Transplantation or removal of the liver surgically is the only cure for this disease. The introduction of hepatitis B virus vaccine led to to the minimal incidence of primary liver cancer.[15]

Nanoparticles are used for imaging and treatment. The traditional methods of liver cancer imaging includes computed tomography (CT) and MRI. However nanoparticles such as iron oxide and superparamagnetic iron oxide nanoparticles are used as contrast agents and gold nanoparticles are used as agents in CT imaging to enhance the imaging of liver cancer.[16]

Polymeric nanoparticles such as Doxorubicin trans-drug formulation showed anti-tumor activity and kills the tumor. Mitoxantrone-loaded polybutylcyanacrylate (PBCA) nanoparticles are slightly efficient than polymeric nanoparticles. Polymeric nanoparticles showed great progress when conjugated with chemotherapy[17]. In mice, Gum Arabic conjugated with gold nanoparticles minimally invades the healthy cells and suppresses the tumor and is a photothermal non-invasive ablation of liver cancer. [18]

VIII.Lung cancer:

Lung cancer is the major reason for most of the cancer deaths with a large genetic and epigenetic variations. There are various treatments and diagnosis available in the clinical field but the diagnosis is possible only after its malignant stage is reached and thus death rate of patients is increased due to unresponsiveness to the treatment. In such cases, nanoparticles come into action where various nanoparticles such as organic, inorganic, metallic and polymeric nanocarriers have been developed for treatment and diagnosis. Combining organic and inorganic nanoparticles into a single system, we are able to produce a hybrid system with enhanced structural, functional and biological properties[19]. These hybrid nanoparticles are able to deliver multiple drugs, target cancer causing agents much more efficiently and the use of photosensitive reagents have brought an enhancement in the sensitivity and the efficiency of detection when compared to the conventional nan carriers. For example:

Gold nanoparticles play a huge role in delivering anti-cancer drugs to the specific tumor sites. Eco-friendly gold nanoparticles have been synthesised using marine bacteria, *Enterococcus* sp. The synthesis was identified by the formation of ruby red in the incubated reaction mixture. When these nanoparticles were studied under TEM, their structure was found to be mostly spherical and their sizes varied from 6 to 13 nm. Thus the final

synthesised gold nanoparticles were found to have more precise anti-cancer activity at a concentration of 100 microgram. These gold nano particles can also be synthesised using cultures of *Pseudomonas aeruginosa, Cyanobacteria, Rhodopseudomonas capsulata, Escherichia coli, Bacillus subtilis*and *Stenotrophomonas maltophilia*. Microorganismscan synthesise nanoparticles in either intercellular or extracellular routes. In this case, the bacteria that we use to synthesise the gold nanoparticles follows an extracellular route[20].

X. Conclusion:

In the treatment of acute lymphocytic leukemia, an alkaloid named Vincristine is used. Vincristine induces cytotoxicity when it binds with tubulin; as a result microtubule depolymerization and metaphase arrest takes place. As a result of these events, apoptosis of cells while undergoing mitosis. Magnetic PLGA nanoparticles and Gd3+ doped amorphous TiO2 nanoparticles detect prostatic cancer cells and oral cancer cells by *Magnetic Resonance Imaging (MRI).* In lungs, drug delivery occurs after challenges like rate of clearance from circulation. In thermal therapy of prostate cancer, nanoparticles are employed in order to induce localized heating. In the chemotherapy treatment done for breast cancer, drugs like doxorubicin and abraxane are used. A natural gum named *Gum Arabic*, promotes adhesion of gold nanoparticles for attracting the tumors in the liver cells.

Nanotechnology in biomedical research has emerged as an interdisciplinary science that has found its own niche in clinical methodology including imaging , diagnostics and therapeutics .It is said that the biochemical applications of nanosized system , is a rapidly developing area within nanotechnology .Several nanotechnology approaches have been used to improve delivery of chemotherapeutic agents to cancer cells with the goal of minimizing toxic effects on healthy tissue while maintaining antitumor efficiency.The use of nanotechnology in cancer encompasses many nanotechnological approaches and it would be impossible to cover this in a single review . It involves the interaction of cellular and molecular components and engineered materials typically smaller particles between 1 and 100nm . Nanotechnology is drug delivery that is progressing in its clinical development in the use of NP to deliver drugs to cancer cells. The biological applications of nanoparticles is rapidly developing area of nanotechnology that raises new possibilities in diagnoses and treatment of human cancers.

References

[1]Aslan, B., Ozpolat, B., Sood, A. K., & Lopez-Berestein, G. (2013). Nanotechnology in cancer therapy. *Journal of drug targeting*, *21*(10), 904-913.

[2] Cuzick, J., Thorat, M. A., Andriole, G., Brawley, O. W., Brown, P. H., Culig, Z., ... & Ilic, D. (2014). Prevention and early detection of prostate cancer. *The lancet oncology*, *15*(11), e484-e492.

[3]Wang, W., Chen, G., & Chen, Y. (2013). Nanotechnology as a Platform for Thermal Therapy of Prostate Cancer. *J Mol Biomark Diagn*, *4*, e117..

[4]Ho, D., Wang, C. H. K., & Chow, E. K. H. (2015). Nanodiamonds: The intersection of nanotechnology, drug development, and personalized medicine. *Science Advances*, *1*(7), e1500439.

[5]Man, H. B., Kim, H., Kim, H. J., Robinson, E., Liu, W. K., Chow, E. K. H., & Ho, D. (2014). Synthesis of nanodiamond–daunorubicin conjugates to overcome multidrug chemoresistance in leukemia. *Nanomedicine: Nanotechnology, Biology and Medicine*, *10*(2), 359-369.

[6]Samir, A., Elgamal, B. M., Gabr, H., & Sabaawy, H. E. (2015). Nanotechnology applications in hematological malignancies. *Oncology reports*, *34*(3), 1097-1105

[7] Meyers, J. D., Doane, T., Burda, C., & Basilion, J. P. (2013). Nanoparticles for imaging and treating brain cancer. *Nanomedicine*, *8*(1), 123-143.

[8]Kang, C., Sun, Y., Zhu, J., Li, W., Zhang, A., Kuang, T., ... & Yang, Z. (2016). Delivery of nanoparticles for treatment of brain tumor. *Current Drug Metabolism*, *17*(8), 745-754.

[9]Meola, A., Rao, J., Chaudhary, N., Sharma, M., & Chang, S. D. (2018). Gold Nanoparticles for Brain tumor imaging: a Systematic Review. *Frontiers in neurology*, *9*.

[10] Reynolds, R. B., & Folloder, J. (2014). Clinical management of pancreatic cancer. *Journal of the advanced practitioner in oncology*, *5*(5), 356.

*[11]*Manzur, A., Oluwasanmi, A., Moss, D., Curtis, A., & Hoskins, C. (2017). Nanotechnologies in pancreatic cancer therapy. *Pharmaceutics*, *9*(4), 39.

[12] Chen, H., Zhen, Z., Todd, T., Chu, P. K., & Xie, J. (2013). Nanoparticles for improving cancer diagnosis. *Materials Science and Engineering: R: Reports*, *74*(3), 35-69.

[13] Wu, D., Si, M., Xue, H. Y., & Wong, H. L. (2017). Nanomedicine applications in the treatment of breast cancer: current state of the art. *International journal of nanomedicine*, *12*, 5879-5892.

[14] Yezhelyev, M. V., Gao, X., Xing, Y., Al-Hajj, A., Nie, S., & O'Regan, R. M. (2006). Emerging use of nanoparticles in diagnosis and treatment of breast cancer. *The lancet oncology*, *7*(8), 657-667.

[15] Bosch, F. X., Ribes, J., Díaz, M., & Cléries, R. (2004). Primary liver cancer: worldwide incidence and trends. *Gastroenterology*, *127*(5), S5-S16.

[16] Moghadam, F. F. (2017). Using nanoparticles in medicine for liver cancer imaging. *Oman medical journal*, *32*(4), 269.

[17] Gad, A., Kydd, J., Piel, B., & Rai, P. (2016). Targeting Cancer using Polymeric Nanoparticle mediated Combination Chemotherapy. *International journal of nanomedicine and nanosurgery*, *2*(3).

[18] Gamal-Eldeen, A. M., Moustafa, D., El-Daly, S. M., Abo-Zeid, M. A., Saleh, S., Khoobchandani, M., ... & Katti, K. V. (2017). Gum Arabic-encapsulated gold nanoparticles for a non-invasive photothermal ablation of lung tumor in mice. *Biomedicine & Pharmacotherapy*, *89*, 1045-1054.

[19] Mottaghitalab, F., Farokhi, M., Fatahi, Y., Atyabi, F., & Dinarvand, R. (2019). New insights into designing hybrid nanoparticles for lung cancer: Diagnosis and treatment. *Journal of Controlled Release*.
[20] Rajeshkumar, S. (2016). Anticancer activity of eco-friendly gold nanoparticles against lung and liver cancer cells. *Journal of Genetic Engineering and Biotechnology*, *14*(1), 195-202.

Acknowledgments

We would like to take this opportunity to thank our college, Kumaraguru College of Technology, for providing us the opportunity to present this review article and the Department of Biotechnology for this wonderful opportunity and we would like to thank Dr.Sivarajasekar for motivating us throughout the process.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

THERMAL BIOSENSORS AND THEIR APPLICATIONS

S.Vasuki, V.Varsha, Mithra, R.Arul Dharshni, S.Abinaya, R.Deva Dharshini, N. Sivarajasekar

Department Of Biotechnology, Kumaraguru College of Technology

Coimbatore-641049, INDIA

vasukisubramaniyam098@gamil.com

Abstract*: In this paper we described the biosensors, their constructions and their various applications in various process. Thermal biosensors measures the heat energy released or absorbed in a biochemical reactions. Thermal biosensing is a universally used method. In recent days, researches are focussed on the removing of organic contaminants from the waste water. By using COD, we can find the organic contaminants in the waste water. COD is abbreviated as Chemical Oxygen Demand. It is defined as the number of oxygen equivalents consumed during the organic contaminant oxidation by strong oxidation agents. This thermal biosensor measures the heat released during this oxidation. Flow injection analysis is used along with the assays. Then other form of biosensor is MEMS Thermal biosensor. MEMS stands for Micro Electo Mechanical Systems. This technology allows integration and batch fabrication of miniaturized devices to create a bioanalytical device at low cost. Thermistor usually makes the systems operational stability to increase.*

Keywords*: Chemical Oxygen Demand; Flow Injection Analysis; Organic contaminants; Oxidation agents; MEMS*

I. Introduction

Thermal biosensors measures the heat energy released or absorbed in a biochemical reaction. Nowadays researches are focussed on purifying the contaminated water. Mainly researches are focussed to remove organic contaminants from the waste water. This is done with the help of thermal biosensor. This is measured by using a parameter called COD. COD stands for Chemical Oxygen Demand. Strong oxidizing agents oxidizes the organic compounds in the waste water. The number of oxygen equivalents consumed in this oxidation is called COD. This COD indicates the amount of organic contaminants present in the waste water. During this oxidation heat energy is released. Here the thermal biosensor measures the heat energy released during oxidation. By using this the amount of organic pollutants in the waste water can be detected. Along with the assay flow injection analysis is used. This thermal biosensor is designed in such a way that it contains a peristaltic pump where its two channels are mounted with a tube, an amplifier, port injection valve, a computer and two thermistor. Using a pH meter, pH values of the solution were determined. The next type of biosensor is MEMS thermal biosensor. MEMS stands for Micro Electro Mechanical Systems. Microelectromechanical systems (MEMS) technology gives low cost integration of miniaturized devices and low cost fabrication. Because of the improved thermal isolation, low thermal mass and sample volume of the MEMS thermal biosensor provides linear range and high sensitivity, low power consumption and low measurement time. There are two forms of thermal biosensors used here. They are thermistors and thermopiles. Thermistors depends on the changes in the electrical resistance with temperature. From this the absolute temperature can be determined. Thermopiles measures the temperature difference between two regions. This biosensor is used only for solid or gas sample and cannot be used for liquid samples. There are multitude of application possibilities of the thermal biosensors, it is used in various bioprocess industries due to its simple design and it is easy to operate and maintain. Recent advancements led to the development of miniaturized thermal biosensors. It increases the system's operational stability. It also increases the robustness and sterile sampling. Thermal biosensors are stable for long term and it is less of cost. But, it's poor reputation is due to its complicated thermostating and it's very weak sensitivity. It also have non-specific heating effects. It has various applications in measurements of enzyme activity, non aqueous, clinical and hybrid parameters and process monitoring.

II. Thermal Biosensor for chemical oxygen demand determination in water

In recent research,most researchers attention is focused on removing the organic contaminants from waste water. These organic wastes highly pollutes the water. Chemical oxygen demand (COD) is a parameter to determine the amount of organic pollutants in waste water. The organic compounds in the waste water is oxidized by strong oxidizing agents such as dichromates and permanganates. The number of oxygen equivalents

consumed in this oxidation is called COD. This indicates the amount of organic pollutants present in the waste water sample. Some other alternative methods are also experimented and proposed, but they are not as efficient as COD.

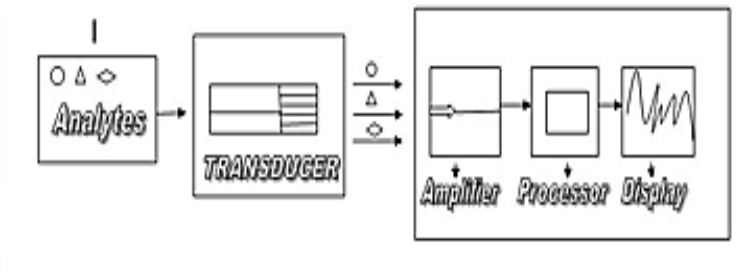


Figure 1. A diagrammatic representation of organic contaminant determination by thermal biosensor

For the determination of COD, a thermal biosensor has been developed. During oxidation of those organic pollutants in the waste water sample, heat is released. This biosensor measures the heat produced during this oxidation process. Along with the assay, flow injection analysis (FIA) technique is used. By using this continous analysis technique, the amount of test water sample and carrier solution can be cotrolled. The change in enthalpy of the organic contents in the waste water is proportional to the thermometric peak height. The thermistor is insensitive to electrochemical, optical and some other properties of the water sample. The degree of interference corresponding to the test water sample characteristics is highly low and the COD values obtained are highly accurate. These thermistors have high detection efficiency.

The designed biosensor consists of a peristaltic pump, where its two channels are mounted with the tube, port injection valve, an amplifier, two thermistor and a computer. The system used is animproved version of the conventional detector, which has only one outlet channel. The signals recorded in voltages. Using a pH meter, the pH values of the solutions were determined. The solutions are degassed in order to remove air bubbles from the reaction column.

III. MEMS Thermal biosensor

Thermal biosensor measures the heat released during the biochemical reaction. Microelectromechanicalsystems(MEMS) technology allows low cost integration of miniaturized devices and low cost batch fabrication. The improved thermal isolation, low thermal mass and sample volume of the MEMS thermal biosensor provides linear range and high sensitivity, low measurement time and low power consumption. Such devices may also offer measurement of multiple samples in parallel.

Temperature detection is the bases of MEMS thermal biosensors. Thermistors or thermopiles are the two forms of temperature sensors. Thermistors depends on the changes in the electric resistance with temperature from which the absolute temperature can be determined but have limited sensitivity. Thermopiles measures the temperature difference between two regions. Thermopiles are the set of thermocouple junctions in series. Thin film thermopiles or fabricated from metals, semiconductors and various substrate semiconductor components. The suspended diaphgrams or bridges was used to minimize thermal mass and maximize thermal isolation. Infrared detections involves thermal components.

These biosensors are used only for solid or gas samples. These type of sensors cannot be used for liquid samples. Research of these biosensors into liquid samples has been limited and involve glucose detection, urine acid detection, insulin detection and studies of living systems include human cell, animal cell , micro organisms and tissues. Investigations has been proceeding on multiple analyte thermal biosensor using MEMS thermopiles.

IV. Recent trends in Thermal biosensors

Due to the universal detection principle, there are multitude of application possibilities of the thermal flow analyzers. In Health care the use of biosensors have increased the interests of chemists. In home monitoring the biosensors are very suitable for monitoring of certain metabolites, like glucose in diabetic control. Recently thermal biosensors are of special interest because they can be simple and easy to operate and maintain.

The thermistor usually makes the system's operational stability to increase in the function. Recent advancements led to the development of miniatured thermal biosensor devices and it is useful in bioprocess

industries. Enzyme thermistor has a current development for bioprocess monitoring with increase in robustness and sterile sampling. In bioprocess and downstream analysis have a special attention to thermal flow analyzers.

Advantages of Thermal biosensors

- **Long -term stability:** As there is no contact between the transducer and the sample, the thermistors are less likely to get damaged.
- **Less cost:** Thermistors are cheap products.
- **Accuracy:** Measurements are not disturbed by varying optical or ionic sample characteristics.

Disadvantages of Thermal biosensors

- **Complexity:** It is due to the complicated thermostating.
- **Sensitivity characteristic:** They have very weak sensitivity.
- **Poor reputation:** It is due to the non-specific heating effects.

V. Conclusion

The organic contaminants in the waste water is detected by a thermal biosensor by using a parameter called chemical oxygen demand (COD). The thermal biosensor measures the heat released during the oxidation of these organic contaminants by strong oxidizing agents. MEMS thermal biosensor provides low cost integration of miniaturized devices and batch fabrication at low cost. Recently miniaturized thermal biosensors are used in various bioprocess industries due to its renowned advantages.

VI. References

[*1]* Yao, N., Wang, J., & Zhou, Y. (2014). Rapid determination of the Chemical Oxygen Demand of water using a thermal biosensor. Sensors, 14(6), 9949-9960.

[2] Kim, Y.-C.; Lee, K.-H.; Sasaki, S.; Hashimoto, K.; Ikebukuro, K.; Karube, I. Photocatalytic sensor for chemical oxygen demand determination based on oxygen electrode. Anal. Chem. **2000**, 72, 3379–3382.

[3] Qu, X.; Tian, M.; Chen, S.; Liao, B.; Chen, A. Determination of chemical oxygen demand based on novel photoelectro—bifunctional electrodes. Electroanalysis**2011**, 23, 1267–1275.

[4] Yu, H.; Wang, H.;Quan, X.; Chen, S.; Zhang, Y. Amperometric determination of chemical oxygen demand using boron-doped diamond (bdd) sensor. Electrochem.Commun. **2007**, 9, 2280–2285.

[5] Zhang, A.; Zhou, M.; Han, L.; Zhou, Q. Amperometric determination of chemical oxygen demand via the functional Combination of three digestion types. Electroanalysis **2010**, 22, 2947–2959

[6] .Wang, L., Sipe, D. M., Xu, Y., & Lin, Q. (2008).A MEMS thermal biosensor for metabolic monitoring applications. Journal of microelectromechanical systems, 17(2), 318-327.

[7] B. Danielsson, "Calorimetric biosensors", *Biochem. Soc. Trans.*, vol. 19, pp. 26-28, 1991.

[8] P. Bataillard, E. Steffgen, S. Haemmerli, A. Manz, H. M. Widmer, "An integrated silicon thermopile as biosensor for the thermal monitoring of glucose urea and penicillin", *Biosens. Bioelectron.*, vol. 8, no. 2, pp. 89-98, 1993.

[9] R. Lenggenhager, H. Baltes, J. Peer, M. Forster, "Thermoelectric infrared sensors by CMOS technology", *IEEE Electron Device Lett.*, vol. 13, no. 9, pp. 454-456, Sep. 1992.

[10] A. W. Vanherwaarden, P. M. Sarro, J. W. Gardner, P. Bataillard, "Liquid and gas micro-calorimeters for (Bio)chemical measurements", *Sens. Actuators A Phys.*, vol. 43, no. 13, pp. 24-30, May 1994.

VII. Acknowledgments

We would like to thank Kumaraguru College of Technology for giving this opportunity under the guidance of Dr. N. Sivarajasekar.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Antimicrobial peptides from plants and their mode of action

Yamuna S, Nithesh Kumar V, Bala Subramaniam D and Kumaravel K

Department of Biotechnology

Kumaraguru College of Technology

Coimbatore, Tamilnadu -641049

kumaravel.k.bt@kct.ac.in

Abstract: Anti-Microbial Peptides (AMPs) are the host defence peptides found in animals, humans, and plants. The AMPs obtained from animals and human cell lines are not only expensive but also tedious to extract. However, AMPs extracted from plant cells are both easier to extract and inexpensive. AMP's mediates the defence mechanism against bacteria by interacting with their cell wall. In the recent years, AMP's were isolated from various plant and their antibacterial activity were tested against many of bacterial species. This article critically reviews about AMPs extracted from various plant sources, mode of action, and their efficacy against bacterial species.

Keywords: Plant AMP's , host defence , antibacterial activity , cell wall, plant sources, action

I. Introduction

Plants, animals and humans are often exposed to the pathogens continuously. The higher organisms naturally have an immune system that protects themselves from the physical, chemical and biological agents such as pollutants, heavy metals, pollens, cold, drought, pathogenic, bacteria, fungi, insects, beetles, larvae [1,2,3]. The plants protect themselves by secreting the phenolic compounds, secondary metabolites and antimicrobial peptides.

Plants produce Anti-Microbial Peptides (AMPs) for defense against pathogenic bacteria and fungi. The antimicrobial peptides size ranges from 2-9 kDa consisting of 10-100 amino acids residues. These plant antimicrobial peptides are divided into many families based on the cysteine residues, their position, length of amino acids, molecular weight [1,3]. Furtermore, such AMPs includes families such as thionins[4-14], defensins[15-23], lipid transfer proteins[23-29], snakins[30], hevein like peptides[31-33]. These AMPs are isolated from different parts of the plant like root , flower, leaf, stem and seeds[1-33] . Isolated plant peptides possess antimicrobial activity against bacteria and fungi. The AMPs interact with the phospolipid bilayer of bacterial cell and hyphae of the fungi. [3]

II. Thionins

Thionin are the antimicrobial pepetides that consist of 45-47 amino acid residues. The molecular weight of the thionin is around 5 kDa. Thionin is divided into two subgroups one with eight cysteine residues connected by four disulfide bonds called 8c residues and the other with six cysteine residue connected by three disulfide bonds called 6c residues. The first isolated thionin was purothionin from endosperm of the wheat. Thionin proven to posses act against *Pseudomonas, Xanthomonas, Corynebacterium ,yeast* and fungi like *Thielaviopsis paradoxa and Dreschesera teres.* In insects thionin exhibit antimicrobial properties when injected in to the haemocele but not when incorporated into the nutrients.

Mode of action of thionin class of AMP's

Thionins are hydrophobic in nature. Thionin interacts with the hydrophobic residues and lyse the cell membrane of the bacteria. Thionins interact directly with the membrane lipids part from interacting with membrane protein receptors. Table 1. represents the Antimicrobial activity of Thionins

Table 1. Antimicrobial activity of Thionins

Name of AMP	Source	Susceptible species	References
Purothionin	Wheat endosperm crude	*Pseudomonas solanacearum* *Xanthomonas phaseoli* *Xanthomonas campestris* *Erwinia amylovora*	[4]

		Corynebacterium fascians *C. flaccumfaciens* *C. michiganese* *C. poinsettiae* *C. sepedonicum*	
α-purothionin	Wheat endosperm	*Rhizoctonia solani*	[5]
Viscotoxin A3 and B	*Viscum album*	*Fusarium solani* *Sclerotinia sclerotiorum* *Phytophtora infestans*	[6]
PR-13 thionins	*Nicotiana attenuate*	*Pseudomonas syringae*	[7]
Thionin	Pearl millet seed	*Sclerospora graminicola*	[8]
WBeta (thionin)	*Triticum aestivum*	*Fusarium solani*	[9],[10]
AX1 thionin	*Beta vulgaris*	*Cercospora beticola*	[11]

III. Defensins

Defensins resemble thionin in their size with 45-5 amino acid residues.the molecular weight of the defensins is 5kDa.the first discovered defensin was considered as thionin and called as s γ-thionin ,later they differ from thionin structurally. Defensin are cationic peptides with 4-5 disulfide bridegs in cyateine residues. Defensins contain two antiparallel α-helices and an antiparallel double stranded β sheets. Defensins are the abundant plant antimicrobial peptide with membranolytic function. Defensins have antibacterial , antifungal activity act as inhibitor for α-amylase and trypsin.

Mode of action of Defensin class of AMP's

efensins are positively charged and amphipathic in nature. Defensins are likely to bind to the bacterial membrane through interaction with the receptors, which results in the influx and efflux of ions ca^{2+} *and* k^{+}. *Defensins also block the* ca^{2+} *ion channel.* Table 2. represents the Antimicrobial activity of Defensins

Table 2. Antimicrobial activity of Defensins

Name of AMP	**Source**	**Susceptible species**	**References**
MsDef1	*Medicago sativa*	*Magnaporthe grisea* *Erwinia carotovora* *Botrytis cinerea*	[18]
WT 1	*Wasabia japonica*	*Magnaporthe oryzae* *Rizoctonia solani*	[19]
Dm-AMP1	*Dahlia*	*Fusarium culmorum*	[20]
Ah-AMP1	*Aesculus hippocastanum*	*Fusarium moniliforme*	[9],[10]
Rs-AFP1	*Raphanus sativus*	*Fusarium culmorum*	[21]
RsAFP2	*Raphanus sativus*	*Baker's yeast* *Candida albicans*	[22]
Hc-AFP1 Hc-AFP2 HcAFP3 Hc-AFP4	*Heliophila coronopifolia*	*Botrytis cinerea* *Fusarium solani*	[23]
HsAFP1	*Heuchera sanguine*	*Aspergillus flavus* *Candida albicans* *Candida krusei*	[24]
Ns-D1 Ns-D2	*Nigella sativa*	*Aspergillus niger* *Fusarium oxysporum* *Fusarium graminearum* *Fusarium culmorum* *Bipolaris sorokiniana* *Botritis cinerea*	[25]

IV. Lipid transfer proteins

Lipid Transfer Proteins (LTP) are the homologous proteins. LTP consist of 90-93 amino acid residues. LTP consist of two families, one with molecular weight of 9kDa (LTPs1 and other with 7kDa (LTPs2). LTP's transfer lipids between membranes *in-vitro. LTP's are defensive against phytopathogens. LTP's are involved in cutin formation , embryogenesis and symbiosis. LTP's are not known for antifungal activity.*

Mode of action of Lipid Transfer Proteins

LTP's not only facilitate transfer of lipids between membranes *in-vitro* but also LTP1s facilitate the transfer of lipids between membranes *in-vivo* as they are extracellular cell wall proteins. Thus LTP promote membrane permeabilization in pathogen. Table 3. represents the Antimicrobial activity of lipid transfer proteins

Table 3. Antimicrobial activity of lipid transfer proteins

Name of AMP	Source	Susceptible species	References
Ace-AMP1	*Allium cepa*	*Fusarium oxysporum*	[28]
Cw18	*Hordeum vulgare*	*Fusarium solani*	[29]
LTP-a1 LTP-a2	*Arabidopsis*	*Fusarium solani*	[30]
LTP-s1 LTP-s2	Spinach	*Clavibacter michiganensis* *Pseudomonas solanacearum*	[31]
Ca-LTP(1)	*Coffea canephora*	*Colletotrichum lindemuthianum Candida tropicalis* Inhibitor of mammalian α-amylase	[32]
Cc-LTP-1	*Coffea canephora*	*Candida albicans* *Candida tropicalis* Inhibitor of mammalian α-amylase	[33]
LTP protein	Wheat	*Rhizoctonia solani* *Curvularia lunata* *Alternaria sp.* *Bipolaris oryzae* *Cylindrocladium scoparium* *Botritis cinerea* *Sarocladium oryzae*	[34]

V. Snakins

Snakins , isolated from the potato tubers comprise of snakinI (StSN1) consisting of 63 amino acid residues and snakin II (StSN2) consisting of 66 amino acid residues. The molecular weight of snakins is 6.9kDa. Snakins consist of six disulfide bridges. Snakins participate in hormonal signalling pathways. Snakins are involved in cell division, cell elongation, transition of flowering and defense.

Mode of action of Snakins

Snakins are involved in hormonal signalling pathways. Thus snakins modulates the level of hormone and its responses. Table 4. represents the Antimicrobial activity of Snakins.

Table 4. Antimicrobial activity of Snakins

Name of AMP	Source	Susceptible species	References
Snakins (StSN1 and StSN2)	*S. tuberosum*	*Botrytis cinerea* *Fusarium solani* *Fusarium culmorum* *Fusarium oxysporum* *Fusarium oxysporum* *Plectosphaerella cucumerina Colletotrichum graminicola Colletotrichum lagenarium Bipolaris maydis* *Aspergillus flavus* *Clavibacter michiganensis* *Ralstonia solanacearum* *Ervinia chrysanthemi*	[35]

VI. Hevein Like Peptides

Hevein like peptides are the basic peptides. Hevein like peptides consist of 29-45 amino acid residues with 3-5 disulfide bonds. The molecular weight of hevein is 4.7 kDa. Hevein like peptides are the most abundant peptide present in the latex of the rubber tree with *in-vitro* antifungal activity. Hevein are the alergen which is involved in the human latex-fruit syndrome.

Mode of action of Hevein Like Peptides

Hevein like peptide are defensive for fungi. Hevein like peptide inhibits the hyphal growth of fungi by binding to the chitin of fungi. . Table 5 represents antimicrobial activity of Hevein like peptides

Table 5. Antimicrobial activity of Hevein Like Peptides

Name of AMP	Source	Susceptible species	References
IWF4	*Beta vulgaris*	*Cercospora beticola*	[36]
Ac-AMP1	*Amaranthus caudatus0*	*Fusarium culmorum*	[37]
EAFP1 EAFP2	*Eucommia ulmoides*	*Phytophthora infestans* *Ascopchyta lycopersici* *Verticillium dahlia* *Giberella zeae* *Alternaria nicotianae* *Fusarium moniliforme* *Fusarium oxysporum* *Colletotrichum gossypii* *Pseudomonas syringae*	[38]
PMAPI	*Broussonetia papyrifera* *Morus papyrifera*	*Trichoderma viride*	[39]
WjAMP1	*Wasabia japonica*	*Botrytis cinerea* *Fusarium solani* *Magnaporthe grisea* *Alternaria alternata* *Escherichia coli* *Agrobacterium tumefaciens* *Pseudomonas cichorii* *P. plantarii (Burkholderia plantarii)* *P. glumae (B. glumae)*	[40]

VII. Conclusion

This article presents the mode of action of three different types of AMPs extracted from various plant sources. In addition, this article also highlights the potential use of Plant-AMPs as efficient antimicrobial agent against many bacterial and fungal species. Furthermore, such AMPs also possess therapeutic properties. However, assessing the efficacy of Plant-AMPs and their mode of administration requires future investigation, nevertheless the plant antimicrobial peptides could play an important role in clinic.

References

[1] Robert Nawrot & Jakub Barylski & Grzegorz Nowicki & Justyna Broniarczyk & Waldemar Buchwald & Anna Goździcka-Józefiak(2013): Plant antimicrobial peptides. Folia Microbiol 59:181–196

[2] James P. Tam , Shujing Wang, Ka H. Wong and Wei Liang Tan :antimicrobial peptides from plants Pharmaceuticals 2015, 8, 711-757

[3] Stintzi, A.; Heitz, T.; Prasad, V.; Wiedemann-Merdinoglu, S.; Kauffmann, S.; Geoffroy, P.; Legrand, M.; Fritig, B. Plant "pathogenesis-related" proteins and their role in defense against pathogens. Biochimie 1993, 75, 687–706.

[4] Fernandez De Caleya R, Gonzales-Pascual B, Garcia-Olmedo F, Carbonero P (1972) Suseptibility of phytopathogenic bacteria to wheat purothionins in vitro. Appl Microbiol 23:998–1000

[5] Oard S, Rush MC, Oard JH (2004) Characterization of antimicrobial peptides agains a US strain ofthe ricepathogen Rhizoctonia solani. J Appl Microbiol 97:169–180

[6] Giudici AM, Regente MC, Villalain J, Pfuller K, Pfuller U, de la Canal L (2004) Misctletoe viscotoxins induce membrane permeabilization and spore death in phytopathogenic fungi. Physiol Plant 121:2–7

[7] Rayapuram C, Wu J, Haas C, Baldwin IT (2008) PR-13/Thionin but not PR-1 mediates bacterial resitance in Nicotiana attenuata in nature, and neither influences herbivore resistance. Mol Plant Microbe Interact 21:988–1000

[8] Chandrashekhara NRS, Deepak S, Manjunath G, Shetty SH (2010) Thionins (PR protein 13) mediate pearl millet down mildew disease resistance. Arch Phytopathol Plant Protect 43:1356–1366

[9] TerrasF,SchoofsH,ThevissenK,OsbornRW,VanderleydenJ,Cammue B,BroekaertWF(1993a)Synergisticenhancementoftheantifungal activity of wheat and barley thionins by radish and oilseed rape 2S albumins and by barley trypsin inhibitors. Plant Physiol 103:1311– 1319

[10] Terras FR, Torrekens S, Van Leuven F, Osborn RW, Vanderleyden J, Cammue BP, Broekaert WF (1993b) A new family of basic cysteine-rich plant antifungial proteins from Brassicaceae species. FEBS Lett 316:233–240

[11] Kragh K, Nielsen J, Nielsen KK, Dreboldt S, Mikkelsen JD (1995) Characterization and localization of new antifungal cysteine-rich proteins from Beta vulgaris. Mol Plant Microbe Interact 8:579–585

[12] Stec, B. Plant thionins--the structural perspective. Cell. Mol. Life Sci. 2006, 63, 1370–1385.
[13] Garcia-Olmedo, F.; Molina, A.; Alamillo, J.M.; Rodriguez-Palenzuela, P. Plant defense peptides. Biopolymers 1998, 47, 479–491.
[14] Mander, L.N.; Liu, H.-W. Comprehensive Natural Products ii Chemistry and Biology; Elsevier Science: Oxford, England, 2010; p. 1.
[15] Florack, D.E.; Stiekema, W.J. Thionins: Properties, possible biological roles and mechanisms of action. Plant Mol. Biol. 1994, 26, 25–37
[16] Osorio e Castro, V.R.; Vernon, L.P. Hemolytic activity of thionin from pyrularia pubera nuts and snake venom toxins of naja naja species: Pyrularia thionin and snake venom cardiotoxin compete for the same membrane site. Toxicon 1989, 27, 511–517
[17] Hughes, P.; Dennis, E.; Whitecross, M.; Llewellyn, D.; Gage, P. The cytotoxic plant protein, beta-purothionin, forms ion channels in lipid membranes. J. Biol. Chem. 2000, 275, 823–827.
[18] Spelbrink RG, Dilmac N, Allen A, Smith TJ, Shah DM, Hockerman GH (2004) Differential antifungal and calcium channel blocking activity among structurally related plant defensins. Plant Physiol 135:2055– 2067
[19] Lay FT, Anderson MA (2005) Defensins—components of the innate immune system in plants. Curr Protein Pept Sci 6:85–101
[20] Zhu YJ, Agbayani R, Moore PH (2007) Ectopic expression of Dahlia merckii defensin DmAMP1 improves papaya resistance to Phytophthora palmivora by reducing pathogen vigor. Planta 226:87–97
[21] De Lucca AJ, Cleveland TE, Wedge DE (2005) Plant-derived antifungal proteins and peptides. Can J Microbiol 51:1001–1014
[22] Thevissen K, De Mello TP, Xu D, Blankenship J, Vandenbosch D, Idkowiak-Baladys J, Govaert G, Bink A, Rozental S, de Groot PW, Davis TR, Kumamoto CA, Vargas G, Nimrichter L, Coenye T, Mitchell A, Roemer T, Hannun YA, Cammue BP (2012) The plant defensin RsAFP2 induces cell wall stress, septin mislocalization and accumulation of ceramides in Candida albicans. Mol Microbiol 84:166–180
[23] De Beer A, Viver MA (2011) Four plant defensins from an indigenous South African Brassicaceae species display divergent activities against two test pathogens despite high sequence similarity in the encoding genes. BMC Res Notes 4:459–476
[24] Thevissen K, Ghazi A, de Samblanx GW, Brownlee C, Osborn RW, Broekaert WF (1996) Fungal membrane responses induced by plant defensins and thionins. J Biol Chem 271:15018–15025
[25] Rogozhin EA, Oshchepkova YI, Odintsova TI, Khadeeva NV, Veshkurova ON, Egorov TA, Grishin EV, Salikhov SI (2011) Novel antifungal defensins from Nigella sativa L. seeds. Plant Physiol Biochem 49:131–137
[26] Kader, J.C. Proteins and intracellular exchange of lipids .1. Stimulation of phospholipid exchange between mitochondria and microsomal fractions by proteins isolated from potato-tuber. Biochem. Biophys. Acta 1975, 380, 31–44.
[27] Kader, J.C.; Julienne, M.; Vergnolle, C. Purification and characterization of a spinach-leaf protein capable of transferring phospholipids from liposomes to mitochondria or chloroplasts. Eur. J. Biochem. 1984, 139, 411–416.
[28] Cammue BP, De Bolle MF, Terras FR, Proost P, Van Damme J, Rees SB, Vanderleyden J, Broekaert WF (1992) Isolation and characterization of a novel class of plant antimicrobial peptides form Mirabilis jalapa L seeds. J Biol Chem 267:2228–2233
[29] Molina A, Segura A, Garcia-Olmedo F (1993) Lipid transfer proteins (nsLTPs) from barley and maize leaves are potent inhibitors of bacterial and fungal plant pathogens. FEBS Lett 316:119–122
[30] Segura A, Moreno M, García-Olmedo F (1993) Purification and antipathogenic activity of lipid transfer proteins (LTPs) from the leaves of Arabidopsis and spinach. FEBS Lett 332:243–246
[31] Diz MS, Carvalho AO, Ribeiro SF, Da Cunha M, Beltramini L, Rodrigues R, Nascimento VV, Machado OL, Gomez V (2011) Characterisation, immunolocalisation and antifungal activity of a lipid transfer protein from chili pepper (Capsicum annuum) seeds with novel α-amylase inhibitory properties. Physiol Plant 142:233–246
[32] Zottich U, Da Cunha M, Carvalho AO, Dias GB, Silva NC, Santos IS, Do Nacimento VV, Miguel EC, Machado OL, Gomes VM (2011) Purification, biochemical characterization and antifungal activity of a new lipid transfer protein (LTP) from Coffea canephora seeds with α-amylase inhibitor properties. Biochim Biophys Acta 4:375–383
[33] Kirubakaren SI, Begum SM, Ulganathan K, Sakthivel N (2008) Characterization of a new antifungal lipid transfer protein from wheat. Plant Physiol Biochem 46:918–927
[34] Cheng CS, Chouabe C, Eyraud V, Rahioui I, Royer C, Soulage C, Bonvallet R, Huss M, Gressent F (2011) New mode of action for a knottin protein bioinsecticide pea albumin 1 subunit b(PA1b) is the first peptidic inhibitor of V-ATP-ase. J Biol Chem 286:36291–36296
[35] Berrocal-Lobo M, Segura A, Moreno M, López G, García-Olmedo F, Molina A (2002) Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. Plant Physiol 128:951–961
[36] Nielsen KK, Nielsen JE, Madrid SM, Mikkelsen JD (1997) Characterization of a new antifungal chitin-binding peptide from sugar beet leaves. Plant Physiol 113:83–91
[37] Broekaert W, Marien W, Terras F, De Bolle M, Proost P, Van Damme J, Dillen L, Claeys M, Rees SB, Vanderleyden J et al (1992) 192 Folia Microbiol (2014) 59:181–196 Antimicrobial peptides from Amaranthus caudatus seeds with sequence homology to the cysteine/glycine-rich domain of chitinbinding proteins. Biochemistry 31:4308–4314
[38] Huang RH, Xiang Y, Liu XZ, Zhang Y, Hu Z, Wang DC (2002) Two novel antifungal peptides distinct with a five-disulfide motif from the bark of Eucommia ulmoides Oliv. FEBS Lett 521:87–90ss
[39] Zhao M, Ma Y, Pan YH, Zhang CH, Yuan WX (2011) A hevein-like protein and a class I chitinase with antifungal activity from leaves of the paper mulberry. Biomed Chromatogr 25:908–912
[40] Kiba A, Saitoh H, Nishihara M, Omiya K, Yamamura S (2003) Cterminal domain of a hevein-like protein from Wasabia japonica has potent antimicrobial activity. Plant Cell Physiol 44:296–303

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Effect of biofertiizer, farmyard manure and vermicompost on seed germination, plant growth and vigor of *Solanum lycopersicum L.* (Tomato)

Kousalya Mangai. S, A. Jenifer, A.S.Agalya, R. Akshara. B, Boobalakannan, K.V.J. Gowsika, S. Abarna and P. Muthukumaran

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu 641049, India
kousalya.16bt@kct.ac.in http://www.kct.ac.in

Abstract*: The purpose of research is to study comparative effect of organic manure on* ***S****olanum lycopersicum L..The soil sample is mixed with the organic manure in the ratio of 3:1 and the treatment is replicated for three times. The data is collected in the basis of root size, number of leaves, shoot length, leaves length and leaves width..This study suggested that the vermicompost served as a potential source of nutrients for plant growth. Supportive study were made in the combination of soil, vermicompost and organic manure in the ratio of 3:1/2:1/2 to determine whether vermicompost is dominating other organic manure like compost, FYM, biofertilizer. Further, with these data statistical analysis(one way anova) was performed and hypotheses were obtained for the growth parameters.*

Keywords*: Biofertilizers;* ***S****olanum lycopersicum L.* *; Vermicompost ; Farmyard manure*

I. Introduction

A good deal of research has been done in India and tomato growing countries in recent year to evaluate the response of tomato to applied organic manures and biofertilizers. However, the information is not up to the mark in relation to the interaction of improved varieties of tomato with certain organic manures like FYM, Vermicompost, Azospirillum and PSB etc., as compared to recommended dose of fertilizers (NPK). This has encouraged generating information's for tomato growers of this region.

B.K.Singh and K.A.Pathak (2010) carried out an experiment on various doses of vermicompost and NPK fertilizer in tomato revealed that fruit density, post-harvest life and TSS are increasing with increase in rate of vermicompost application. Additionally, the increased amount of humus in soil through application of vermicompost and decomposition of organic mulches by earthworms would certainly help favourable change in physical, chemical and biological properties of soil, and in enhancing the water-holding capacity. Jenny and Malliga (2016)revealed that application of different concentrations of organic manure showed a significant effect on the biochemical contents when compared to control plant Furthermore, 20g of minimum particle size of organic manure applied plant fruits exhibited rich source of nutrient contents than the other treatments and control. This could be due to the presence of micro and macro nutrients in the optimized concentration of organic manure can be easily assimilated by the plants.

Reshid (2014) reported the application of vermicompost at 10, 20, 30 and 40% level had significantly improving effects on the accumulation of macro-nutrients, N, P, K, organic matter in the soil and soil texture. All doses of vermicompost (10, 20, and 30%) produced significantly higher length of tape root of the tomato plants than those tomato plants treated with 0 and 40% vermicompost. However, tomato plants grown in 20% doses of vermicompost had significantly higher dry and fresh weight of leave and also had significantly higher root, stem, number of fruit and yield than those grown in pots treated with 0 and 10% vermicompost. Athokpam and ShabirHussain (2016) significantly revealed that vermicompost and boron is an essential nutrients and plays a pivotal role in the earliness, nutrient content, reduced fruit cracking, yield, and better fertility of soil. Among the vermicompost application, maximum level of vermicompost (20q/ha) was found to be the best in increasing the yield and fertility of soil.

AbulKashem and AshokaSarker (2015) reported that in terms of plant growth and soil health, vermicompost plays an important role in improving soil texture, aeration, soil compaction and thus enhances more water and nutrients uptake by plants from their surrounding areas of root zone. There is much evidence that the activity of earthworms accelerates organic matter mineralization, decomposition of polysaccharides, increase the humus in the soil, and oppositely reduce the availability of toxic heavy elements to plants. Singh et al. (2010) examined that increased plant height, fruit weight, fruit yield, fruit density, post-harvest life and TSS of tomato, when application of vermicompost @ 7.5 tonnes ha-1 + RDF (NPK @ 60:30:30 kg ha-1).

Shankar et al. (2012) studied the effect of organic farming on nutritional profile, quality characteristics and toxic parameters of tomato. Four organic manure treatments consisting vermicompost, poultry manure, farm yard

manure, cow dung and recommended dose of chemical fertilizers, i.e., conventional farming as control. Application of organic manures was found to be significantly influence the nutrient content (micro-nutrients, TSS, lycopene etc.) compared to conventional fertilizers application.

The main objectives of this research are to study the effect of organic manures such as vermicompost, farm yard manure, biofertilizer on the growth of *Solanum lycopersicum L*.

II. Materials and methods

This chapter embodies concise dissertation of the method adopted and materials used during the course of investigation. The experiments Effect of organic manures and biofertilizers on growth of tomato (*Solanum lycopersicum L.*) was conducted at Department of Biotechnology, Kumaraguru College of Technology, Coimbatore- 641 69, Tamil Nadu, India.

Experimental site

The experiment was carried out under pot culture experiment in laboratory, Kumaraguru College of Technology, Coimbatore- 641 69, Tamil Nadu, India.

Nursery rising:

The seed of tomato variety was obtained from authorized dealer. The soil was manured with different organic manures as mentioned above. Then the seeds were sown in cups. The seedlings were raised in cups of length 10 cm and 5 cm diameter. All intercultural operations were done as and when required.

Observation and its procedure

Ten seeds were sown and two plants in each cup were selected randomly for recording observations successive and following characters such as height of plant, Root length, numbers of leaves, Length and width of leaves were noted for study purpose during stage of growth. The observations were taken at regular interval of 7 days.

III. Results and discussion

The experimental observations were recorded on growth characters and growth from various treatments. The data were statistically computed for different characters. Thereafter, these are presented in this chapter with suitable diagrams.



(a)Control



(b)Vermicompost

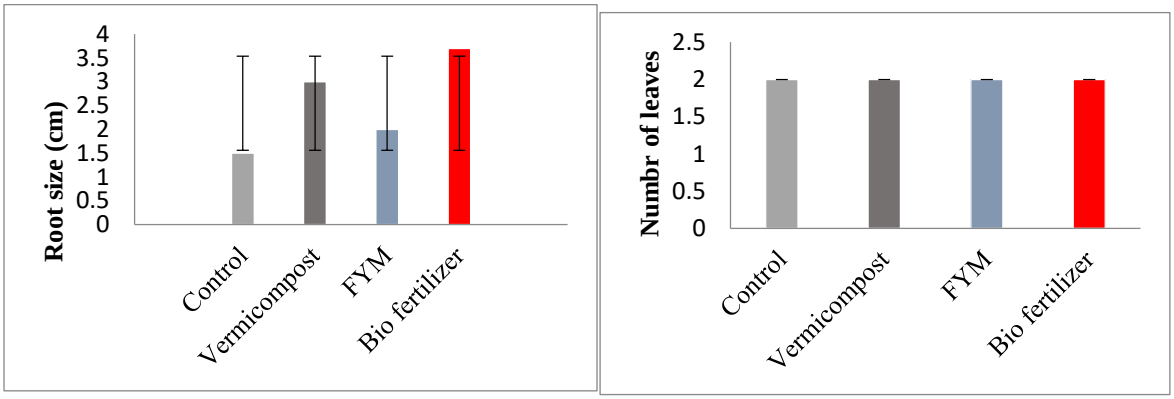


(c) F Y M

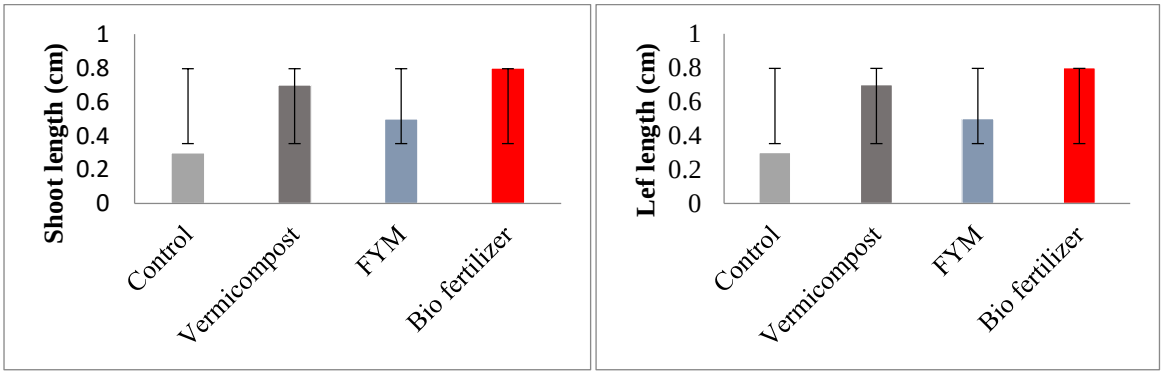


(d)Biofertilizers

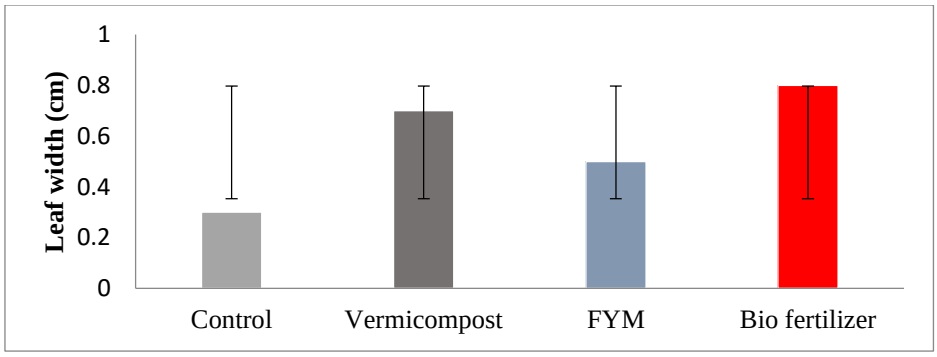
Figure 1: Application of organic manures on *Solanum lycopersicum.L*



(a) **(b)**



(c) **(d)**



(e)

Figre 2: Effect of organic manures on growth of *Solanum lycopersicum.L*

(a) Root size (b) No. of leaves (c) Shoot length (d) Leaf length (e) Leaf width

The growth parameters of tomato plant were analysed for organic manures like vermicompost, biofertilizers and farmyard manure. Triplicates were done and out of that data were obtained. The different growth parameters like shoot length, root length, leaf length, no. of leaves and leaf width for different organic manures were tested. From the figure 2, it was analysed that biofertilizers showed good results for root size, leaf length and leaf width. Similarly, vermicompost showed better growth in shoot length and leaf width. Thus, it can be concluded that both biofertilizers and vermicompost produced good results in the growth of plant.

V. Conclusion

On the basis of results recorded from this experiment, the following recommendations can be generalized; among various combinations used, t0t3 showed better results regarding most of the parameters. Moreover the application of vermicompost and biofertilizers increases availability of N, P, K and Organic Carbon, which exerts positive effect on growth, development of tomato. It can be concluded that vermicompost application to soil increased growth parameters of tomato plants (*Solanum lycopersicum L.*) over the inorganic fertilizer (NPK fertilizer) and hence could be useful for different crops production instead of inorganic fertilizers. The use of vermicompost and biofertilizers could be a better option and farmers need to be educated about the importance of vermicomposting. More field trials with different crops need to be conducted in the future.

References

[1] EmineOrhan, AhmetEsitken, SezaiErcisli, MetinTuran, and FikrettinSahin. Effects of plant growth promoting rhizobacteria on yield,growth and nutrient contents in organically growing raspberry. ScientiaHorticulturae, 111(1):38–43, 2006.

[2] RM Atiyeh, CA Edwards, S Subler, and JD Metzger. Pig manure vermicompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth. Bioresource technology, 78(1):11–20, 2001.

[3] RM Atiyeh, S Lee, CA Edwards, NQ Arancon, and JD Metzger. The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Bioresource technology, 84(1):7–14, 2002.

[4] RM Atiyeh, N Arancon, CA Edwards, and JD Metzger. Influence of earthwormprocessed pig manure on the growth and yield of greenhouse tomatoes. Bioresource Technology, 75(3):175–180, 2000.

[5] Mohammad Akhtar and Abdul Malik. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresource Technology, 74(1):35–47, 2000.

[6] LR Bulluck III and JB Ristaino. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. Phytopathology, 92(2):181–189, 2002.

[7] LE Drinkwater, DK Letourneau, FAHC Workneh, AHC Van Bruggen, and C Shennan. Fundamental differences between conventional and organic tomato agroecosystems in california. Ecological applications, 5(4):1098–1112, 1995.

[8] Cong Tu, Jean B Ristaino, and Shuijin Hu. Soil microbial biomass and activity in organic tomato farming systems: Effects of organic inputs and straw mulching. Soil Biology and Biochemistry, 38(2):247–255, 2006.

[9] David J Walker and M Pilar Bernal. The effects of olive mill waste compost and poultry manure on the availability and plant uptake of nutrients in a highly saline soil. Bioresource technology, 99(2):396–403, 2008.

[10] Rola M Atiyeh, Jorge Domínguez, Scott Subler, and Clive A Edwards. Changes in biochemical properties of cow manure during processing by earthworms (*Eiseniaandrei, bouché*) and the effects on seedling growth. Pedobiologia, 44(6):709–724, 2000.

[11] PA Abbasi, J Al-Dahmani, F Sahin, HAJ Hoitink, and SA Miller. Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. Plant Disease, 86(2):156–161, 2002.

[12] Alam, M.N., Jahan, M.S., Ali, M.K., Islam, M.S. and Khandaker, S.M.A.T. Effect of Vermicompost and NPKS Fertilizers on Growth, Yield and Yield Components of Red Amaranth. Australian Journal of Basic and Applied Sciences, 1, 706-716, 2007.

[13] Joshi, R. and Vig, A.P. Effect of Vermicompost on Growth, Yield and Quality of Tomato (*Solanum lycopersicum L.*). African Journal of Basic Applied Sciences, 2, 117-123, 2010.

[14] Ersahin, Y.S. The Use of Vermicompost Products to Control Plant Diseases and Pests. In: Karaca A. (ed.) Biology of Earthworms, Soil Biology Springer-Verlag Berlin Heidelberg, 2011.

[15] Ibrahim, M.H.,Quaik, S. and Ismail, S.A. Vermicompost, Its Applications and Derivatives. In Katheem KS et al., (eds.) Prospects of Organic Waste Management and the Significance of Earthworms (pp. 201-230), 2016. Springer, Switzerland.

Acknowledgments

We would like to thank the Kumaraguru College of Technology, Coimbatore, Tamil Nadu for providing research facilities.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Transglutaminase Cross-Linked With Casein and Gelatin Coating for Fruits and Vegetables

Sowndharya G[1] S Daniel Robert[2] Lakshmi Anand[3]

[1]Department of Nutrition and Dietetics, Dr. N.G.P Arts and Science College, Coimbatore.

[2]Assistant Professor, Department of Nutrition and Dietetics, Dr. N.G.P Arts and Science College, Coimbatore.

[3]Head Quality Controller, Senthil Papain Food Products (P) Limited, Coimbatore.

India

sowndhi21221@gmail.com, http://http://www.drngpasc.ac.in

Abstract: *There is an increased demand for extended shelf life, fresh and minimally processed fruits and vegetables by the consumers. Hence the purpose of the present study was to examine the effect of the edible coating prepared with transglutaminase enzyme, casein and gelatin on selected fruits and vegetables. In the present study mangos, tomatoes and carrots were used as samples. Three types of samples were prepared. Sample one (S1) was coated with the TGE alone (0.1%), sample two (S2) was coated with TGE (0.1%), casein (2.5%) and gelatin (7.5%) and sample three (S3) was coated with TGE (0.1%) and gelatin (10%). The reference food sample (R) was not coated with any substance. All samples were then stored at room temperature and observed for 15 days. On 15th day it was noticed that S1coated with TGE alone was found to have better weight retention and organoleptic properties than S2, S 3 and R. It is concluded that TGE based edible coating enhances the freshness of fruit and vegetables.*

Key words: *Edible coating, Transglutaminase, Shelf life, Organoleptic, Weight*

I. Introduction

Fruits and vegetables are good sources of antioxidants, phytochemicals, dietary fibers and vitamins, and minerals such as beta-carotene, vitamin C and potassium. Fruits and vegetables provide enormous health benefits. However vegetables and fruits are highly perishable as they contain 80–90% water by weight. Hence fruits and vegetables require coordinated activity by growers, storage operators, processors and retailers to maintain quality and reduce food loss and wastage. The Food and Agriculture Organization estimated that in the year 2009, 32% (weight basis) of all food produced in the world was lost or wasted during the post-harvest period [1]. Fruits and vegetables undergo many damages during the post-harvest period due to microbes, insects, respiration and transpiration. In addition spoilage of fruits and vegetables can be due to external factors such as atmospheric composition of oxygen, carbon-dioxide, ethylene ratios, temperature, and stress factors and the internal factors include the species, cultivar and its growth stage. On the other hand biochemical deterioration of fruits and vegetables such as browning, off flavor and texture breakdown will as well decrease the quality of the fruits and vegetables [2].

The important quality factors of fresh products contributing to the market are texture, color, appearance, flavor, nutritional value and microbial safety [3, 4]. These quality factors are measured by plant variety, ripening stage, and maturity stage, pre-harvest and post-harvest conditions. The post-harvest losses of fruits and vegetables are serious problem because it deteriorates them rapidly during handling, storage and transportation [5]. Edible coatings are thin layers of edible material applied to the product surface in addition to or as a replacement for natural protective waxy coatings and to provide a barrier to moisture, oxygen, and solute movement for the food [6-11]. Edible coatings have a high potential to carry active ingredients such as anti-browning agents, colorants, flavors, nutrients, spices, and antimicrobial compounds that can extend product shelf life and reduce the risk of pathogen growth on food surface [12].

Over the last two decades the development and use of edible coatings to prolong the shelf life and improve the quality of fresh products has received increased attention. However, industrial implementation is still incipient. Enzyme based edible coating technology is an environmentally friendly method. This method does not produce any harm to the mankind. Earlier studies have showed that enzyme based edible coated fruits and vegetables can be safely eaten as they do not produce unfavorable properties to the foods. TGE is an enzyme that catalyzes the formation of isopeptide bonds between proteins. Its cross-linking property is widely used in various processes

which include cheese manufacturing and other dairy products, in meat processing, to produce edible films and to manufacture bakery products. Transglutaminases are enzymes that crosslink proteins together by linking the epsilon amino group of lysine in one protein to the carboxyl group of aspartic or glutamic acids in another protein. Previous studies state that transglutaminase enzyme (TGE) potentially improves the firmness, viscosity, elasticity and water binding capacity of food products [13, 14]. Another study found that TGE enzyme when coated on apples and potatoes, exhibited good water vapor and gas barrier properties and reduced weight loss and microbial growth [15-17].

In this study mangoes, carrots and tomatoes were coated with TGE and TGE cross linked with casein and gelatin and were examined for its effect on visual quality, color, firmness, taste and weight retention.

II. Materials and Methods.

Mangos (S1, S2, S3), tomatoes (S1, S2, S3) and carrots (S1, S2, S3) were used as samples for this study. Sample one (S1) was coated with TGE alone (0.1%), sample two (S2) was coated with TGE (0.1%), casein (2.5%) and gelatin combination (7.5%) and sample three (S3) was coated with TGE (0.1%) and gelatin mixture (10%). The coating was done by immersion method [18]. Table 1 shows the concentrations of different coating materials. The reference food sample (R) (mangos, tomatoes and carrots) was not coated with any substance.

Table: 1 Concentrations of different eible coating materials

Test Samples	Coating material	Percentage of coating material /1000ml water (%)
Sample 1 (S1)	TGE *	0.1
Sample 2 (S2)	TGE Casein Gelatin	0.1 2.5 7.5
Sample 3 (S3)	TGE Gelatin	0.1 10

* Transglutaminase enzyme

All samples were then stored at room temperature and observed for 15 days. In this experiment TGE from microbial source was used. Transglutaminases are involved in protein cross-linking by catalyzing the formation of gamma-glutamyl-lysine isodipeptide bonds between adjacent polypeptides. TGE was cross linked with casein/gelatin blend by means of enzymatic mechanism in order to improve the water-vapor barrier as well as the mechanical properties and resistance to proteolysis [19, 20]. All the samples were then analyzed for their $_{P}H$, total soluble solids and acidity. The $_{P}H$ was recorded using digital $_{P}H$ meter. Total soluble solids were determined by using refractometer and the specific gravity of the different samples are determined by weighing the empty bottle and then with distilled water and finally with the sample.

Titratable acidity of the coating materials were estimated by titrating the coating samples against 0.1N NaOH. Titration was carried out by taking 1ml of the sample and made up to 100 ml using distilled water. This solution was then titrated against 0.1N NaOH. Phenolphthalein was used as an indicator. The end point was the appearance of a pale pink colour. The acidity was calculated by using the given formula.

$$\text{Acidity} = \frac{(\text{ml NaOH used}) \times (0.1\text{ N NaOH}) \times (\text{mile equivalent factor}) \times 100}{\text{Sample (g)}}$$

All the samples were then coated by dipping method and were placed on separate trays for drying. Later these trays containing the respective samples were kept on a table and observed for 15 days, at room temperature. Every 5th day all the samples were tested for its sensory parameters like visual quality, firmness, color and taste. For the sensory evaluation 10 volunteers were recruited. Each volunteer were given a scorecard to rate the test samples and the reference samples, on which a score of 5 was “Excellent”; 4 was “Very good”; 3 was “Good”;

2 was "Fair"; and 1 was "Poor". After taking the initial weight of all the samples, each sample was weighed every 5th day until the end of the storage period ie 15th day using a digital balance.

III. Results and Discussion

Table : 2 Mean scores of the sensory evaluation

	Criteria	Reference	Sample 1 (TGE)	Sample 2 (TGE, casein , gelatin)	Sample 3 (TGE , gelatin)
Mango	Visual Quality	1.01±0.3	4.64±0.3	4.40±0.5	4.52±0.4
	Firmness	1.66±1.0	4.5±0.4	4.28±0.5	4.46±0.5
	Colour	2.76±0.3	4.58±0.4	4.40±0.5	4.52±0.4
	Taste	1.01±0.3	4.52±0.4	3.22±0.6	3.65±0.5
Tomato	Visual Quality	3.15±0.5	4.64±0.3	4.64±0.3	4.58±0.4
	Firmness	3.21±0.5	4.52±0.4	4.21±0.5	4.40±0.5
	Colour	4.35±0.7	4.64±0.3	4.28±0.5	4.52±0.4
	Taste	2.79±0.7	3.89±0.4	2.96±0.5	3.28±0.5
Carrot	Visual Quality	1.01±0.3	3.03±0.6	2.34±0.5	2.40±0.5
	Firmness	1.08±0.3	3.04±0.7	1.86±0.9	2.40±0.5
	Colour	1.15±0.4	2.17±0.8	1.41±0.6	1.90±0.5
	Taste	1.01±0.3	2.97±0.7	1.80±0.8	2.46±0.5

Score Ratings : Excellent-5; Very good-4; Good-3; Fair-2; Poor-1

Table 2 shows the mean scores of the sensory evaluation. Results of the sensory evaluation indicates that S1 of all foods coated with TGE obtained the highest score when compared with all S2 and S3 samples. The reference samples obtained the lowest score. The visual quality, firmness colour and taste of S1/S2/S3 (Mangoes and Tomato) obtained scores between 4 -5 whereas S1/S2/S3 (Carrots) obtained scores between 1- 3. This means that TGE and TGE crosslinked with either caesin / gelatin coatings reacted well with manogoes and tomatoes than with carrots.

Table : 3 Physicochemical characteristics of the test samples

Samples	pH value	TSS* (%)	Specific gravity	Acidity (%)
S1(Mango)	5.53	15.75	1.03	0.17
S1(Tomato)	4.32	5.07	1.21	0.48
S1(Carrot)	6.32	11.10	1.09	0.21
R†(Mango)	8.07	20.10	0.47	0.72
R(Tomato)	5.17	6.32	1.18	0.79
R(Carrot)	6.98	13.15	1.09	0.85

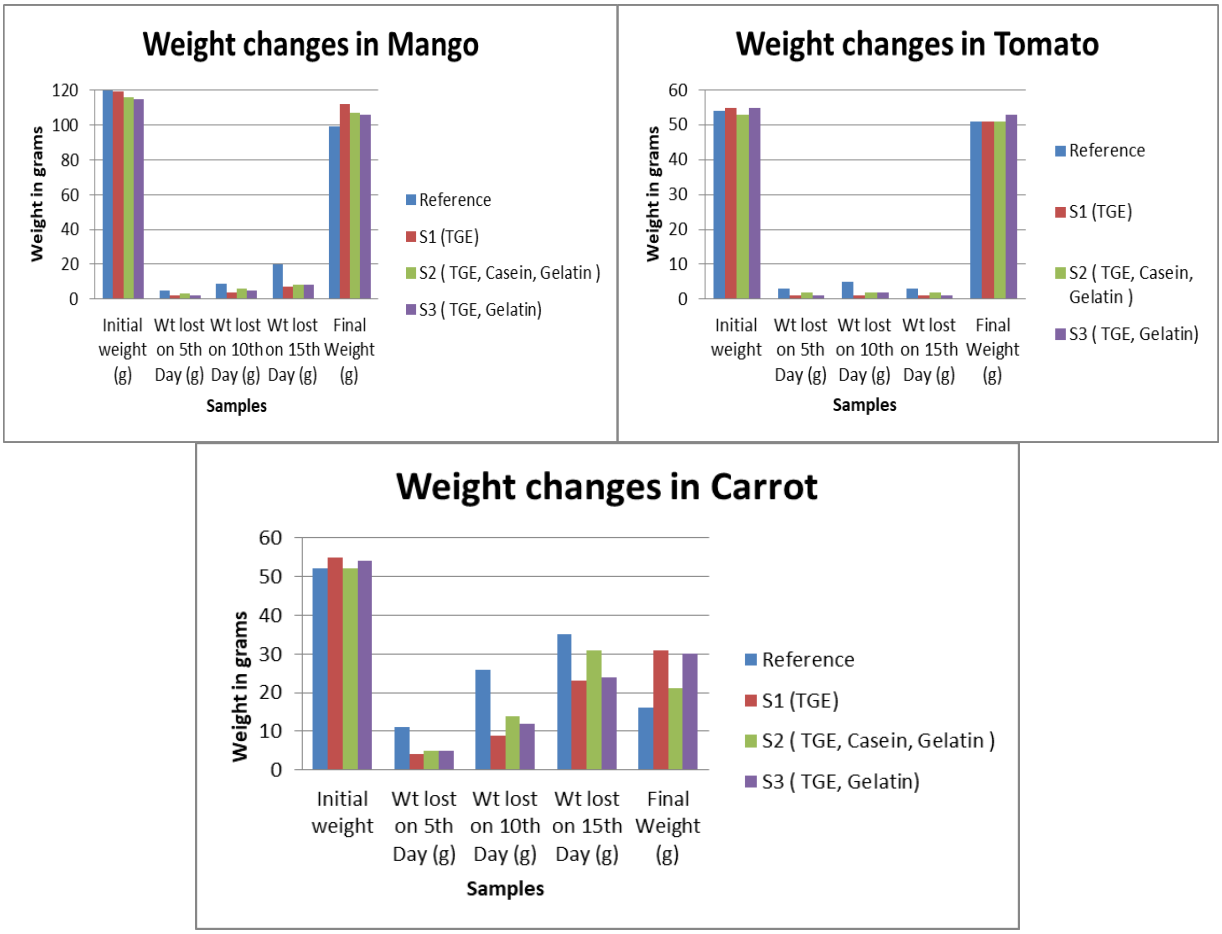
*Total soluble solids

† Reference sample

Table 3 indicates the results of the physicochemical analysis that was done after 15 days. As the sensory evaluation results showed that S1 category of test samples had obtained better results than S2 and S3, the physicochemical properties of S1 category only was analysed. The pH value, total soluble solids ,specific gravity and acidity of S1(Mangoes/ Tomatoes/carrots) was lower than their respective reference samples. This shows that samples coated with TGE has a delayed ripenning process which is essesential to prevent early decaying of the fruits and vegetables and can be due to reduced the activity of some acid-producing bacteria such as Alicyclobacillus acidoterrestris [21].

Weight changes of the samples compared with their respective reference samples is shown in figure 1. It was noted that the final weight of all the test samples coated with TGE were higher when compared with their reference samples. After 15 days , it was noticed that S1 (Mango) lost 6% of its weight and S2 and S3 lost 7% each of its weight whereas reference (Mango) lost 17% of its weight. The weight loss after 15 days in R and S1 (Tomato) was 6% and S2 was 4% and that of S3 was only 3%. In the case of carrot , it was found that after 15 days the percentage of weight loss in R was 68% and that of S1, S2and S3 was 43%, 59% and 45% respectively.

This shows that TGE coating on mangoes and carrots acted as an effective barrier to water vapour . However it was noticed that tomatoes coated with TGE,caesin and gelatin had better weigt retention than TGE alone.The good coating performance could be explained by the formation of transglutaminase film barrier that reduced the interstitial space within the film network and consequently increased film water vapor and gas barrier properties [22].



Fifure : 1 Weight changes of test samples compared with the reference sample

IV. Conclusion

The current study was conducted to determine the physicochemical properties, sensory evaluation, shelf life and weight retention of mangoes, tomatoes and carrots. In conclusion, mangoes, tomatoes and carrots coated with TGE and TGE crosslinked either with casein/gelatin reduced decay and had better sensory and physicochemical properties and lesser weight loss when compared with that of the reference samples. Further research should be done with variety of fruits and vegetables in order to confirm the findings of this study. It is therefore recommended that transglutaminase/ casein/gelatin edible coating can be used to extend the shelf life of mangoes, tomatoes and carrots.

References

[1] Lipinski B, Hanson C, Lomax J, Kitinoja L, Waite R, Searchinger T. 2013 Reducing Food Loss and Waste. Working Paper, Installment 2 of Creating a Sustainable Food Future. Washington, DC, USA.

[2] Harris, L. J., Farber, J. N., Beuchat, L. R., Parish, M. E., Suslow, T. V., Garrett, E. H., and Busta, F. F. (2003). Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and freshcut produce. Compr. Rev. Food Sci. F. (Supplement) 2 (Chapter 3):78–141.http://www.ift.org.

[3] Rouphael, Y., Schwarz, D., Krumbein, A., & Colla, G. (2010). Impact of grafting on product quality of fruit vegetables. Scientia Horticulturae, 127, 172–179. http://dx.doi.org/10.1016/j.scienta.2010.09.001

[4] Zhang, B., Huang, W., Li, J., Zhao, C., Fan, S., Wu, J., & Liu, C. (2014). Principles, developments and applications of computer vision for external quality inspection of fruits and vegetables: A review. Food Research International, 62, 326–343. http://dx.doi.org/10.1016/j.foodres.2014.03.012

[5] Buzby, J. C., Farah-Wells, H., & Hyman, J. (2014). The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. USDAERS Economic Information Bulletin 121.

[6] Smith, S., Geeson, J., and Stow, J. (1987). Production of modified atmospheres in deciduous fruits by the use of films ad coatings. Hortic. Sci. 22:772–776.
[7] Nisperos-Carriedo, M. O., Baldwin, E. A., and Shaw, P. E. (1992b). Development of an edible coating for extending postharvest life of selected fruits and vegetables. Proc. Annual Meeting Fla State Hortic. Soc. 104:122–125.
[8] Guilbert, S., Gontard, N., and Gorris, L. G. M. (1996). Prolongation of the shelf life of perishable food products using biodegradable films and coatings. Lebensm. Wiss. Technol. 29:10–17.
[9] Lerdthanangkul, S. and Krochta, J. M. (1996). Edible coating effects on post harvest quality of green bell peppers. J. Food Sci. 61:176–179.
[10] Avena-Bustillos, R. J., Krochta, J. M., and Saltveit, M. E. (1997). Water vapor resistance of red delicious apples and celery sticks coated with edible caseinate-acetylated monoglyceride films. J. Food Sci. 62:351–354.
[11] McHugh, T. H. and Senesi, E. (2000). Apple wraps: A novel method to improve the quality and extend the shelf life of fresh-cut apples. J. Food Sci. 65:480–485.
[12] Pranoto, Y., Salokhe, V., and Rakshit, K. S. (2005). Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. Food Res. Int. 38:267–272.
[13] Marx CK, Hertel TC, Pietzsch M (2008) Random mutagenesis of a recombinant microbial transglutaminase for the generation of thermostable and heat sensitive variants. J Biotechnol 136:156–162.
[14] Trespalacios P, Pla R (2007) Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. Food Chem 100:264–272.
[15] Porta, R., Mariniello, L., Di Pierro, P., Sorrentino, A., & Giosafatto, C. V. L. (2011). Transglutaminase crosslinked pectin and chitosan-based edible films: A review. Critical Reviews in Food Science and Nutrition, 51, 223e238.
[16] Di Pierro, P., Rossi Marquez, G., Mariniello, L., Sorrentino, A., & Porta, R. (2013). Effect of transglutaminase on the mechanical and barrier properties of whey protein/ pectin films prepared at complexation pH (pHc). Journal of Agricultural and Food Chemistry, 61, 4593e4598.
[17] Shon, J., & Choi, Y.-H. (2011). Effect of edible coatings containing soy protein isolate (SPI) on the browning and moisture content of cut fruit and vegetables. Journal of Applied Biological Chemistry, 54, 190e196.
[18] Okcu, Z., Yavuz, Y. ve Kerse, S. (2018). Edible Film and Coating Applications in Fruits and Vegetables. Alınteri Journal of Agriculture Sciences, 33(2): 221-226. doi: 10.28955/alinterizbd.368362.
[19] Cuq, B., Gontard, N., Guilbert, S. (1998). Proteins as Agricultural Polymers for Packaging Production. Cereal Chemistry, 75 (1), 1–9.
[20] Bourtoom, T. (2009). Protein edible film: Properties enhancement. International Food Research Journal, 16 (1), 1-9.
[21] Jagannath, J. H., Napjappa, C., Das Gupta, D., & Bawa, A. S. (2006). Studies on the stability of an edible film and its use for the preservation of carrots (Daucus carota). International Journal of Food Science and Technology, 41, 498e506.
[22] Di Pierro, P., Rossi Marquez, G., Mariniello, L., Sorrentino, A., & Porta, R. (2013). Effect of transglutaminase on the mechanical and barrier properties of whey protein/ pectin films prepared at complexation pH (pHc). Journal of Agricultural and Food Chemistry, 61, 4593e4598.

Acknowledgments

The authors wish to thank Mr. Mohankumar S, Plant Manager, Senthil Papain and Food Products (P) Ltd., Coimbatore, for permitting us to conduct this study at his institution. We are grateful to all the participants who prticipated in the sensory evaluation of the samples.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Effect of drying methods and infusion of functional ingredients on the quality characteristics of coconut based snack

[1]Dr.M. Sivasakthi, [2]Dr.Narayanasamy Sangeetha,

[1]Assistant Professor, Department of Nutrition and Dietetics, Dr.N.G.P. Arts and Science College, Coimbatore-641 048, India.

[2]Assistant Professor, Department of Food Science and Technology, Pondicherry University, Puducherry, India

Email ID: sivasakthiphd2015@gmail.com, praba_sangeen@yahoo.com

Abstract: *Natural versatile ingredients like beet root, carrot, ginger and mint have been chosen for the recent study in terms of their effective bioactive composition as osmotic infusions in the form of filtrates. Sliced matured coconut samples of thickness 0.8±0.1mm were subjected to osmotic dehydration for a period from 0 min to 720 min at room temperature and the slices were dried in a hot-air oven at temperature 45-60°C for about 6-7 hours and freeze drying at temperature (-40 to 30°C) for a duration of 14-16 hours. Osmotic medium without the infusion of filtrates of functional ingredients serves as the control. The dehydrated samples were packed in Aluminium foil laminated LDPE pouches with infusion of 100% nitrogen gas composition and stored at ambient temperature till analyzing the phytochemical characteristics. The phytochemicals detected in the sample impregnated with filtrate of Mentha piperita were caffeic acid, rosmarinic acid, hesperedin, apigenin and luteolin. The samples impregnated with Zingiber officinale were possessing phytonutrients like myricetin (T_2-43.1; T_1-38), apigenin (T_2 -28.8; T_1-21.6), caffeic acid (T_2 -20.9; T1-18.4), curcumin (T_2-7.1; T_1-6.5), quercetin (T_2-2.9; T_1-2.8), cineole (T_2-2.0; T_1-1.89) and gingerol (T_2-0.93; T_1-0.78). The presence of phytochemicals in the sample impregnated with Beta vulgaris filtrate were found to be betanin, isobetanin, betacyanin, betaxanthin and vulgaxanthin. The order of presence of phytochemicals in the sample impregnated with Daucus carota filtrate were kaempferol (T_2-12.6; T_1-11.6), luteolin (T_2-4.1; T_1-3.2), quercetin (T_2-3.4; T_1-3.3), lutein (T_2-3.6; T_1-3.2) and zeaxanthin (T_2-0.07; T_1-0.06). Freeze dried samples were found to contain comparatively greater pack of phytochemicals than hot air oven dried samples due to its conservative effects which was observed significant at $p \leq 0.05$ level when compared with control.*

Keywords: *Osmotic dehydration, coconuts, phytonutrients, impregnation, antioxidants*

I. Introduction

Consumption of nuts is inversely related to the prevalence of cardiovascular diseases, cancer, diabetes and chronic diseases. Boston [1] suggested that diets with good fats are required in small portions for a healthy well-being. The healthy fats include extra-virgin olive oil, coconut oil, flax seed oil etc., are generally obtained from plant sources such as nuts, oil seeds, avocados and coconuts. Among nuts, coconuts (*Cocus nucifera*) play a foremost role in our daily life, possess traditional distinctiveness, acts as a functional food and also own biologically active components thereby enhancing health and well being [2]. Coconuts provide a nutrition source of meat, juice, milk, oil and rich in fibre, vitamins and minerals which has fed and nourished populations around the world for many generations. It is classified as a functional food because it provides many health benefits beyond its basic nutritional content.

Most of the plant foods are rich in micronutrients, functional components and bioactive phytochemicals etc., which are mandatory for the physical and mental health of mankind [3]. The major source of nutraceuticals and functional foods are fruits, vegetables, tubers, roots, green leafy vegetables, nuts & oils seeds etc., with phenolics, carotenoids, vitamins and minerals, possessing antioxidant properties. FAO/WHO (2002) reported that, daily consumption of fruits and vegetables (<400g/d) resulted in reduction of ischemic heart diseases, gastro-intestinal cancers, stroke and many chronic ailments.

Osmotic dehydration is a pre-treatment given to solid foods used to improve the nutritional, sensorial and functional properties of food without changing its integrity [4]. It partially dehydrates solid foods and instantaneously impregnates it with solutes that modify its functional, structural and nutritional properties [5]. Various osmotic agents such as fructose, glucose, corn syrup, sodium chloride, sucrose and other combinations

have been used for osmotic dehydration. The pre-treatment osmotic dehydration not only enables water removal but also improves the taste of the final product [6].

Incorporation of physiologically active compounds have been taken place during osmotic dehydration such as minerals, phenolic compounds and vitamins from plant produce into food tissue without abolishing the initial food matrix have already been attempted by many researchers [7]. The physiologically active components have been observed in plant foods like greens leafy vegetables, roots and tubers are essential for a healthy diet in reducing the risk of major degenerative diseases and various ailments [8].

Green leafy vegetables are the cheapest and locally available micronutrient and antioxidant rich sources. *Mentha piperita* (Peppermint) called as hybrid mint has been used as an ancient folk popular remedy. It acts as an excellent gastric stimulant as it has pleasant taste [9]. Mint finds culinary application in making jellies, beverages, syrups, candies and often used in the preparation of ice cream, flavoring in tea, chewing gum and tooth paste [10].

Ginger (*Zingiber officinale*) is one of the most widely used venerable medicinal root herb consists of several bioactive compounds like gingerols, shogaols and curcumin with health promoting characteristics due to its greatest antioxidant activity [11]. The red beet roots (*Beta vulgaris*) and carrots (*Daucus carota*) are considered as a wonderful food ingredient and colorant, accepted universally due to the presence of red violet betacyanins, yellow betaxanthins and orange carotenoids with tremendous antioxidant powers and used in preparation of various dairy products such as sherbet, ice cream, yogurt, dry soft drink mixes, soups, confectionary items, tomato and bacon products.

The incorporation of these plant extractions, herbal infusions into the basic foods have been concentrated as pre-treatments in the emerging food processing sectors to obtain healthy nutritious foods with enriched nutraceuticals and functional components which are beneficial in general. The drying methods are followed after pre-treatments of various foods using osmotic agents, blanching methods etc., to obtain dehydrated foods with nutritional retention without any enzymatic deterioration with good shelf-life.

Among the various drying methods, hot air oven drying is one of the most common methods used to preserve fruits and vegetables. Freeze drying gives high-quality products after dehydration among various drying techniques. Moreover, freeze drying preserves minerals, vitamins, original flavor and aroma of the food products.

In the emerging trend, consumer with increased awareness on health, consumption of potentially healthy foods has been arrived. In addition to that, consumer view on saving time and money, ready-to-eat foods have been popularized much in the market and are being developed with the assistance of dehydration technologies for better quality products with extended shelf-life. Coconuts are being consumed by people in various forms as ready-to-eat products or as an ingredient in many food products such as fruit ice cream, pie filling and other intermediate moisture food (IMF) products are obtained by osmotic dehydration with assisted drying methods.

The present study emphasis on osmotic dehydration of coconut slices with the impregnation of functional ingredients such as *Beta vulgaris*, *Daucus carota*, *Zingiber officinale* and *Mentha piperita* which holds immense nutritional and antioxidant properties prior to osmotic dehydration by using its filtrates as osmotic medium followed by assisted drying methods namely hot air oven drying and freeze drying. The shelf-life of the product is highly improved due to efficient moisture removal with lowest water activity at which physico-chemical, nutritional and microbial deteriorations are retarded. With this as a background, the study emphasis on the effect of drying methods and impregnation of filtrate of functional ingredients on the phytochemical characteristics of the ready-to-eat coconut based snack.

II. Methodology

The methodology pertaining to the study entitled, “Effect of functional ingredient infusions on the quality characteristic of coconut based snack” is discussed under the following heads: Selection and pre-processing of raw materials, Process involved in the formulation of ready-to-eat coconut based snack, Finalised process parameters, Quality analysis of the ready-to-eat coconut based snack and Statistical interpretation of the data.

Selection and pre-processing of raw materials:

Fresh and matured good quality coconuts were purchased from Pazhamudir Nilayam, Puducherry prior to each set of experiments for osmotic dehydration. The coconuts were processed which involves selection of matured coconut of 10-12 months old, followed by removal of husk, breaking into 2 halves, separation of endosperm from shell and removal of testa with the help of a knife. Finally the processed coconut pieces were standardised to obtain optimum thickness of slices using slicers.

The effect of osmotic dehydration depends on the thickness of the food materials such as fruits and vegetables. Torreggiani (1993) reported that the rate of osmotic dehydration decreases as the thickness of the slices increases which leads to difficulty of penetration of the solute inside the food materials from the solution. The pre-processed coconut pieces were sliced into varying thickness from 0.6 mm to 1.0 mm using screw gauge. The sliced coconut pieces were kept in vessel containing water before commencement of experiments to avoid microbial contamination. The sliced coconuts were subjected to steam blanching at 80 to 90°C for 5 minutes duration to inactivate enzymatic actions. Further the pre-processed coconut slices were subjected to osmotic dehydration using sugar as the main osmotic agent, as it is easily available and cost-effective with increased diffusion rate possessing good mass transfer characteristics as reported by Lerici *et al*., (1985).

The experiments were carried out using sugar as a major component in the osmotic medium infused along with filtrates of functional ingredients namely *Beta vulgaris*, *Daucus carota*, *Zingiber officinale* and *Mentha piperita* obtained from Pazhamudir Nilayam, Puducherry. The functional ingredients were subjected to pre-processing steps like washing, peeling, grating, grinding into fine paste and finally filtered to obtain filtrates using 0.08 mm sieve.

The filtrates of functional ingredients have been taken in the range from every 5% addition upto 100% and impregnated with sugar as the osmotic medium (100%). The thickness of coconut slices, sweetness of sugar and concentration of filtrates were taken in three ratios such as 1:1:1, 1:2:2 and 1:3:3 for standardisation purpose.

Process involved in the formulation of ready-to-eat coconut based snack:

The osmotic dehydration of the coconut slices was carried out with or without the impregnation of filtrates of functional ingredients for a period from 0 min to 720 min. The duration of the osmotic dehydration was finalised based on the physico-chemical and physical properties of the samples such as maximum reduction in brix, weight of the osmotic dehydrated slices and the degree of impregnation of functional characteristics.

Dehydration is one of the preservation process of food materials for indefinite periods by effective removal of moisture, thereby growth of micro organisms are prevented. Dehydration is considered as best among the oldest food preservation methods, most commonly used to extend the shelf-life of the food products. The pre-processed foods were dehydrated to remove the moisture content of the foods especially fruits and vegetablesto attain desirable moisture content, in spite of the considerable reduction in quality of foods. It results in significant decrease in volume and mass, minimise packaging, storage and transportation costs and makes convenient storage under room temperatures. Hot air drying and freeze-drying were adopted in the present study.

The coconut samples were dehydrated in hot air oven dryer by allowing hot air which flushed inside the chamber and the food gets exposed to hot air, the desired moisture content achieved due to removal of moisture up to 2-3%. The desired moisture content was attained with standardised temperature 45-50°C and time 6-7 hours which was dependent on the nature of the product. Whereas most of the fruits and vegetables are dehydrated in the temperature range 50-60°C to get dried foods without much degradation reactions [12].

Freeze drying is one of the sophisticated methods of drying foods used extensively in industrial pharmacological preparations and manufacture of functional foods especially to increase the shelf-life of the products with meagre chemical deteriorations during storage time. In spite of its highest instrumentation cost, finds wider application in improving the mass transfer kinetics of foods such as higher gain of solutes in shortest times, better conservational effects on color, taste and texture of the products. The physico-chemical, nutritional and sensory traits of the food products affected with respect to the type of packaging materials used during storage.

The coconut samples were dehydrated using freeze dryer (Model: Del Vac) by spreading the samples uniformly in the trays and kept inside the chamber where all the valves are closed and checked whether the instrument working under vacuum condition. The low temperature -40°C to 30°C was applied over a period of time 14-16 hours, which found to preserve the color and texture of the samples through thermostat adjustment. The dehydrated coconut samples obtained from both the drying methods were packed in Aluminium Foil Laminated LDPE pouches with the infusion of 100% nitrogen gas composition and stored at ambient temperature (35°C) for further analysis. The process involved in the formulation of ready-to-eat coconut based snack is given in Plate1.

Finalised process parameters:

After several permutations and combinations, the process parameters were finalised from the above methodology and represented in the ratio 1:2:2. The thickness of coconut slices, sweetness of sugar and concentration of filtrates from the functional ingredients were standardized and arrived with the above ratio for further processing.The sugar infusion and impregnation of filtrates of functional ingredients at 100% concentration gave the coconut based snack with most acceptable taste, texture, crispiness and sweetness,

henceforth this proportion was selected finally.Whereasthe osmotic dehydration of the coconut slices without the impregnation of filtrates of functional ingredients serves as the control.

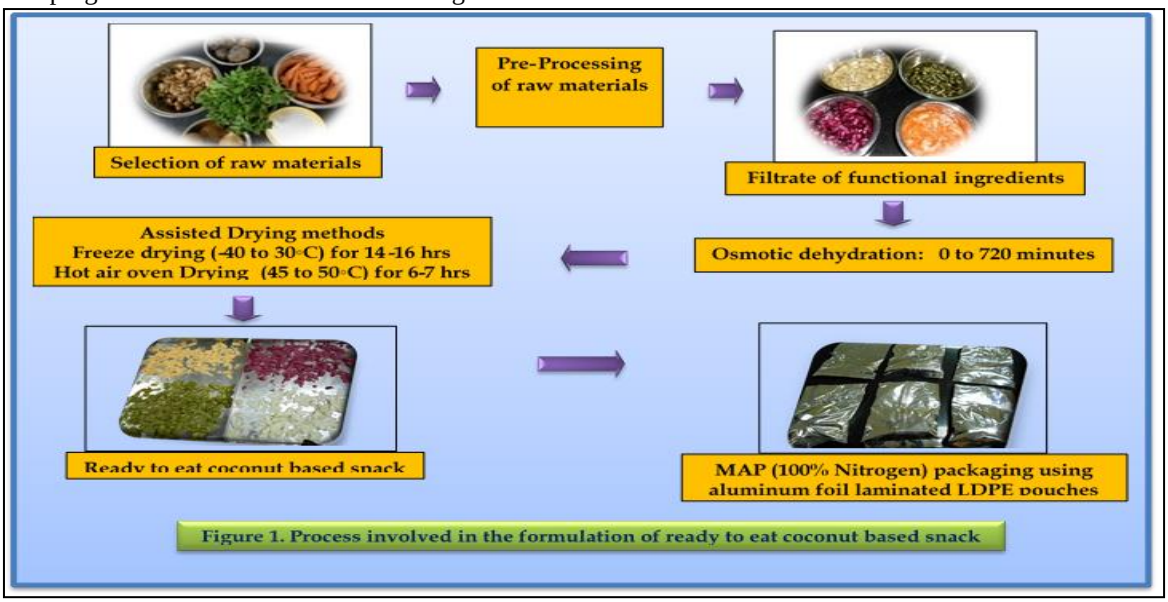


Plate 1. Process involved in the formulation of ready-to-eat coconut based snack

Analysis of the phytochemical composition of the ready to eat coconut based snack:

The bio active components possess a number of biological effects. Tannin content: Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungsto molybdic acid by tannin like compounds in alkaline solution [13]. Steroid (mg) content: Gas chromatography was used to analyse the steroid content in the sample. Alkaloid (mg) content:The determination of alkaloids by HPLC consisted of mobile phase by dissolving 9.93 g of monobasic potassium phosphate in 730 ml of distilled water. [14]. Saponin (mg) content:Saponins were extracted according to the method of [15]. Total flavonoid (mg) content:Flavonoids were extracted according to the methods of [16]. Total phenol (mg GAE) content:The TPP content was determined according tothe procedure of Folin-Ciocalteu method with slight modifications. Betalain (mg) content:The samples (500 mg) were analysed using HPLC [14]. Curcuminoid (mg) content:The curcuminoids in the samples were estimated based on ASTA procedure [17]. β-carotene (µg) content: The filtered aliquot of the extract was subject to HPLC analysis. Gingerol (mg) content:The determination of 6-gingerol content was made according to the procedure of Pharmacopeial forum.

Statistical analysis of the data: Data analysed using statistical tool 'student t test' of SPSS version-18.

III. Results and discussion

a) Phenolic compounds (mg) of the ready-to-eat coconut based snack infused with *Mentha piperita*

Table 1. Phenolic compounds (mg) of the ready-to-eat coconut based snack infused with *Mentha piperita*

Phenolic compounds	Samples	Osmotic medium	T_1	% gain	T_2	% gain	*p-value*
Caffeic acids (mg)	Control	ND	ND	ND	ND	ND	-
	Sample A	21.4	9.3±0.05	43.45	11.8±0.20	55.14	0.000*
Rosmarinic acid (mg)	Control	ND	ND	ND	ND	ND	-
	Sample A	978.4	393.2±0.02	40.19	421±2.0	43.03	0.000*
Hesperedin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample A	10.98	4.98±0.34	45.35	7.43±0.09	67.66	0.000*
Apigenin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample A	9.6	5.43±0.18	56.56	5.8±0.39	60.42	0.001*
Luteolin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample A	13.7	7.86±0.50	57.37	10.67±0.2	77.88	0.001*

All values are means of triplicate determinations± standard deviation (SD), T_1- Hot Air Oven Drying, T_2- Freeze Drying *Mentha piperita* filtrate impregnated coconut based snack, ND-Not Detected, NS- Not Significant,*Significant at 5% level

Among dark green leafy vegetables, *Mentha piperita* (Peppermint), has been used as an ancient folk popular remedy for various ailments and it acts as an effective anti-fungal, anti-viral, anti-microbial and insecticidal, antioxidant and anti-amoebic agents. The major phytochemical present in *Mentha piperita* is menthol found as a

greatest amount in oil of the leaves. The peppermint composed of other phytochemicals are menthol, menthyl acetate and also phenolic compounds which are present in wider sense with potential health outcomes.

The phytochemicals present in the sample impregnated with filtrate of *Mentha piperita* are caffeic acid, rosmaric acid, hesperedin, apigenin and luteolin. Rosmaric acid is abundantly present in filtrate of *Mentha piperita*. The highest concentration of rosmaric acid was present in the sample subjected to freeze drying was 421 and hot air oven drying was 393.2. Among the phytochemical present, least concentration of hesperidin (T_2-7.43 & T_1-4.98) was present, which was statisticaly significant at $p \leq 0.05$ when compared with two different drying methods. The slightly decreased values in hot air oven dried sample was due to the wet thermal processing which disintegrates the cell integrity leading to migration of components and chemical reactions induced by light, enzymes and oxygen.

In the present study, the phenolic compounds were found higher in T_2 samples than T_1 samples which could be due to heat treatments related to the results of [9] who reported that prolonged drying resulted in oxidation of phenolic compound by enzyme phenol oxidases in yacon chips and exhibited a lowest antioxidant activity at 40-60°C.

a) **Phenolic Compounds and Cineole (mg) of the ready-to-eat coconut based snack infused with *Zingiber Officinale***

Figure 1 reveals the phenolic and volatile compounds of sample impregnated with filtrate of *Zingiber officinale*.The order of presence of phytochemicals in the samples impregnated with *Zingiber officinale* were

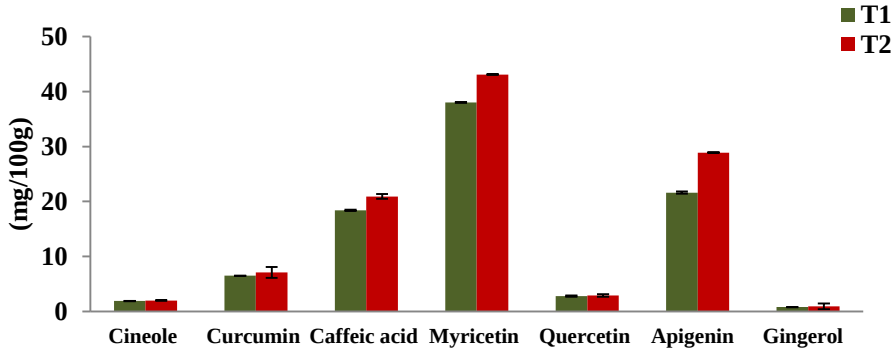


Figure 1. Phenolic compounds and cineole (mg) of the ready-to-eat coconut based snack infused with *Zingiber officinale*

myricetin (T_2-43.1, T_1-38), apigenin (T_2-28.8, T_1-21.6), caffeic acid (T_2-20.9, T_1-18.4), curcumin (T_2-7.1, T_1-6.5), quercetin (T_2-2.9, T_1-2.8), cineole (T_2-2.0, T_1-1.89) and gingerol (T_2-0.93, T_1-0.78). On comparison, the freeze dried samples possessed slightly increased values than hot air oven dried samples due to the eservation effect of freeze drying. The finding of the present study was in par with [9] who reported that the percent degradation of phenolic flavonoids like quercetin was estimated to be 73.5% by freeze drying and 97% by oven drying methods. Generally flavonoids destruction by air-oven drying was observed around temperature 45ºC from 76 to 97% and by freeze drying from 31 to 73%. The flavonoid destruction is mainly due to harsh drying effects and wet thermal processing which disintegrates cell integrity leading to migration of components and chemical reactions induced by light, enzymes and oxygen.

It is reported that decrease in gingerol content due to drying which increases the terpene hydrocarbons and conversion of monoterpenes alcohols to acetates. The oily phenyl propanoid derivative gingerol was not affected by freeze drying methods [11]

b) **Flavonoids and carotenoids (mg) of the ready-to-eat coconut based snack infused with *Daucus carota* in sample C**

Figure 2 details on the flavonoids and carotenoids of sample impregnated with filtrate of *Daucus carota*.Carrots possess excellent nutritional qualities with therapeutic significance. The precursor to vitamin A, a class of phytochemicals with higher amount of carotenoids is present in carrots which are good for eyes. Orange carrots contain greatest α and β-carotenes. The order of presence of phytochemicals in the sample impregnated with *Daucus carota* filtrate were kaempferol (T_2-12.6; T_1-11.6), luteolin (T_2-4.1; T_1-3.2), quercetin (T_2-3.4; T_1-3.3), lutein (T_2 -3.6; T_1 -3.00), zeaxanthin (T_2-0.07; T_1-0.06). Freeze dried sample was found to contain comparatively higher values than hot air oven dried sample. The carotenoids and beta carotenes in foods gets depleted when exposed to oxygen and temperature beyond 57 ºC with approximately 54 % of loss.

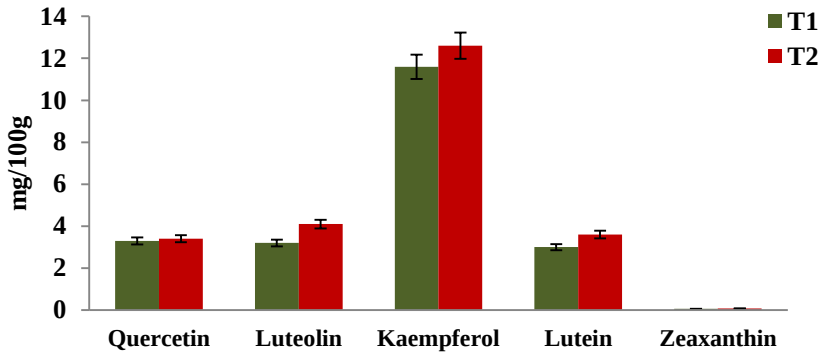


Figure 2. Flavonoids and carotenoids (mg) of the ready-to-eat coconut based snack infused with *Daucus carota*

c) **Phenolic compounds (mg) of the ready-to-eat coconut based snack infused with *Beta vulgaris***

Table 2 represents the phenolic compounds of sample impregnated with filtrate of *Beta vulgaris.*

The plant phenolics, the antioxidant components in fruits, vegetables and roots are suggested as the protective foods considered more advantageous over chronic diseases [1]-[3]. reported that beetroots are the potent antioxidant rich vegetables with greatest presence of phenolic derivatives. The red beet root concentrate is considered as a wonderful food ingredient and colorant accepted universally (red violet betacyanins and yellow betaxanthins).

The presence of phytochemicals in the sample impregnated with *Beta vulgaris* filtrate were found to be betanin, isobetanin, betacyanin, betaxanthin and vulgaxanthin. The highest amount of the phytonutrient was observed in freeze dried sample and hot air oven dried samples was betanin (T_2-190 mg, T_1-153 mg) respectively. Whereas other bio-active components were found relatively in lesser amounts than betanin content. It is reported that betacyanin and betaxanthin degrades at 70 to 90ºC. The betanin degradation is a result of hydrolytic cleavage.

Table 2. Phenolic compounds (mg) of the ready-to-eat coconut based snack infused with *Beta vulgaris*

Phenolic compounds	Samples	Osmotic medium	T_1	% gain	T_2	% gain	*p-value*
Betanin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample D	505	153±2.7	30.3	190±4.70	37.62	0.000*
Isobetanin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample D	4.3	1.5±0.07	34.88	1.9±0.58	44.18	0.000*
Betacyanin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample D	330	95±8.60	28.78	117±7.46	35.45	0.000*
Betaxanthin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample D	355	115.4±2.0	32.39	160.6±1.3	45.24	0.001*
Vulga-xanthin (mg)	Control	ND	ND	ND	ND	ND	-
	Sample D	420	136.3±1.0	32.3	169.21±0.3	40.28	0.001*

All values are means of triplicate determinations± standard deviation (SD), T_1- Hot Air Oven Drying, T_2- Freeze Drying, *Daucus carota* filtrate impregnated coconut based snack, *Significant at 5% level

IV.Conclusion

The infusion of functional filtrate ingredients showed a significant increment in the bioactive components in the coconut based snack, the assistance of drying methods had contributed a good quality of snack for better shelf life extension of the snack. The snack could be supplemented for all age group of people which have therapeutic roles.

V. References

[1] Boston, MA. (2005). Eating nuts promotes cardiovascular health, Harvard Health Publications, Harvard Medical School, www.health.harvard.edu/press_relases/ benefits_eatings_nuts.

[2] Kabara, J. J. (1984). Antimicrobial agents derived from fatty acids. *Journal of the American Oil Chemists' Society*, 61(2): 397-403.

[3] Madrau, M. A., Piscopo, A., Sanguinetti, A. M., Del Caro, A., Poiana, M., Romeo, F. V., & Piga, A. (2009). Effect of drying temperature on polyphenolic content and antioxidant activity of apricots. *European Food Research and Technology*, 228(3): 441-448.

[4] Torreggiani, D. (1993). Osmotic dehydration in fruit and vegetable processing. *Food Research International*, 26(1): 59-68.

[5] Alzamora, S. M., Salvatori, D., Tapia, M. S., López-Malo, A., Welti-Chanes, J., & Fito, P. (2005). Novel functional foods from vegetable matrices impregnated with biologically active compounds. *Journal of Food Engineering*, 67(1): 205-214.

[6] Fito, P., Chiralt, A., Betoret, N., Gras, M., Cháfer, M., Martınez-Monzó, J., & Vidal, D. (2001). Vacuum impregnation and osmotic dehydration in matrix engineering: Application in functional fresh food development. *Journal of Food Engineering*, 49(2): 175-183.

[7] Torringa, E., Esveld, E., Scheewe, I., van den Berg, R., & Bartels, P. (2001). Osmotic dehydration as a pre-treatment before combined microwave-hot-air drying of mushrooms. *Journal of Food Engineering*, 49(2):185-191.

[8] McKay, D. L., & Blumberg, J. B. (2006). A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). *Phytotherapy Research*, 20(8): 619-633.

[9] Kiremire, B. T., Musinguzi, E., Kikafunda, J. K., & Lukwago, F. B. (2010). Effects of vegetable drying techniques on nutrient content: A case study of south-western Uganda. *African Journal of Food, Agriculture, Nutrition and Development*, 10(5): 2587-2600.

[10] Gracindo, L. A. M. B., Grisi, M. C. M., Silva, D. B., Alves, R. B. N., Bizzo, H. R., & Vieira, R. F. (2006). Chemical characterization of mint (Mentha spp.) germplasm at Federal District, Brazil. *Review of Plant Medicine*, 8: 5-9.

[11] Puengphian, C., & Sirichote, A. (2008). (6)-gingerol content and bioactive properties of ginger (Zingiber officinale Roscoe) extracts from supercritical CO2 extraction. *Asian Journal of Food and Agro-Industry*, 1(1): 29-36.

[12] Demirel, D., & Turhan, M. (2003). Air-drying behavior of Dwarf Cavendish and Gros Michel banana slices. *Journal of Food Engineering*, 59(1): 1-11.

[13] Ranganna S. (2000). Vitamins In *Hand book of Analysis and Quality Control for Fruit and Vegetable Products*. (2nd) edition. Tata McGraw Hill publishing Co.ltd, New Delhi, 113-117.

[14] Lawrence Evans Ph.D., Senior Scientific Associate Expert Committee (2007), (DSN) *Dietary Supplements*: Non-Botanicals, USP27–NF22, P. 2063, Pharmacopeia Forum.

[15] Huhman, D. V., Berhow, M. A., & Sumner, L. W. (2005). Quantification of saponins in aerial and subterranean tissues of Medicago truncatula. *Journal of Agricultural and Food Chemistry*, 53(6): 1914-1920.

[16] Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantitative analysis of flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agricultural and Food Chemistry*, 45: 590-595.

[17] ASTA: American Spice Trade Association Method (1985). Determination of colour in turmeric. *In Official analytical methods of the American Spice Trade Association*. NJ: Englewood Cli.

VI. Acknowledgments

The authors thank the Department of Food Science and Technology of Pondicherry University for the successful completion of the research work and UGC, New Delhi for having rendered JRF fellowship and financial support to conduct the same.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Optimization of Process Parameters for The Osmotic Dehydration of Banana in Sugar Solution

P.Manivannan*, G.Kalaiarasi , M.Rajasimman

Department of Chemical Engineering, Annamalai University, Annamalainagar-608002,

**manivannanpetchi@yahoo.com (Corresponding Author)*

Abstract*: In this work, osmotic dehydration of banana was carried out using sugar solution. Response surface methodology was used to determine the optimum processing conditions that yield maximum water loss and weight reduction and minimum solid gain during osmotic dehydration of banana in sugar solution. The experiments were conducted according to Central Composite Design (CCD). The independent process variables for osmotic dehydration process were temperature (25 - 45℃), processing time (30 -150 minutes), sugar concentration (20 - 60% w/w) and solution to sample to ratio (5:1 - 25:1 w/w).The osmotic dehydration process was optimized for water loss, solute gain, and weight reduction. The optimum conditions were found to be: temperature - 37℃, immersion time – 100 min, sugar concentration - 45% w/w and solution to sample ratio 14.5:1 w/w. At this optimum point, water loss, solid gain and weight reduction were found to be 34.2 (g/100 g fresh sample), 9.2 (g/100 g fresh sample) and 25(g/100 g fresh sample) respectively.*

Keywords: *Optimization, Osmotic dehydration, Banana, Weight loss, Solid gain*.

I. Introduction

Banana is the common name for herbaceous plants of the genus *Musa*. Bananas come in a variety of sizes and colors when ripe, including yellow, purple, and red. The botanical name of red banana is Musa acuminata. It is a plant from Ziziberaceae family. In popular culture and commerce, "banana" usually refers to soft, sweet "dessert" bananas. They are native to tropical Southeast Asia. Today, they are cultivated throughout the tropics.They are grown in at least 107 countries and primarily for their fruit, and to a lesser extent to make fiber and as ornamental plants.

Individual banana fruits (commonly known as a banana or 'finger') average 125 grams, of which approximately 75% is water and 25% dry matter. Bananas are rich in vitamin C. They are good source of Calcium, Magnesium and Potassium. Bananas are naturally slightly radioactive, more so than most other fruits, because of their high potassium content and the small amounts of the isotope potassium-40 found in naturally occurring potassium. Proponents of nuclear power sometimes refer to the banana equivalent dose of radiation to support their arguments. Bananas are eaten deep fried, baked in their skin in a split bamboo, or steamed in glutinous rice wrapped in a banana leaf. Bananas can be made into jam. Banana pancakes are popular amongst backpackers and other travellers in South Asia and Southeast Asia. Banana chips are a snack produced from sliced dehydrated or fried banana or plantain, which have a dark brown color and an intense banana taste. Dried bananas are also ground to make banana flour. Extracting juice is difficult, because when a banana is compressed, it simply turns to pulp. Banana fried with batter is a popular dessert in Malaysia, Singapore, and Indonesia. A similar dish is known in the United States as banana fritters.

Osmotic dehydration is widely used to remove water from fruits and vegetables by immersion in aqueous solution of sugars and/or sugars at high concentration. Two major simultaneous counter-current flows occur during osmotic dehydration: water flow out of the food into the solution and transfer of solute from the solution into the food [1]. Osmotic dehydration is used as a pretreatment for many processes used to improve nutritional, sensorial and functional properties of food without changing its integrity [2]. It generally precedes process such as freezing, freeze-drying, vacuum drying or air drying. It also increases sugar to acid ratio, and improves texture and stability of pigments during dehydration and storage [3]. It is effective around ambient temperatures, so heat damage to texture; colour and flavour can be minimized. The other major application is to reduce the water activity of food materials so that microbial growth will be inhibited. Since most food materials contain large amount of water, it is cost intensive to ship, pack and store [4]. Osmotic dehydration is acknowledged to be an energy efficient method of partial dehydration, since there is no need for a phase change. There are numerous studies on osmotic dehydration of vegetables [5]-[10] and some work has been carried out on optimization of vegetables by RSM method [11]-[15]. However, no information is available on the statistical

modeling of radish drying by osmotic dehydration. The present study aims at (i) modeling the influence of the temperature, processing time, sugar concentration, and solution to sample ratio on changes in mass of the radish and (ii) to determine the optimal conditions of temperature, processing time, sugar concentration, and solution to sample ratio for the water loss and solute gain in radish during osmotic dehydration using response surface methodology.

II. Materials and Methods

Fresh bananas purchased locally, were thoroughly washed with water to remove adhering soil and other debris. Then, they were cut into circular pieces of 30 mm diameter and 3 mm thickness. The average moisture content of the banana was found to be 76% on a wet basis. Sugar, the osmotic agent, was purchased from a local supermarket. The osmotic solution is prepared by mixing the sugar with proper amount of pure water.

Response surface methodology (RSM) was used to estimate the main effects of osmotic dehydration process on water loss (WL) and solid gain (SG) in banana slices. A central composite design was used with independent process variables viz. temperature (25 – 45℃), processing time (30 –150 minutes), sugar concentrations (20 – 60% w/w) and solution to sample to ratio (5:1 – 25:1) (Table 1). For the generated 30 experiments by central composite design, RSM is applied to the experimental data using Design Expert 7.1.5.

Table 1 Levels of different process variables in coded and un-coded form for the osmotic dehydration of bananas using sugar solution

Independent variable	Range and levels				
	-2	-1	0	+1	+2
Temperature (A, ºC)	25	30	35	40	45
Processing time (B, min)	30	60	90	120	150
Sugar concentration (C, %w/w)	20	30	40	50	60
Solution to sample ratio (D, w/w)	5:1	10:1	15:1	20:1	25:1

The osmotic dehydration is conducted in a 500 ml Erlenmeyer flask, which is placed in a thermostatically controlled water bath shaker. Bananas were cut into small pieces and weighed and then placed into dehydrating vessel containing the osmotic agent solution of varying concentrations. The vessel was placed in the water bath at a constant temperature. The solution to sample ratio was varied between 5:1 and 25:1. At each sampling time (0.5–2.5 hrs), the sample slices were taken out and then gently blotted with adsorbent paper and weighed. The effect of temperature was also investigated and the experiments were conducted between temperatures of 25 - 45ºC. The average moisture and dry matter content of the samples are determined by tray drying at 70ºC. The moisture content of banana was found to be 76%. In each of the experiments fresh osmotic syrup was used. All the experiments were done in triplicate and the average value was taken for calculations. Agitation is necessary to improve mass transfer, maintain uniform concentration, temperature profile and prevent the formation of a dilute solution film around the samples. For each experiment the agitation speed is maintained constant at 200 rpm.

In order to follow adequately the osmotic dehydration kinetics, individual analysis for each sample was carried out and from which, weight reduction (WR), solid gain (SG) and water loss (WL) data were obtained, according to the expressions

$$WR = \left(\frac{M_o - M}{M_o} \right) \quad \text{....1}$$

$$SG = \left(\frac{m - m_o}{M_o} \right) \quad \text{.....2}$$

$$WL = WR + SG \quad \text{.....3}$$

where, M_0 - initial mass of sample (g), M - mass of sample after dehydration (g), m_0 - initial mass of the solids in sample (g), m - mass of the solids in sample after dehydration (g).

III. Results and Discussion

A Model F-value of 46.37, 14.4 and 27.46 for WL, SG and WR implies respectively that the model is significant for the osmotic dehydration of banana in sugar solution. The Lack of Fit F-value of 35.50, 10.21 and 37.73 for WL, SG and WR implies the Lack of Fit is significant.

The coefficient of determination (R^2) is calculated to be 0.9774, 0.9307 and 0.9624 for WL, SG and WR respectively. This implies that more than 93% of experimental data are compatible with the data predicted by the model (Table 2) and only less than 7% of the total variations are not explained by the model. The value of

the Adj R^2 (0.9563 for WL, 0.8661 for SG, 0.9274 for WR) is also high to advocate for a high significance of the model. Here in this case the adjusted R^2 value is lesser than the R^2. The Pred R^2 for WL - 0.8699, SG - 0.7815 and WR - 0.8338 are in reasonable agreement with the Adj R^2. The value of CV is also low as 6.71, 12.2 and 9.82 which indicate that the deviations between experimental and predicted values are low. Adeq precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this work the ratio is found to be >13, which indicates an adequate signal.

The experimental results are analyzed through RSM to obtain an empirical model for the response. The mathematical expression of relationship to the response with variables are shown below

$$Y_1 = 33.42 + 0.43\,A + 3.32\,B + 3.66\,C + 0.84\,D + 0.25\,AB + 1.20\,AC + 0.20\,AD - 0.34\,BC - 0.46\,BD - 0.22\,CD - 2.63\,A^2 - 2.73\,B^2 - 4.51\,C^2 - 3.26\,D^2 \quad \ldots 4$$

$$Y_2 = 9.25 + 0.43\,A + 0.32\,B + 1.01\,C + 0.89D + 0.25\,AB - 0.39\,AC - 0.85\,AD - 0.31\,BC + 0.025\,BD + 0.23\,CD - 0.25\,A^2 - 0.73\,B^2 - 1.44\,C^2 - 0.68\,D^2 \quad \ldots 5$$

$$Y_3 = 24.16 + 0.86\,A + 3.00\,B + 2.65\,C + 0.050\,D - 0.0025\,AB + 1.59\,AC + 1.05\,AD - 0.029\,BC - 0.48\,BD - 0.44\,CD - 2.38\,A^2 - 2.00\,B^2 - 3.07\,C^2 - 2.58\,D^2 \quad \ldots 6$$

where Y_1,Y_2, Y_3 are water loss (%), solid gain (%) and weight reduction (%) respectively, and A, B, C, and D are the coded values of the test variables, temperature (^oC), processing time (min), sugar concentration (%w/w), and solution to sample ratio (%w/w) respectively.

Table 2 Experimental conditions and observed response values of CCD for osmotic dehydration of banana in sugar solution

Run No	Experimental			Predicted		
	WL, %	SG, %	WR, %	WL, %	SG, %	WR, %
1	22.65	4.88	17.77	21.95	4.54	17.41
2	22.11	6.29	15.82	22.09	5.80	16.28
3	21.31	7.68	13.63	22.06	8.32	13.74
4	33.43	9.21	24.22	33.42	9.28	24.14
5	20.35	5.62	14.73	22.69	5.49	17.20
6	27.45	6.68	20.77	29.13	6.96	22.17
7	15.30	5.27	10.03	15.85	5.66	10.19
8	19.56	8.52	11.04	22.05	9.12	12..93
9	17.23	4.74	12.49	18.72	4.78	13..94
10	20.46	8.15	12.31	20.38	8.04	12.33
11	14.70	5.14	9.56	14.35	5.08	9.27
12	33.40	9.32	24.08	33.42	9.28	24.14
13	23.01	8.32	14.69	22.76	7.61	15.15
14	20.29	8.64	11.65	20.63	7.90	12.73
15	23.45	8.62	14.83	23.34	7.95	15.39
16	33.37	9.30	24.07	33.42	9.29	24.14
17	30.10	7.20	22.9	30.12	8.09	22.04
18	19.52	4.75	14.77	18.88	4.03	14.85
19	8.15	0.65	7.50	8.05	1.45	6.60
20	33.46	9.27	24.19	33.42	9.28	24.14
21	33.43	9.21	24.22	33.42	9.28	24.14
22	10.73	1.49	9.24	11.99	2.22	9.77
23	33.40	9.20	24.20	33.42	9.28	24.14
24	12.53	6.35	6.18	11.91	6.19	5.722
25	20.81	4.47	16.34	19.55	5.33	14.22
26	28.66	4.91	23.75	27.50	5.12	22.38
27	24.01	7.31	16.70	23.76	7.39	16.38
28	20.64	7.48	13.16	18.78	8.37	10.42
29	14.38	6.22	8.16	13.63	4.51	9.13
30	29.54	8.11	21.43	26.76	7.53	19.24

The results of multiple linear regressions conducted for the second order response surface model are given in Table 2. The significance of each coefficient is determined by Student's *t*-test and *p*-values, which are listed in Table 3. The larger the magnitude of the *t*-value and smaller the *p*-value, the more significant is the corresponding coefficient. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case, B, C, D, AC, A^2, B^2,C^2, D^2 and A, B, C, AC, AD, A^2, B^2,C^2, D^2 are significant model terms for WL and WR respectively. Table 3 also indicates that the model terms A, C, D, AD, B^2, C^2, D^2 have significant influence on SG.

Table 3 Analysis of variance (ANOVA) for response surface quadratic model for the osmotic dehydration of banana using sugar solution

Source	Water Loss				Solid Gain				Weight Reduction			
	Coefficient	Sum of Squares	F value	p-value	Coefficient	Sum of Squares	F Value	p-value	Coefficient	Sum of Squares	F Value	p-value
Constant	33.42	1536.73	46.37	< 0.0001	9.25	137.43	14.40	< 0.0001	24.16	967.45	27.46	< 0.0001
A	0.43	4.42	1.87	0.0919	0.43	4.47	6.56	0.0217	0.86	17.78	7.07	0.0179
B	3.32	264.54	111.76	< 0.0001	0.32	2.52	3.70	0.0736	3.00	215.40	85.58	< 0.0001
C	3.66	321.49	135.83	< 0.0001	1.01	24.52	35.97	< 0.0001	2.65	168.43	66.92	< 0.0001
D	0.84	16.77	7.08	0.0178	0.89	18.83	27.62	< 0.0001	0.050	0.060	0.024	0.8794
AB	0.25	0.96	0.41	0.5337	0.25	0.98	1.44	0.2491	-0.0025	1.00E-004	3.97E-005	0.9951
AC	1.20	23.18	9.80	0.0069	-0.39	2.37	3.48	0.0818	1.59	40.39	16.05	0.0011
AD	0.20	0.62	0.26	0.6151	-0.85	11.53	16.91	0.0009	1.05	17.51	6.96	0.0186
BC	-0.34	1.82	0.77	0.3941	-0.31	1.53	2.24	0.1555	-0.029	0.013	5.25E-003	0.9432
BD	-0.46	3.33	1.41	0.2540	0.025	1.00E-002	0.015	0.9052	-0.48	3.71	1.47	0.2437
CD	-0.22	0.77	0.33	0.5758	0.23	0.81	1.19	0.2929	-0.44	3.17	1.26	0.2795
A^2	-2.63	189.39	80.02	< 0.0001	-0.25	1.71	2.51	0.1337	-2.38	155.07	61.61	< 0.0001
B^2	-2.73	204.45	86.38	< 0.0001	-0.73	14.82	21.73	0.0003	-2.00	109.19	43.38	< 0.0001
C^2	-4.51	558.26	235.86	< 0.0001	-1.44	57.27	84.01	< 0.0001	-3.07	257.92	102.48	< 0.0001
D^2	-3.26	290.87	122.89	< 0.0001	-0.68	12.54	18.40	0.0006	-2.58	182.61	72.55	< 0.0001
Lack of fit		35.50				10.21				37.73		
R^2		0.9774				0.9307				0.9624		
Adj R^2		0.9563				0.8661				0.9274		
Pred R^2		0.8699				0.7815				0.8338		
PRESS		204.48				58.85				217.35		
CV, %		6.71				12.20				9.82		
Std.Dev		1.54				0.83				1.59		
Adeq Precision		23.317				13.363				16.439		

The response surface curves for WL, SG and WR are shown in Figures. 1 - 18. From the figurers it is observed that the elliptical nature of the contour in 3D-response surface graphs depict the mutual interactions of all the variables. There is a relative significant interaction between every two variables, and there is a maximum predicted yield as indicated by the surface confined in the smallest ellipse in the contour diagrams.

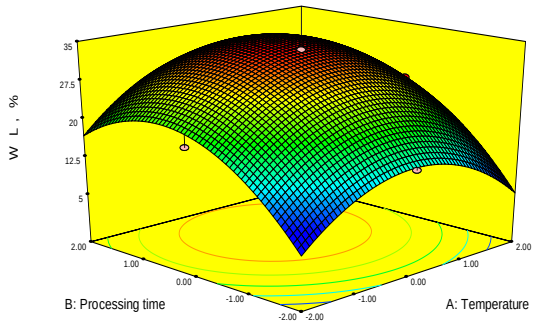


Figure.1. 3D plot of the combined effect of the temperature and processing time on WL

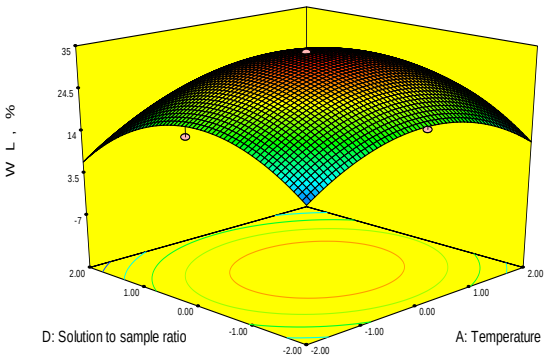


Figure.2. 3D plot of the combined effect of the of the temperature and solution to sample ratio on WL

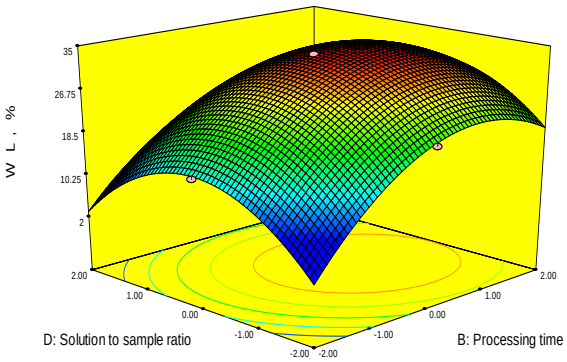


Figure.3. 3D plot of the combined effect of the processing time and solution to sample ratio on WL

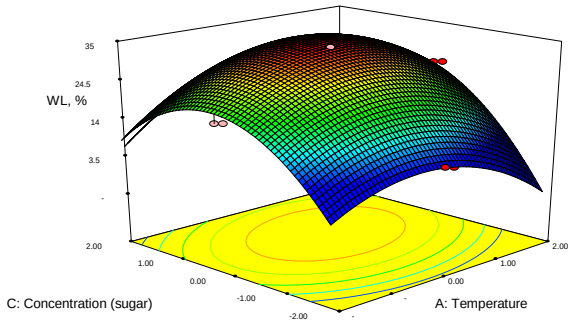


Figure.4. 3D plot of the combined effect of the temperature and sugar concentration on WL

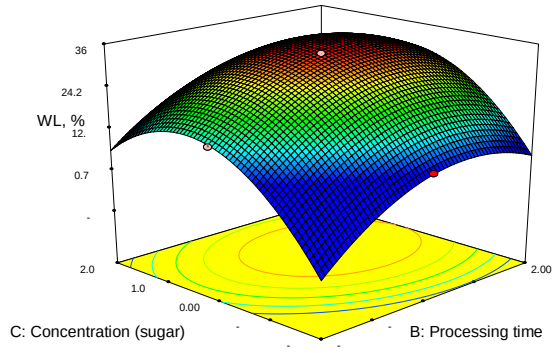


Figure.5. 3D plot of the combined effect of the processing time and sugar concentration on WL

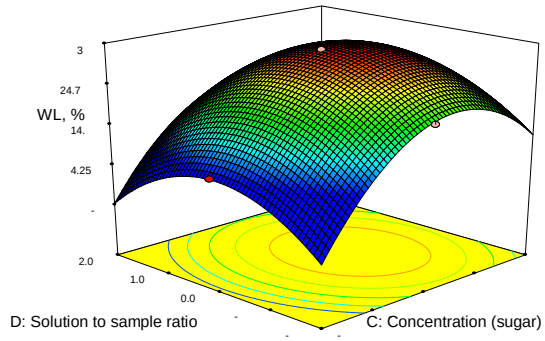


Figure.6. 3D plot of the combined effect of the sugar concentration and solution to sample ratio on WL

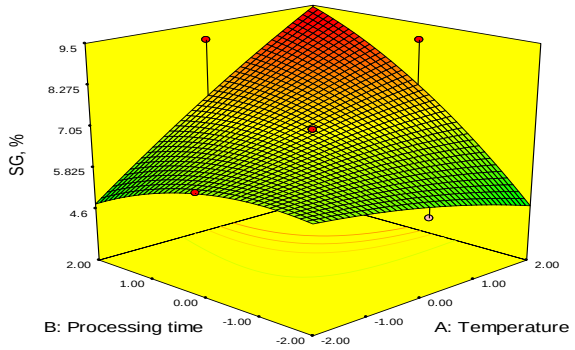


Figure.7. 3D plot of the combined effect of the temperature and processing time on SG

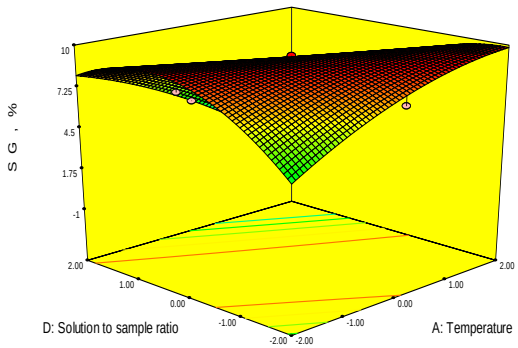


Figure.8. 3D plot of the combined effect of temperature and solution to sample ratio on SG

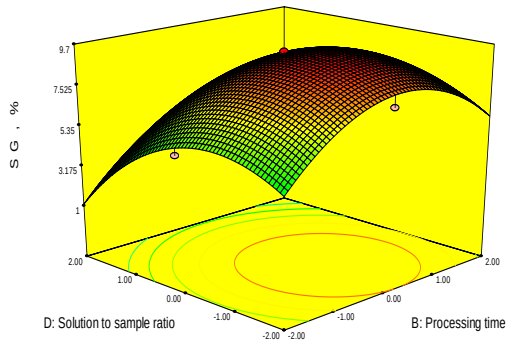


Figure.9. 3D plot of the combined effect of the processing time and solution to sample ratio on SG

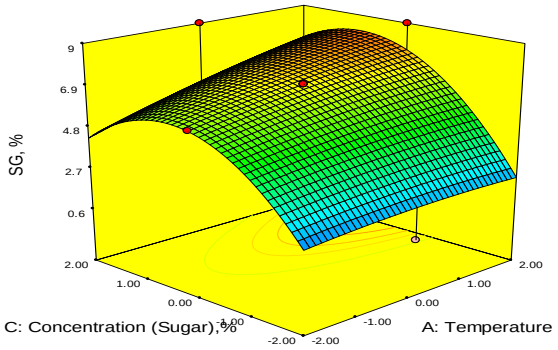


Figure.10. 3D plot of the combined effect of the temperature and sugar concentration on SG

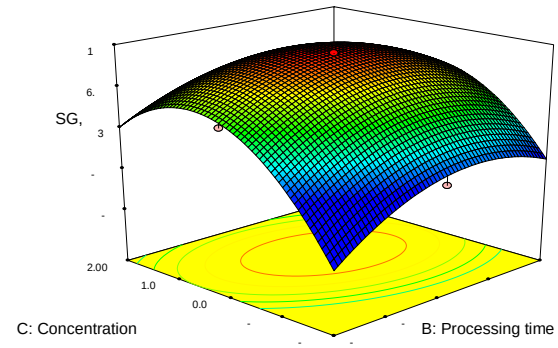


Figure.11. 3D plot of the combined effect of the processing time and sugar concentration on SG

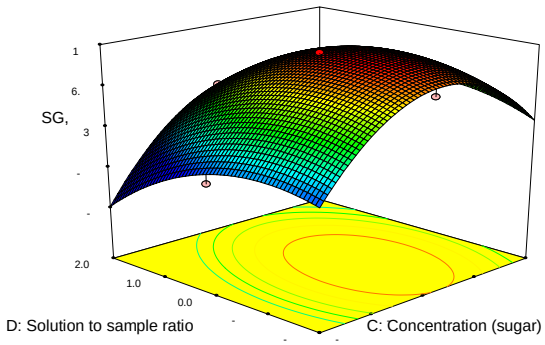


Figure.12. 3D plot of the combined effect of the sugar concentration and solution to sample ratio on SG

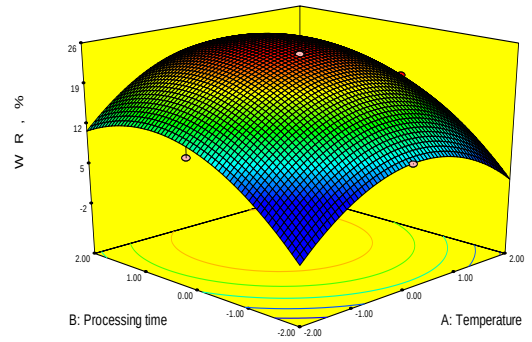


Figure.13. 3D plot of the combined effect of the temperature and processing time on WR

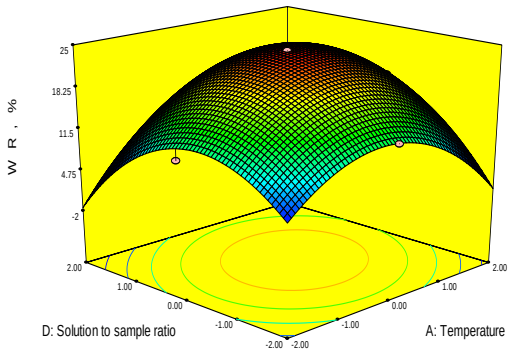


Figure.14. 3D plot of the combined effect of temperature and solution to sample ratio on WR

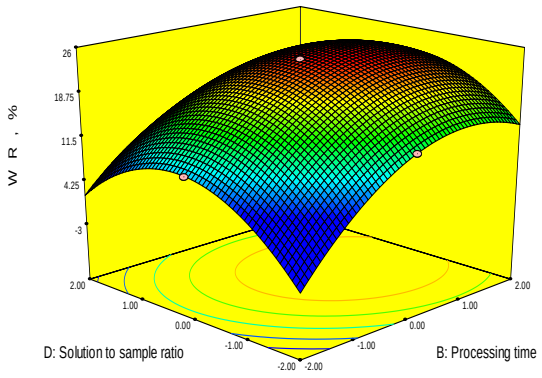


Figure.15. 3D plot of the combined effect of the processing time and solution to sample ratio on WR

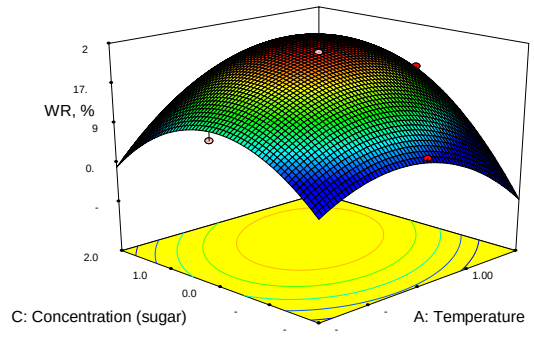


Figure.16. 3D plot of the combined effect of the temperature and sugar concentration on WR

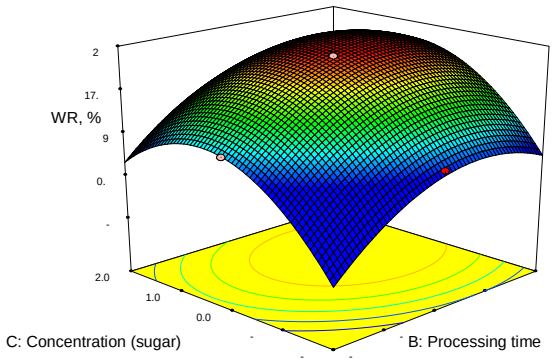


Figure.17. 3D plot of the combined effect of the processing time and sugar concentration on WR

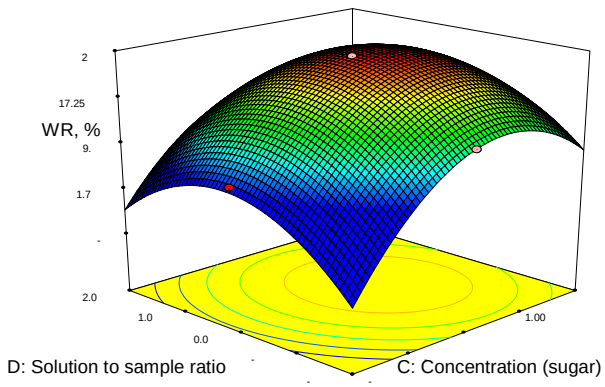


Figure.18. 3D plot of the combined effect of the sugar concentration and solution to sample ratio on WR

The magnitude of coefficient factors in Table 3 indicates the maximum positive contribution of temperature, processing time, sugar concentration and solution to sample ratio on the water loss during osmotic dehydration. It implies increased water loss with increase in temperature, processing time, sugar concentration and solution to sample ratio. This is clearly depicted in Figures. 1 – 6. The quadratic terms of temperature, processing time, concentration and solution to sample ratio have negative effect on water loss. Further, the interactions of A-B,

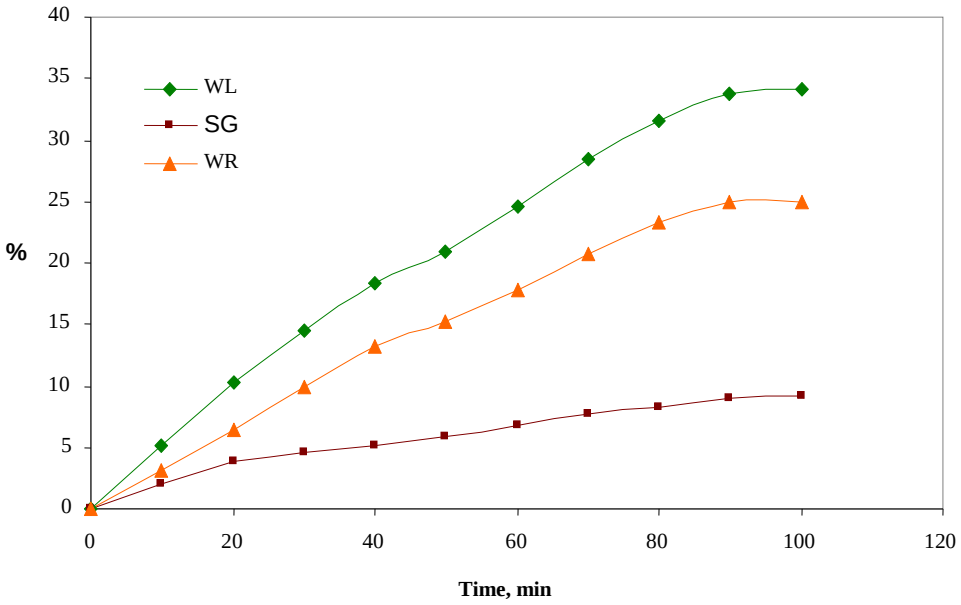
A-C and A-D have positive effect, whereas the interactions of B-C, B-D and C-D have negative effect on water loss.

From the figures, it is observed that, increase in temperature leads to increase in WL. This may be due to: increasing diffusion coefficients and decreasing viscosity of salt solution. However, there are limitations using high temperatures because of lower WL/SG ratio and lower product quality. WL increases with time. On the other hand, WL increases gradually with sugar concentration. It is also found that, increasing the solution to sample ratio, resulted in more water loss (and solid gain) but increasing the volume means more costs arising from more materials to be used and greater volumes to operate with.

The linear effects of all variables show positive effect on solid gain. It implies increased solid gain with increase of process variables (Figures. 7 - 12). The quadratic terms of temperature, processing time, sugar concentration and solution to sample ratio have negative effect on solid gain. The interactive effects of A-B, B-D and C-D have positive effect on solid gain, whereas the interactive effects of A-C, A-D and B-C have negative effect.
The SG is increasing with temperature. As it was explained for WL, temperature has an effect on the cell membrane permeability that could allow solutes to enter by losing its selectivity. Decrease of solution viscosity at higher temperatures may influence SG due to fact that lower viscosity decreases the resistance to diffusion of solutes into the plant tissue. Also, higher solid gains at higher temperatures may be due the destruction to cell membrane structure. The SG increases gradually with processing time and salt concentration. Increasing the solution to sample ratio resulted in more solid gain. This behavior can be explained, in part, by the increase of moisture loss of the samples, what would allow solutes to enter the fruit.

The magnitude of coefficient factors in Table 3 gives the maximum positive contribution of temperature, processing time, solution concentration and solution to sample ratio on the weight reduction during osmotic dehydration. It implies increased weight reduction with increase in process variables. This is clearly shown in Figures. 13 – 18. The quadratic terms of temperature, processing time, sugar concentration and solution to sample ratio have negative effect on weight reduction. Further, the interactions of A-C and A-D have a positive effect, whereas the interactions of A-B, B-C, B-D and C-D have negative effect on weight reduction. The studies of the contour plot (Figures. 1 - 18) also reveal the optimum region of the process conditions for the osmotic dehydration of banana using sugar solution.

Figure.19. Water loss, solid gain and weight reduction during the osmotic dehydration of banana in sugar solution at optimum condition



Optimum conditions for the osmotic dehydration of banana are determined to obtain maximum water loss and weight reduction and minimum solid gain. Second order polynomial models obtained in this study are utilized for each response in order to determine the specified optimum conditions. The sequential quadratic programming in MATLAB 7 is used to solve the second-degree polynomial regression equation 4, 5 and 6. The optimum values obtained by substituting the respective coded values of variables are: temperature – 37ºC, processing time – 100 min, sugar concentration – 45% w/w, solution to sample ratio – 14.5:1w/w. At these conditions, water loss, solid gain and weight reduction is found to be 34.2 (g/100 g fresh sample), 9.2 (g/100 g fresh sample), 25 (g/100 g fresh sample), respectively. This is clearly depicted in Figure.19. These values are in accordance with an efficient osmotic dehydration process in which 40–60% water loss and <10% solid gain are mostly aimed. The value of solid gain 9.2 (g/100 g fresh sample), which is hard to obtain in common osmotic dehydration processes, is achieved.

IV. Conclusion

In this study, RSM was used to determine the optimum operating conditions that yield maximum water loss and weight reduction and minimum solid gain in osmotic dehydration of banana. Analysis of variance has shown that the effects of all the process variables including temperature, time, sugar concentration and solution to sample ratio were statistically significant. Second order polynomial models were obtained for predicting water loss, solid gain and weight reduction. The optimal conditions for maximum water loss and weight reduction and minimum solid gain correspond to temperature of 37ºC, processing time of 100 min, sugar concentration of 45% and solution to sample ratio of 14.5:1w/w in order to obtain water loss of 34.2 (g/100 g fresh sample), solid gain of 9.2 (g/100 g fresh sample) and weight reduction of 25(g/100 g fresh sample).

V. References

[1] P.S.Madamba,. Thin layer drying models for osmotically predried young coconut. *Dry. Technol.*, 21, 1759-1780, (2003).

[2] D.Torrenggiani, Osmotic dehydration in fruits and vegetable processing. *Food Res. Int.*, 26, 59-68, (1993).

[3] A.L.Raoult-Wack, Advances in osmotic dehydration. *Trends Food Sci. Technol.*, 5, 255-260, (1994).

[4] R.N.Biswal, and M..Le Maguer, Mass transfer in plant material in contact with aqueous solution of ethanol and sodium chloride: equilibrium data. *J. Food Proc. Eng.*, 11, 159-176, (1989).

[5] G.S.Mudahar, R.T.Toledo, J.D.Floros, and J.J.Jen, Optimization of carrot dehydration process using response surface methodology. *J. Food Sci.*, 54, 714-719, (1989).

[6] R.N.Biswal, K. Bozorgmehr, F.D.Tompkins, and X.Liu, Osmotic concentration of green beans prior to freezing. *J. Food Sci.*, 56, 1008-1012, (1991).

[7] N.K.Rastogi, and K.S.M.S.Raghavarao, Water and solute diffusion coefficients of carrot as a function of temperature and concentration during osmotic dehydration. *J. Food Eng.*, 34, 429-440, (1997).

[8] A.Kar, and D.K.Gupta, Osmotic dehydration characteristics of button mushrooms. *J. Food Sci. Technol.*, 38, 352-357, (2001).

[9] B.I.O.Ade-Omowaye, N.K.Rastogi, A.Angersbach,. and D.Knorr, Osmotic dehydration behavior of red paprika (*Capsicumannuum* L.). *J. Food Sci.*, 67, 1790-1796, (2002).

[10] P.P.Sutar, and D.K.Gupta, Mathematical modeling of mass transfer in osmotic dehydration of onion slices. *J. Food Eng.*, 78, 90-97, (2007).

[11] M.B.Uddin, P.Ainsworth, and S.Ibanoglu, Evaluation of mass exchange during osmotic dehydration of carrots using response surface methodology. *J.Food Eng.*, 65, 473-477, (2004).

[12] O.Corzo, and E.R.Gomez, Optimization of osmotic dehydration of cantaloupe using desired function methodology. *J.Food Eng.*, 64, 213-219, (2004).

[13] I.Eren, and F.K.Ertekin, Optimization of osmotic dehydration of potato using response surface methodology. *J. Food Eng.*, 79, 344-352, (2007).

[14] B.Singh, P.S.Panesar, A.K.Gupta, and J.F.Kennedy, Optimization of osmotic dehydration of carrot cubes in sugar-salt solutions using response surface methodology. *Eur. Food Res.Technol.*, 225, 157-165, (2007).

[15] B.Singh, P.S.Panesar, V. Nanda, and M.B.Bera, Optimization of osmotic dehydration process of carrot cubes in sodium chloride solution. *Int. J. Food Eng.*, 4, 1-24, (2008).

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Quality Assessment and Shelf Life Evaluation of *Momordica charantia* Based Candies

P. Divya*, M. Priyadharshini, S. Tamilarasu, S. Poornima, B. Mythili Gnanamangai, K.S. Ghanapathy Ram, M. Sanjeev Kumar, R.C. Gowtham

Department of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode - 637 215, Tamil Nadu INDIA, p.divya.biotech@gmail.com

Abstract*: Candies were prepared with four different combinations of Trial - 1 (M. charantia blanched with water), Trial - 2 (M. charantia blanched with beet root juice), Trial - 3 (M. charantia in powdered form) and Trial - 4 (M. charantia treated with honey). To establish the best product, the prepared candies were assessed by organoleptic evaluation through Nine-point Hedonic scale. Out of the four trials, two best candies of interest was selected and stored at room temperature (35°C) to evaluate its shelf life. The microbial growth in the prepared sample was analyzed at regular intervals for every ten days for a period of two months. The experimental results showed that Trial - 3 and 4 were the most preferred candies by the panel members. Moreover, to preserve the food products from the environmental pathogens, the product was packed for the consumer's better interest. The changes in consumer preference for safe food had led to innovation in packaging technologies. Packaging method was designed with the usage of instant gel powder along with glycerin, which helped to make the foods safer with better shelf life.*

Keywords: *Momordica charantia, Bitter gourd, Candy, Organoleptic evaluation, shelf life*

I. Introduction

Momordica charantia (*M. charantia*), known as bitter melon, bitter gourd, bitter squash or balsam pear. It is a tropical and subtropical flowering vine of the family Cucurbitaceae, widely cultivated in Asia, Africa and the Caribbean for its edible fruit. Its many varieties differ substantially in the shape and bitterness of the fruit [1]. Over the decade, research into nutrition and dietary supplements has focused heavily on plant-derived nutraceuticals. The nutrient profile of *M. charantia* shows that it has twice the β-carotene found in broccoli, twice the calcium of spinach and twice the potassium of bananas [2].

The clinical significance of *M. charantia* shows that its extract and components have the efficiency to exert anti-diabetic and hypoglycemic effects via various biochemical and pharmacological mechanisms [3]. Moreover, the medicinal uses of *M. charantia* includes anti-cancerous and anti-leukemic activity against various cell lines [4], anti-inflammatory property on lipopolysaccharide stimulated macrophages [5], anti-oxidant effects [6], anti-viral activity against Epstein-Barr, Herpes and HIV viruses [7] and hypolipidemic property [8].

Candy is a sweet food product prepared from fruits or vegetables by impregnating with sugar syrup and developing into a dried confectionary product with longer shelf life [9]. The matured fruit or vegetable can be harvested and processed to improve their shelf life and its availability in the most presentable form.

The present study was focused on the preparation of confectionary candy using *M. charantia* and its shelf life evaluation. *M. charantia* based candy could attract a wide category of consumers and thereby these candies can carry over the nutritional and pharmacological property to the users. So the candy was prepared after assessing its organoleptic results and the final products were packed through instant gel packs, an eco-friendly and an innovative method.

II. Materials and Methods

A. Cultivation on M. charantia

M. charantia seeds were brought commercially and germinated for the cultivation of plant using organic manure. The organic manure was prepared using *Eichhornia crassipes* (Water hyacinth). Water hyacinth was collected from Pallipalayam, Cauvery river side, Erode. The collected Water hyacinth was shade dried and powdered and mixed with soil in the ratio of 1:1 and maintained for a period of one month [10].

M. charantia seeds were grown on the prepared manure and harvested after *M. charantia* reached the preferred stage. The disease free vegetables were graded and washed to remove the dirt and further processed for the candy preparation.

B. Preparation of candies

The harvested *M. charantia* were graded to remove the seeds and diced into pieces. The blanching temperature was uniformly maintained as 60ºC for 5 min for the trials. The candy was prepared with 75% of sugar and the consistency of the sugar syrup was 75º Brix [11]. The syrup was finally drained and dehydrated in Hot air oven at 70ºC for 1 hr [12].

The candy was prepared with four different combinations as follows.

Trials

Trial - 1 = T_1 = *M. charantia* blanched with water + 75% sugar
Trial - 2 = T_2 = *M. charantia* blanched with beet root juice + 75% sugar
Trial - 3 = T_3 = *M. charantia* in powdered form + 75% sugar
Trial - 4 = T_4 = *M. charantia* + 75% honey

C. Organoleptic evaluation

The prepared candy of different trials was evaluated by the panel of 20 members. The organoleptic parameters include color, flavor, taste, texture and overall acceptability [13]. The panel members evaluated the candies using Nine-point Hedonic scale.

D. Packing of candy

The candies were packed in wrappers which was prepared using gelatin powder and Glycerin. The degradability of the wrappers was tested to ensure it properties.

E. Storage and Shelf life evaluation

The two most preferred candy trials were finally selected for its shelf life evaluation. The prepared candies were stored at ambient temperature (35ºC) for a period of two months. The organoleptic evaluation and microbial growth in the product was analyzed for every ten days.

F. Statistical analysis

All the data regarding to the trials and shelf life evaluation was analyzed using Randomized Complete Block Design (RCBD) as recommended by Gomez & Gomez, 1984 [14] and the means were divided by applying LSD test to check the level of significance at 5% probability ($p<0.05$).

III. Results and Discussion

A. Organoleptic properties of candies

M. charantia based candies were prepared and the organoleptic evaluation was conducted using Nine-point Hedonic scale of Larmond (1977). The results obtained were tabulated in Table I.

Table I. Overall oraganoleptic evaluation results of the four different trials

Trial	Organoleptic evaluation					Average
	Colour	Flavour	Taste	Texture	Overall acceptability	
T_1	6.6	5.2	5.8	7.2	7.4	**6.4**
T_2	7.0	6.4	6.2	7.6	7.8	**7.0**
T_3	7.8	7.6	7.8	7.8	7.8	**7.8**
T_4	8.0	7.8	7.8	8.0	8.2	**8.0**

Based on the results obtained, *M. charantia* added in powdered form (T_3) and *M. charantia* treated with honey (T_4) was the most preferred candies by the panel members with highest score of 7.8 and 8.0 respectively. Thus the further studies were carried out with T_3 and T_4.

B. Packing and storage

The wrappers prepared using gelatin powder and Glycerin, was subjected to biodegradability studies. The results showed the complete degradation of the wrapper within 24 hrs when buried in soil. This eco-friendly wrapper was further used in the packing of *M. charantia* candies. The packed candies were stored in air tight containers.

The study of the color, flavor, taste, texture and overall acceptability of the packed candies was conducted for every ten days for a period of ten days.

C. Color

The mean score of the panel members for color was found to be significantly ($p<0.005$) decreased from 7.9 to 1.2 during the storage of *M. charantia* candies at ambient temperature as tabulated in Table II. For the trials, the maximum and minimum score was observed to be 4.89 and 4.51 after the storage of 60 days. Both the trials had attractive color when stored at ambient temperature. But the significant decrease in the color of the candies was noted as the storage period increases.

Sharmerz et al (2013) observed the similar results in the organoleptic evaluation of the candies prepared from the citrus peel. The mean score of the sensory evaluation results showed a significant decrease from 8.83 to 4.84. Similar results were reported by Durrani et al (2011) in the quality assessment of candies prepared with the different combination of carrot and honey. The research commented that addition of honey could find a way to improve the quality of the candies.

Table II. Effect of storage period on color of *M. charantia candies* stored at room temperature

Trial	Storage period (days)							% Decrease	Mean
	0	10	20	30	40	50	60		
T_3	7.8	6.8	6.0	4.8	3.0	2.2	1.0	87.18	**4.51d**
T_4	8.0	7.2	6.6	5.2	3.4	2.4	1.4	82.50	**4.89d**
Mean	**7.9a**	**7.0b**	**6.3c**	**5.0d**	**3.2e**	**2.3f**	**1.2g**		

*Means followed by small letters (a-g) are significantly different (p<0.05)

D. Flavour

Flavour is one of the important factors that decide the quality of the food product. The panel members responsed to *M. charantia* candies and they noted the changes in the flavour. The mean score of the results was observed to be significantly (p<0.005) decreased from 7.7 to 3.3 as showed in Table III. Decrease in the quality of flavor was notes as the storage period increases with percentage decrease in the flavour of T_3 and T_4 was 57.89 and 56.41 respectively.

The results of Zeeshan et al (2017) reported that the flavour would determine the attraction of the consumer towards the food product and the mean score of the judges of flavour was observed to be declined from 8.47 to 5.4 in the sensory evaluation of Dhakki dates candy.

Table III. Effect of storage period on flavour of *M. charantia candies* stored at room temperature

Trial	Storage period (days)							% Decrease	Mean
	0	10	20	30	40	50	60		
T_3	7.6	7.2	6.8	6.0	4.8	4.8	3.2	57.89	**5.77d**
T_4	7.8	7.4	6.8	6.4	5.2	5.2	3.4	56.41	**6.03d**
Mean	**7. 7a**	**7.3b**	**6.8c**	**6.2d**	**5.0e**	**5.0f**	**3.3g**		

*Means followed by small letters (a-g) are significantly different (p<0.05)

E. Taste

The taste is the most vital factor that attracts the customers. The mean score of the panel members for the taste of *M. charantia* candies was analyzed to be significantly (p<0.005) decreased from 7.8 to 3.6 as showed in Table IV. Similar results were reported by Jothi et al (2014), in the production of candy from the pineapple (*Ananas comosus*). The Duncan's Multiple Test results revealed that mean score of the samples had the higher score of 8.43 and lower score of 6.94.

Table IV. Effect of storage period on taste of *M. charantia candies* stored at room temperature

Trial	Storage period (days)							% Decrease	Mean
	0	10	20	30	40	50	60		
T_3	7.8	7.4	6.6	6.0	4.6	4.0	3.4	56.41	**5.69d**
T_4	7.8	7.6	6.8	6.4	5.4	4.8	3.8	51.28	**6.08d**
Mean	**7.8a**	**7.5b**	**6.7c**	**6.2d**	**5.0e**	**4.4f**	**3.6g**		

*Means followed by small letters (a-g) are significantly different (p<0.05)

F. Texture

The mean score of the panel members for evaluation of *M. charantia* candies for the texture was observed to be significantly (p<0.005) decreased from 7.9 to 4.6 when stored at ambient temperature as noted in Table V. For the trials, the percentage decrease in texture was found to 43.58 and 40.00 for T_3 and T_4.

The research findings of Zeeshan et al (2017), in the sensory evaluation of Dhakki dates candy prepared with 70% sugar concentrations showed 32.22% decrease in the texture when stored in cool airtight boxes for 180 days.

Table V. Effect of storage period on texture of *M. charantia candies* stored at room temperature

Trial	Storage period (days)							% Decrease	Mean
	0	10	20	30	40	50	60		
T_3	7.8	7.4	6.8	6.2	5.4	5.2	4.4	43.58	**6.17d**
T_4	8.0	7.6	7.0	6.4	6.0	5.6	4.8	40.00	**6.49d**
Mean	**7.9a**	**7.5b**	**6.9c**	**6.3d**	**5.7e**	**5.4f**	**4.6g**		

*Means followed by small letters (a-g) are significantly different (p<0.05)

G. Overall acceptability

The results on the overall acceptability of *M. charantia* based candies gradually decreased from 8.0 to 4.3, as the storage period increased upto 60 days which was represented in Table VI.

As discussed by Zeeshan et al (2017), the reduction in the mean score of the candies would be due to the decline in score of color, flavour, taste and texture. Similar results were observed by Sharmerz et al (2013) in the sensory evaluation of citron peel candies, where the mean score of the judges varied from 8.70 to 5.00.

Table VI. Effect of storage period on overall acceptability of *M. charantia candies* stored at room temperature

Trial	Storage period (days)							% Decrease	Mean
	0	10	20	30	40	50	60		
T_3	7.8	7.2	6.8	6.4	5.8	5.0	4.2	46.15	**6.17d**
T_4	8.2	7.8	7.0	6.6	6.2	5.2	4.4	46.34	**6.49d**
Mean	**8.0a**	**7.5b**	**6.9c**	**6.5d**	**6.0e**	**5.1f**	**4.3g**		

*Means followed by small letters (a-g) are significantly different ($p<0.05$)

H. Shelf life studies

The shelf life of *M. charantia* based candies packed using gelatin powder and Glycerin, had significant reduction in the overall score of the organoleptic evaluation results when stored at room temperature for a period of two months. The decrease in the score would be due to the growth of the microorganisms and increase in the moisture content in the container.

Thus, *M. charantia* based candies were found to be stable till 50 days for storage period which was clearly tabulated in Table VII.

Table VII. Consolidated report on effect of storage period on quality of *M. charantia candies*

Storage period (days)	Trial	Initial value	Organoleptic evaluation					Average	% Decrease	Fungal Growth
			Colour	Flavour	Taste	Texture	Overall acceptability			
10	T_3	7.8	6.8	7.2	7.4	7.4	7.2	7.2	7.69	Absence
	T_4	8.0	7.2	7.4	7.6	7.6	7.8	7.5	6.25	Absence
20	T_3	7.8	6.0	6.8	6.6	6.8	6.8	6.6	15.38	Absence
	T_4	8.0	6.6	6.8	6.8	7.0	7.0	6.8	15.00	Absence
30	T_3	7.8	4.8	6.0	6.0	6.2	6.4	5.8	25.64	Absence
	T_4	8.0	5.2	6.4	6.4	6.4	6.6	6.2	22.50	Absence
40	T_3	7.8	3.0	4.8	4.6	5.4	5.8	4.7	39.74	Absence
	T_4	8.0	3.4	5.2	5.4	6.0	6.2	5.2	35.00	Absence
50	T_3	7.8	2.2	4.8	4.0	5.2	5.0	4.2	46.15	Absence
	T_4	8.0	2.4	5.2	4.8	5.6	5.2	4.6	42.50	Absence
60	T_3	7.8	1.0	3.2	3.4	4.4	4.2	3.2	58.97	Presence
	T_4	8.0	1.4	3.4	3.8	4.8	4.4	3.6	55.00	Presence

IV. Conclusion

The study indicated a good attempt to prepare the candies from *M. charantia*. This idea would attract a wide variety of customers to consume *M. charantia* in the form of candies, thereby to carry over the nutritional and pharmacological properties to them. Moreover, the results of the research concluded that *M. charantia* added in powered form (T_3) and *M. charantia* added with honey, instead of sugar (T_4), provided good results and could be packed and stored in air tight container for a period of 50 days at room temperature without any visible microbial growth. Further study is necessary to investigate the percentage hold of the chemical composition and nutrients of *M. charantia* in candies, to make the product successful.

V. References

[1] Madhu Gupta, Sushil Sharma, K. Ajay Gautam and Rekha Bhadauria, "*Momordica charantia linn. (Karela):* Nature's silent healer", International Journal of Pharmaceutical Sciences Review and Research, vol. 11, no. 1, 2011, pp. 0976-044X.

[2] V. Trivedi Rashmi, J. Waldher Kamlesh, B. Taksande Yayashri and J. Umekarr Milind, 'Bitter melon: A bitter body with a sweet soul", International Journal of research in Ayruveda & Pharmacy, vol. 2. no. 2, 2011, pp. 443-447.

[3] Baby Joseph and D. Jini, "Antidiabetic Effect of *Momordica charantia* and Its Medicine Potency", Asian Pacifi Journal of Tropical Disease vol. 3, no. 2, 2013, pp. 93-102.

[4] J.K. Grover and S.P. Yadav, "Pharmacological actions and potential uses of *Momordica charantia:* A reviw", Journal of Ethnopharmacology, vol. 93, no. 1, 2004, pp. 123-132.

[5] C.K.Lii, H.W.Chen, W.T.Yun and K.L. Liu, "Supressive effects of wild bitter gourd (*Momordica charantia* Linn. Var. abbreviate ser.) fruit extracts on inflammatory respponses in RAW 264.7 macrophages, Journal of Ethnopharmacology, vol. 122, 2009, pp. 227-233.

[6] J. Kubola and S. Siriamornpun, "Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf stem and fruit fracion extarcts in vitro, Food Chem., vol. 110, 2008, pp. 881-890.
[7] Q.C. Zhang, "Prelimianry reprt on the use of *Momordica charantia* extract by HIV patients, J Naturopath Med., vol. 3, 1992, pp. 65-69.
[8] R. Noguchi, Y. Yasui, R. Suzuki, M. Hosokawa, K. Fukunaga and K.Miyashita, "Dietary effects of bitter gourd oil on blood and liver lipids of rats", Arch Biochem Biophys, vol. 396, pp. 207-212.
[9] U. Mehta and S. Bajaj, "Changes in the chemical composition and organoleptic quality of citrus peel candy during preparation and storage", J Food Science and Technology, vol. 21, 1984, pp. 422-424.
[10] Sharda Vidya and Lakshmi Girish, "Water Hyacinth as a green manure for organic farming", International Journal of Research in Applied, Natural and Social Sciences, vol. 2, 2014, pp. 65-72.
[11] Anisa Musarath Durrani, P.K. Srivastava and Sangeeta Verma, "Development and quality evaluation of honey based carrot candy", Journal of Food Science Technology, vol. 48, no. 4, 2011, pp. 502-505.
[12] Jakia Sultana Jothi, Monirul Islam, Md. Serajul Islam, Md. Ramim Tanver Rahman and Shireen Akther, "Development and shelf life prediction of pineapple preserve and candy", International Journal on Innovation and Science Research, vol. 10, no. 1, 2014, pp. 77-82.
[13] E. Larmond, "Laboratory methods of snesory evaluation of foods', Canada. Deptt. Agric. Pub. No. 1284, 1977, pp. 36-37.
[14] K.A. Gomez and A.A. Gomez, "Statistical procedure for Agricultural Research", 2nd Edition, 1984, pp. 8-22.
[15] Baber Shamrez, Samra Aftab, Muhammad Junaid, Nazeer Ahmed and Saeed Ahmed, "Preparation and Evaluation of Candies from Citron Peel", Journal of Environmental Science, Toxicology and Food technology, vol. 7, no. 4, 2013, pp.21-24.
[16] M. Zeeshan, S.A. Saleem, M. Ayub, M.Shah and Z. Jan, "Physicochemical and Sensory Evaluation of Dhakki Dates Candy", Journal of Food Processing & Technology, vol. 8, no. 3, 2017, pp. 1-4.

Acknowledgments

The authors are thankful to, The Management and The Principal of K.S.Rangasamy College of Technology, Tiruchengode, Tamil Nadu, India and also gratefully acknowledge DST FIST (Fund for Infrastructure for Science and Technology) FIST No: 368 (SR/FST/College - 235/2014) and DBT STAR (BT/HRD/11/09/2018) for their instrumentation support to carry out this work.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Production and Quality Evaluation of Herbal Wine

[1]Dr.M. Sivasakthi, [2]M.Rajeswari

[1]Assistant Professor, [2]PG student, Department of Nutrition and Dietetics, Dr.N.G.P. Arts and Science College, Coimbatore-641 048, India.

Email ID: sivasakthiphd2015@gmail.com,

Abstract: *Nature provides profuse package of nutrients, antioxidants and when incorporated with novel foods contributes increased therapeutic value. Versatile plant based produce like aloe Vera, grapes, palm sugar and fermenting agents like yeast is required to develop wine in different variations such as V1(0.5:3:1:0.3), V2(0.75:3:1:0.3) with different ratio of aloe Vera, grapes, palm sugar and wheat grass with 5g of yeast to each. Sensory panel members have selected the best product variation V2 than V1 and control in terms of nutritional, physico-chemical, anti-oxidant and shelf-life analysis. Aloe Vera and grapes were pre-processed, the gel is extracted from aloe Vera and blended with grape, sugar and then supplemented with additives to maintain the pH for favourable yeast growth during fermentation. Later the ageing of wine was done for a certain period in an oak wood barrel to develop the maturation of wine. The study, it was observed that V2 wine has highest sensory score (4.8). It was found statistically significance difference existed between control and variation-2 considering nutritional parameters at 5% level. Whereas, antioxidants like vitamin C content (41.1mg) and antioxidants activity of V2 (70.2) was greater than the control (14mg and 21.3). The variation was also found to be high stable at room temperature without any preservatives. From the study, it was concluded that variation-2 was selected as the best of other variation and control. The wine was good in term of TSS, acidity, ash, moisture and alcohol. Proximate nutrient like energy, carbohydrates and minerals possessed almost similar values with control. The final total microbial count of aloe Vera grapes wine was 1×10^2(cfu/ml) respectively on the 90 days of the storage life comparatively lesser than the control. This is highly stable in room temperature without any preservatives. The product wine V2 has met standards and recommendation of FSSAI and has the potential on commercialisation worldwide.*

Keywords: *aloe vera, grapes, fermentation, wine*

I. Introduction

Wine is a natural product resulting from a number of biochemical reactions, which begin during ripening of the fruits and continue during harvesting, throughout the alcoholic fermentation, clarification and after bottling [1]. Wine is the product of a complex biological and biochemical interaction between grapes (grape juice) and different microorganisms (fungi, yeasts, lactic acid bacteria and acetic acid bacteria) and the mycoviruses and bacteriophages affecting them [2].

The process starts in the vineyard, continues through fermentation and maturation, and concludes at packaging. It is affected by the various viticultural and oenological practices available to the grape-grower and winemaker, respectively [3]. Of the microorganisms involved, it is the yeasts that play the most important role; they conduct the alcoholic fermentation (conversion of grape sugar to ethanol and CO2).

Furthermore, although wine flavour is directly determined by grape variety, yeasts also affect wine flavour and quality by the production and excretion of metabolites during growth and through autolysis [4]. In some instances, yeasts can also act as spoilage organisms during wine production (including maturation) and after packaging [5].

Yeasts present during fermentation are derived from grapes and the vineyard, the equipment used in the cellar, cellar surfaces and external sources such as selected cultures that are added to facilitate the fermentation process.The production of wine involves standardization of initial sugar concentration, inoculum level and temperature of the must prepared during fermentation [6].

Aloe Vera has been used therapeutically.certainly, since roman times and perhaps long before [7]. different properties being ascribed to the inner, colourless,leaf gel and to the exudate from the outer layers.aloevera (L.) burm.f.gel [8] popular interest and use of the gel have increased dramatically.in this country it is now a familiar ingredient in a range of healthcare and cosmetic product widely available advertised in shops [9].There are more than 200 species of aloe vera plants. The aloe plant has yellow flower.in 1851 it was discovered that the potency

of aloe was the result of aloin, a bitter juice that dried to a yellow powder and functioned as a cathartic medicine [10].

Grapes have a long and abundant history. During the ancient Greek and Roman civilizations, grapes were revered for their use in winemaking. Nowadays, there are three main species of grapes: European grapes (Vitis vinifera), North American grapes (Vitis labrusca and Vitisrotundifolia) and French hybrids. Grapes are classified as table grapes, wine grapes (used in viniculture), raisin grapes, and so on, with edible seeds or seedless. Grape fruit contains various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals. The phenolic compounds mainly include anthocyanins, flavanols, flavonols, stilbenes (resveratrol) and phenolic acids [11].

Sugar palm is one of the most versatile palm species because almost all parts of the tree can be used, with the palm sap being the most important product. Apart from the palm sap, other significant products that can be produced from sugar palm include palm neera, fresh juices, traditional sugar blocks, toddy, crystal and brown sugar ,vinegar, bio-ethanol, starch from trunk, sea water resistant fibre, edible heart fruits, leaves for roofing, brooms, matting, baskets, cigarette papers, cattle feeds and its starch inside the stem can be processed to make biopolymer.

The palm sugar can be consumed fresh or can be cooked to make traditional sugar blocks as ingredient in making cakes, desserts and food coating. It can also be processed to crystal and brown sugar and used as an alternative to the commercialisedsugarcane granular sugar [12].

In the present study,versatile plantbased produce like aloe vera,grapes,palm sugar and fermenting agent like yeast in required quantity was utilised to develop value added fermented product wine with enriched nutrient profile on account of its antioxidant,antimicrobial and anticancer properties.with this background the following objectives framed: formulation of value added wine using aloe vera, grapes, palm sugar, fermenting agent yeast and analysis of organoleptic profile, nutritional, physic-chemical and shelf life quality of the wine [13]-[14].

II. Methodology

The methodology pertaining to the study entitled, “Production and Quality evaluation of Herbal Wine” is discussed under the following heads.

Procurement of ingredients and preparation of herbal wine :

Aloe vera plants are well known for their medicinal and healing properties from centuries. palm sugar is rich in iron, zinc, calcium, sodium and potassium, dietary fiber and inulin possess varying therapeutic properties. In wine grapes, tannins include a range of polyphenolic compounds ranging from small oligomeric forms to large proanthocyanidin polymers, also known as condensed tannins(Downey *et al.*,2006). Other protective compounds in whole wheat grains include phytate, phyto-oestrogens such as lignan, plant stanols and sterols, and vitamins and minerals. It helps to improvements in biomarkers with whole-grain consumption, such as weight loss, blood-lipid improvement, and antioxidant protection. It has isolated nutrients and phytochemical (Slavin, 2004). Hence the ingredients were selected for preparation of wine.These ingredients are widely available in all over the places in Indian markets, farms, local fruits and vegetable shops.

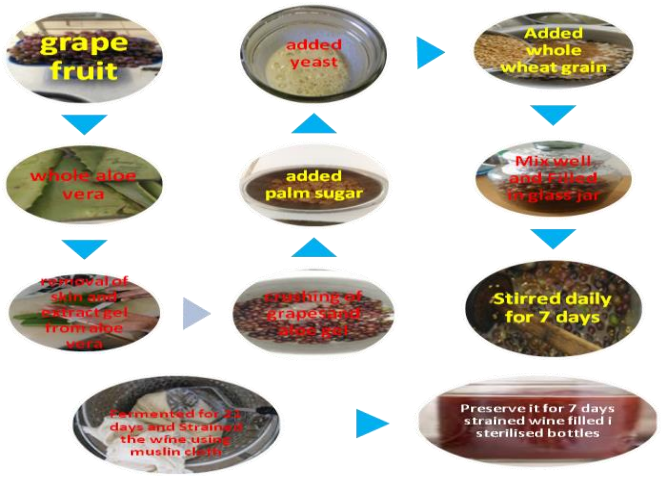


Figure -1:Steps involved in processing of wine

Standardization of the wine

Table 1:Composition Of The Wine

PRODUCT	ALOEVERA	GRAPE	SUGAR	PALM SUGAR	WHEAT GRAIN	YEAST
CONTROL	-	2kg	1/2kg	-	200g	3g
VARIATION 1	1/2kg	3kg	-	1kg	300g	5g
VARIATION 2	3/4kg	3kg	-	1kg	300g	5g

Wine was prepared under the above mentioned proportions and combinations and made comparison with the control.

Organoleptic evaluation of the wine

Sensory evaluation is the process of evaluating according to the knowledge acquired via human senses like sight, taste, touch, and smell(yager,2000).The sensory characteristics like appearance, flavour, taste, consistency and overall acceptability were evaluated through 5 point hedonic scale from 25 panel members.

Physiochemical analysis of the wine

The pH,Total soluble solids,acidity, and moisture for all the selected products were estimated by using standard procedures.The percentage of ethanol was evaluated for prepared wine.

Nutrient analysis of the wine

The energy,carbohydrate, protein, vitamin c,calcium,iron and total antioxidant activity were estimated for selected best aloe vera grape infused wine by using standard procedures (AOAC, 2000).

Storage stability of the wine

Preservation method is followed when the fruits are available in abundant amount and to be kept for future use without effecting its nutritional quality, taste, and acceptability and to stored in room temperature for long time. The prepared wine were stored in glass bottles at room temperature for 90 days.the sensory attributes were evaluated for every 15th day during the study period(90 days) to find out the storage stability. The storage stability(1st to 90th) was assessed through microbial analysis, *i.e.* total plate count, yeast and mold count.

Statistical interpretation of the data

The results obtained were tabulated and statistically analysed. The statistical tools like mean, standard deviation, T test were used for interpreting the results.

III. Results and Discussion

Sensory analysis of wine

The quality has been defined as degree of excellence and includes colour, consistency, taste, appearance, flavour and nutritional content. A five-point scale was used to elicit the sensory attributes.

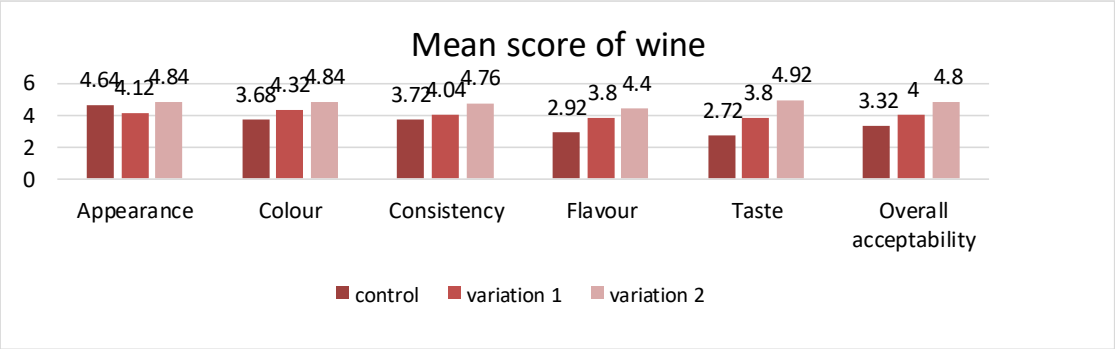


Figure -1 Mean sensory score of wine

Physico-chemical properties of wine

Table 2. Comparison of physico chemical properties between control and variation 2

S.no	Parameters	control	Variation 2	*p value*
1	pH	4.2	4.0	
2	Total soluble solids (%)	30.1	21.5	0.369232*
3	Titrable acidity (%)	0.68	0.67	
4	Ash content(g)	2.06	3.62	
5	Moisture (%)	30.2	29.2	
6	Alcohol (%)	7	6.06	
7.	Total sugars (%)	20.0	25.5	

The values are expressed in mean ±standard deviation, *significant difference

The statistical attributes like pH, Total soluble solids,Titrable acidity,Ash content, Moisture, Alcohol,Total sugars, showed slight significant difference **($p \leq 0.05$)** between control and variation 2 at 5%.

Nutrient Composition of Wine

The nutritive value parameters like energy, carbohydrate,Total antioxidant activity, protein Vitamin c, Iron,calcium showed slight significant difference ($p \leq 0.05$) between control and variation 2 at 5%.

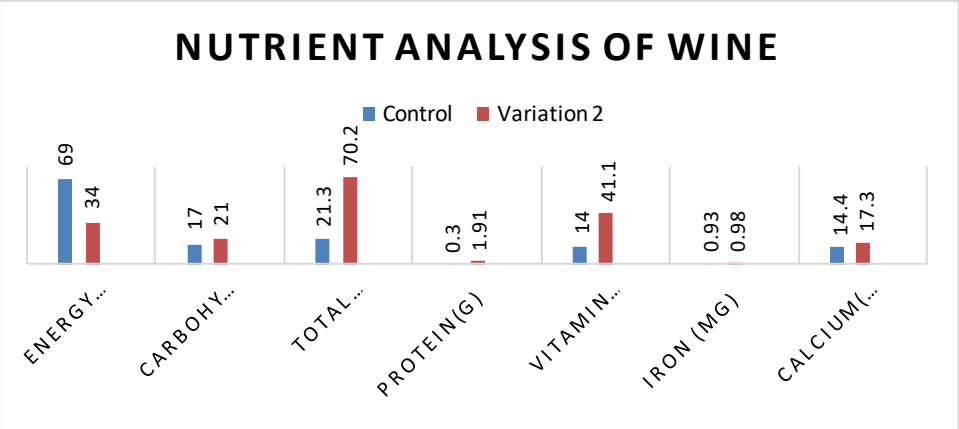


Figure-2. Nutrient analysis of wine

Storage Stability of Wine

Table 3 .Mean Score of Sensory Attributes on Storage Comparison

S. no	Product	15days	30days	45days	60days	75days	90days
1.	Variation 2	4.5±0.42	4.3±0.42	4.2±0.42	4.1±0.42	4.0±0.42	3.9±0.33
2.	Control	4.3±0.39	4.3±0.49	4.2±0.42	4.1±0.38	4.0±0.42	3.7±0.33

The organoleptic evaluation of selected product was done in every 15 days for analysing the storage stability and acceptability of the products. The acceptability of sensory attributes of the product were good even on 90th day but the mean score was found to be less when compared to initial.

Microbial analysis of selected wine:

Table 4.Microbial Analysis of Wine

S.no	Product	Total plate count (cfu/ml)		Yeast and mold count (cfu/ml)	
		Initial	Final	Initial	Final
1.	Variation 2	<10	1×10^3	600	1×10^3
2	Control	<10	2×10^3	600	3×10^3

The final total microbial count of aloe vera grape wine are 1×10^3(cfu/ml) respectively on the 90 days of storage. There was 1×10^3 of yeast and mold count till 90 days. This was highly stable at room temperature without the addition of any preservatives.

IV. Conclusion

The prepared wine from aloe vera and grapes were found to have high acceptability. It was found to be the good source of nutrients and antioxidants and hence it could be used for regular consumption at minimum level at 1to 2 teaspoon per day. Hence it could be concluded that the widely available aloe vera and grapes could be extensively exploited by processing in various forms.

V. References

[1] Arnous, A., & Meyer, A. S. (2008). Comparison of methods for compositional characterization of grape (Vitis vinifera L.) and apple (Malus domestica) skins. *food and bioproducts processing*, *86*(2), 79-86.

[2] Attri, B. L. (2009). Effect of initial sugar concentration on the physico-chemical characteristics and sensory qualities of cashew apple wine.

[3] Calhelha, R. C., Andrade, J. V., Ferreira, I. C., &Estevinho, L. M. (2006). Toxicity effects of fungicide residues on the wine-producing process. *Food microbiology*, *23*(4), 393-398.

[4] Chemat, F., & Khan, M. K. (2011). Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonicssonochemistry*, *18*(4), 813-835.

[5] Choi, S., & Chung, M. H. (2003, March). A review on the relationship between Aloe vera components and their biologic effects. In *Seminars in integrative medicine* (Vol. 1, No. 1, pp. 53-62). WB Saunders.

[6] Christaki, E. V.,**&Florou-Paneri**P. C. (2010). Aloe vera: a plant for many uses. *J Food Agric Environ*, *8*(2), 245-249.

[7] Corral-Aguayo, R. D., Yahia, E. M., Carrillo-Lopez, A., & González-Aguilar, G. (2008). Correlation between some nutritional components and the total antioxidant capacity measured with six different assays in eight horticultural crops. *Journal of Agricultural and Food Chemistry*, *56*(22), 10498-10504.

[8] criteria in Penicillium taxonomy and a synoptic key to Penicillium subgenus Penicillium. In *Modern Concepts in Penicillium and Aspergillus Classification* (pp. 373-384). Springer, Boston, MA.

[9] Crosswhite, F. S., &Crosswhite, C. D. (1984). Aloe vera, plant symbolism and the threshing floor. *Desert plants*.

[10] Devi, N. S., HariPrasad, T., Ramesh, K., &Merugu, R. (2015). Antioxidant Properties of Coconut Sap and its Sugars. *International Journal of PharmTech Research*, *8*(1), 160-162.

[11] Downey, M. O., Dokoozlian, N. K., &Krstic, M. P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture*, *57*(3), 257-268.

[12] Downey, M. O., Harvey, J. S., & Robinson, S. P. (2003). Synthesis of flavonols and expression of flavonol synthase genes in the developing grape berries of Shiraz and Chardonnay (Vitis vinifera L.). *Australian Journal of Grape and Wine Research*, *9*(2), 110-121.

[13] Du Toit, M., & Pretorius, I. S. (2000). Microbial spoilage and preservation of wine: using weapons from nature's own arsenal–a review. *S. Afr. J. Enol. Vitic*, *21*, 74-96.

[14] Farahi, A., Kasiri, M., Sudagar, M., Soleimani, I. M., &Zorriehzahra, S. M. J. (2012). Effect of dietary supplementation of Melissa officinalis and aloe vera on hematological traits, lipid oxidation

VI.Acknowledgement

The authors thank the Management, Principal and the Department of Nutrition and Dietetics of Dr.N.G.P.ASC for having offered the laboratory facilities to undertake the project in a successful manner.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Review on Non-Dairy Based Ice Cream with Fortification

S. Mythra[1], A. Sangeetha[2], C. Raagul[3], T. Surya[4]

Department of Food Technology, Kongu Engineering College, Perundurai, Erode – 638052

INDIA, mythrashanmugam98@gmail.com

Abstract*: Ice cream is a composite food colloid that comprises of an unfrozen serum phase, air bubbles and ice crystals and fat globules. The main ingredients of ice cream are milk, solid-not-fat, fat, sucrose, emulsifiers and stabilizers. But dairy based milk cannot be consumed by people with lactose intolerance. The effects such as stomach pain, bloating, diarrhea, head ache, etc are caused by lactose mal-absorption. Other alternative for the dairy based milk has been reviewed and concluded with Cocos nucifera milk and Arachis hypogaea milk . Cocos nucifera milk has comparatively high fat content with increased taste, texture and viscosity. Arachis hypogaea milk has higher amounts of minerals andvitamins. The aim of this research is to formulate a non-dairy based ice cream using peanut and coconut milk and to fortify it with herbs, as ice cream will be a good carrier for herbs to increase the nutrient content and to study the physico-chemical properties, microbiological quality of the ice cream.*

Keywords*: Non-dairy milk, Cocos nucifera, Arachis hypogaea, fortification,physiochemical properties*

I. Introduction

Ice cream is a delicious and nutritious processed milk product which is consumed by all age groups all over the globe. Soy milk is rich in protein and hard fibre and unsaturated fat, minerals and vitamins, polysaccharide, amino acids, lecithin as well as bioactive polyphenols such as isoflavones, phenolic acids, saponins and tannins [1]. Coconut (*Cocos nucifera*) milk contains large amounts of vitamins (C, E and many B vitamins), minerals (particularly calcium, phosphorus, potassium, selenium and sodium) and antioxidants. Coconut milk contains large quantity of oleic acid and lauric acid content and thus have antiviral,antibacterial,and antiprotozoal properties[2]. Peanut (Arachis hypogaea) milk is a non-dairy drink produced using peanuts and water, it does not contain any lactose and hence advisable for lactose intolerance people [3]. Chickpea milk is a good source of essential vitamins such as riboflavin, niacin, thiamine, folate and the vitamin A precursor β-carotene. Hence, Peanut milk and coconut milk are economical sources that comprise of high nutritional quality and health benefits. Thus, it could be a suitable choice in ice cream making [4]

II. Ice cream

Ice cream is made by a mixture of air, water, dairy fat or nondairy fats, milk solids-not-fat (MSNF), sweeteners, stabilizers, emulsifiers and flavors. An ice cream mix is the unfrozen combination of the ingredients, except the air and flavoring materials [5]. To match the standards of United States Food & Drug Administration, ice cream should contain at least 10% dairy fat by weight. Besides contributing the ice cream with the creaminess and smooth texture,fats join with proteins, emulsifiers, and air bubbles to create a three-dimensional structure that prevents the ice cream from rapidly breaking up when it melts. Milk proteins interconnect with fat globules in order to suspend and disperse them in the ice cream and thus stable emulsion is formed. Further emulsifiers—for example, mono glycerides or diglycerides disrupt the fats and combine partially, forming a network of fat globule clusters which supports air bubbles throughout the ice cream. The whipping also encourages the ice crystals to disperse ice crystals all through the ice cream mix. Once the freezing process ends up, around 75% of the water in ice cream is freezed and the remaining forms a liquid phase that comprises dissolved sugars and other ingredients and all the way through which the fat globules air bubbles, and ice crystals are suspended. Another main element of the serum phase is stabilizing gum—like locust bean or guar gum —which controls generation and growth of ice crystals. Repeated thawing and refreezing of ice cream can cause ice crystals to grow faster and larger, which lends rough and grainy texture. Stabilizers help in preventing this and keep water molecules in the serum from moving together and forming crystals.

III. Non-dairy supplements

Cow's milk holds a magnificent nutrient profile. It is loaded with high-quality protein and predominant vitamins and minerals, including calcium, phosphorus and B vitamins. However, cow's milk is not an appropriate choice for everyone. There are various reasons to go for a substitute, including milk sensitivity, lactose intolerance and dietary limitations. Milk sensitivity can cause various symptoms, including rashes, vomiting, diarrhea and acute anaphylaxis in 2–3% of children under the age of three are sensitive to cow's milk (about 80% of children outgrow this sensitivity by age 16). Approximately 75% of the globe's community is intolerant to lactose, the sugar present in milk. This situation occurs when people have a deficiency in lactase, the enzyme present in the body which digests lactose. It causes various effects such as stomach pain, bloating, diarrhea, head ache etc. With respect to dietary limitations, some people wish to eliminate the animal products from their diets for moral or health reasons. There are many non-dairy options available [6]

Owen Densel and Haley Burtch tested for taste, texture and viscosity with 3 variables of ice cream, one was made from coconut milk, the other was made from 2% dairy milk and the another was made from half coconut milk and half 2% dairy milk. The result shown that coconut milk had greater fat content, smoothest texture and the greatest viscosity compared to dairy milk. Thus it was concluded that when dairy milk was replaced by coconut milk in ice cream, a smoother and more preferred ice cream, along with a greater viscosity was obtained. It was observed that there was no statistical difference in taste between coconut milk and dairy milk [7]

Prajakta Nande and Priti Tapadia compared ice cream made from animal milk and soy milk. The result shown that, comparatively, soy milk had the lowest fat content, highest solids-not-fat and highest acidity level. But the shelf life of soy milk was poor. As the nutritive value was taken into account, soy milk had the lower energy (in kcal), protein (in g), fat (in g), carbohydrates (in g) and calcium (in mg). It was concluded that ice cream made from soymilk yielded less amount of energy, fat and carbohydrates and thus reduce the risk of diabetics and heart disease. Also it was said that soymilk was cheaper than cow milk and can act as an asset for those who suffer from lactose intolerance [8]

Kaushal Kishor and John David, studied the nutritional composition of chickpea milk. Chick pea milk enhanced children nutritive status and their blood parameters. Chickpea is rich in carbohydrates and proteins, which together comprises of about 80% of the total dry seed mass. The starch content of chickpea has been described to vary from 41% to 50%. Chickpea milk was highly convincing in the controlling of untiring diarrhea and comprises natural protein. There was noticeable increase in total protein, red blood cell, haemoglobin and hematocrit values in children. Pea nut milk has excellent effects on some of the major human diseases such as cardiovascular disease, Type 2 diabetes, digestive problems and some cancers. It was said that chickpea milk can be employed to develop nutritious value added products [9]

Although other milk substitutes were used in ice cream, coconut milk is chosen its fat content and texture, chick pea milk for its nutritional content.

IV. Fortification

Hemali H. Patel and Bijal K. Amin fortified ice cream with guava pulp for its high ascorbic acid content, extraordinary digestive and nutritive value, pleasing flavor, high palatability and plenty availability at moderate price. It is a good source of certain minerals like calcium, phosphorus and iron which are essential for human health. The appearance, consistency and over all acceptability were all good [10]

Filiz Yangılar formulated the ice cream which was fortified with peach fibre. Peach (Prunus persica L.) fruits have high profitable and nutritional value. It is loaded with carbohydrates, minerals, dietary fiber and organic acids. Peach fruits have therapeutic properties and are used for the treatment of duodenum ulcers. It was concluded that Peach fiber alone or with ice cream stabilizers could be used victoriously in ice cream production [11]

Shukla Sudha and Daniel Madhvi developed ice cream fortified with beetroot green pulp because of its high vitamin A content. Beet roots are a rich source of folic acid, potassium and dietary fiber. It was concluded that there is a significant increase in vitamin A, protein, calcium, fat, iron and fiber [12]

Studies have shown that ice cream can also be fortified with herbs in order to increase its nutrient content and its quality.

V. Formulation

There are numerous variations of formula, dairy components, sweeteners, stabilizers and emulsifiers, flavors, colors, freezing method, sizes, shapes and other variables that would create opportunities for the development of

a wide variety of products. These components can be combined in varying proportions within acceptable ranges. The composition of ice cream varies in different countries and in different localities [13]

Table 1 shows Preliminary trials for sensory Evaluation

Trial	Ingredients used	Result	Inference
1	Full cream milk, sugar, flavour, stabilizer		Good taste Smooth and creamy texture Less crystal formation
2	Full cream milk, sugar, herbs, egg		Good texture and taste Less crystal formation Bitterness and flavour change after few days
3	Peanut milk, coconut milk, sugar, stabilizer		Improper texture Thick consistency Strong flavour

VI. Conclusion

According to the reviews discussed, objectives of the work have been framed to develop a non dairy based ice-cream fortified with herbs. The work has been divided into 2 phases. The first phase is to develop ice cream with milk extracted from coconut and peanut and to study its physio- chemical properties. Next phase is to fortify the formulated ice cream with suitable herbs based on nutritional properties and sensory acceptance.

References

[1] M. Abdullah, Saleem-ur-Rehman, H. Zubair, H.M. Saeed, S. Kousar and M. Shahid, "Effect of Skim Milk in Soymilk Blend on the Quality of Ice Cream", Pakistan Journal of Nutrition 2(5): 305-311,2003

[2] Aboulfazli. Fatemeh, Shori. Amal Bakr, Baba. Ahmad Salihin, "Effects of the replacement of cow milk with vegetable milk on the count of probiotics and changes in sugar and amino acid contents in fermented ice creams", February 2016

[3] Perugu Balachandra Yadav, "Studies on the Preparation of Peanut Milk and Milk Powder", July 2016

[4] Kaushal Kishor, John David, Snehlata Tiwari, Anoop Singh and Bhole Shankar Rai, "Nutritional Composition of Chickpea Milk", International Journal of Chemical Studies 2017; 5(4): 1941-1944
[5] H.D. Goff and R.W. Hartel, Ice Cream "Compositions and Formulations," DOI 10.1007/978-1-4614-6096-1_2, 2013
[6] Emma Hiolski, special to C&EN, "Innovation in Food Science continually Improve on this Classic Sweet Treat", volume 96, Issue 31
[7] Owen Densel and Haley Burtch, "Coconut Milk as Dairy Replacer in Ice Cream", NUTR 453 Research Project, 12/01/2014
[8] Prajakta Nande, Priti Tapadia, Kruti Jain, Frenal Lodhaya and Sabiha A.Vali, "A Study On Soymilk As A Substitute For Animal Milk," J. Dairying, Foods & H.S.,Vol. 27, No. 1, 2007
[9] Kaushal Kishor, John David, Snehlata Tiwari, Anoop Singh and Bhole Shankar Rai, "Nutritional Composition of Chickpea (Cicer arietinum) Milk," IJCS 2017
[10] Hemali H. Patel and Bijal K. Amin, " Formulation and Standardization of Different Milk Ice-Cream Fortified with Pink Guava Pulp," International Journal of Dairy Science, 10: 219-227 , 2015
[11] Filiz Yangılar, "Production and evaluation of mineral and nutrient contents, chemical composition, and sensory properties of ice creams fortified with laboratory-prepared peach fibre," Food and nutrition research,2016,vol 60:31882
[12] Shukla Sudha and Daniel Madhvi, "Vitamin A Fortification of Vanilla Ice Cream Using Beetroot Green Pulp," IJW, Vol. 1, No.1, 2014
[13] H.D. Goff and R.W. Hartel, Ice Cream "Compositions and Formulations," DOI 10.1007/978-1-4614-6096-1_2, 2013

Acknowledgments

We take immense pleasure to express our heartfelt thanks to our beloved project guide Ms. A. Sangeetha M.Tech., Assistant Professor, Kongu Engineering for her constant encouragement and support provided all through the project.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Influence of wall material on microencapsulation of *Nigella Sativa* Seed Oil

Gothainayagi[1], Josephine Nirmala Many[2]
*Ph.D Scholar[1], Associate Professor[2]
PG & Research Dept of Home Science
Bharathidasan Govt College for Women (Autonomous)
Affiliated to Pondicherry University, Puducherry, India.
e-mail ID: gothaifsn789@gmail.com, nirmalamany@gmail.com

Abstract: Encapsulation is a process by which small particles of core products are packaged within a wall material to form microcapsules. *Nigella Sativa* seed oil have a wide variety of biological activities and therapeutic properties, microencapsulation process by freeze drying using different wall material will protect the functional component of *Nigella Sativa* seed oil from chemical and biological degradation. Wall material maltodextrin and gum acacia was used as encapsulating material in the ratio of oil-wall material was 1:1 and 1:1.5. The microencapsulation effectiveness was determined in base of process yield and the microencapsulation efficiency. Highest encapsulation was higher in maltodextrin (92.6±0.23%) and oil was encapsulated at 67%. The impact of wall material on *Nigella Sativa* oil was determined and the prepared emulsion was characterized through zeta potential particle size analyzer and viscosity measurements and a morphological characterization of microcapsules was performed by scanning electron microscope.

Keywords: Microencapsulation; Freeze Drying; Maltodextrin; SEM; *Nigella Sativa* Oil

I.Introduction

Microencapsulation is a process by which an active core material is encapsulated in a shell of limited permeability that will protect active core material from external environment until the right stimulus encountered. This technique allow liquid or solid such as drugs, proteins, hormones, fertilizers and other small particle encased in a uniform shell. The composition of the shell material may vary depending on the method used and can be formed from natural type material such as starch, gelatin, modified starch and cellulose, acrylates and other synthetic polymers. Microencapsulation of bioactive molecules has been efficiently used in increasing of bioavailability of the drugs and nutrients [3]. The microencapsulation strategy will preserve their physical stability during processing, storage and prevent from deleterious interaction with food matrix and the environment [4,5]. Moreover, microencapsulation technique has capable to increase bioactivity owning to their micrometer size range.

Functional oils from agricultural or marine origin are those which contain the biologically active omega-3, omega-6 or omega-9 fatty acids. These oils are frequently used in culinary or as dietary supplements. Microencapsulation of these oils in powder form using different drying medium for ease of handling and protection against oxidation. Freeze-drying, also known as lyophilization or cryodesiccation, is a simple process used for the dehydration of almost all heat-sensitive materials and aromas like oils. Before drying, the oil is dissolved in water and frozen (between −90 °C and −40 °C) and then the surrounding pressure is reduced and enough heat is added to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase [1].

Seeds of *Nigella sativa* is considered among the functional oils due to its rich content of omega-6 and omega-9 fatty acids including linoleic (54.0–70.0%) and oleic (15.0–24.0%), respectively[2]. Crude oil from *nigella sativa* seeds showed a protective effect onnerve cells beside other biological activities.*Nigella sativa* crude oil also contains a small fraction of a volatile oil that has functional properties due to its major biologically active constituent, thymoquinone [8,9]. Due to these functional properties and advantages, microencapsulation of *Nigella sativa* crude oil in freeze dried powder is required as an approach to facilitate handling and application in different food and nutraceutical products in addition to potential protection against oxidation. Encapsulating agent Gum Acacia(GA) and Maltodextrin (MD) were used at the composition at 1:1 and 1:1.5 weight ratios [18]. The emulsion characteristics such as the droplets size, viscosity,as well as powder properties such as particle size, surface oil, the morphology were investigated to determine the influence of wall materials used.

II.Materials & Methods

A. Materials:

A commercial 100% cold –pressed *Nigella Sativa* Seed Oil was used in the study (Herbal Store. Puducherry). Maltodextrin (DE 10) and gum acacia were purchased from Sigma Aldrich Inc. St Louis, MO, USA.

B. Methods:

1.Emulsion preparation:

Aqueous solution of wall material containing maltodextrin and gum acacia were prepared by dispersing them in hot distilled water at 50°C. *Nigella Sativa* Seed Oil was then added to the dissolved wall materials at oil/wall material ratios 1.1 and 1:1.5. Emulsion was prepared by homogenizing the above mixture at a speed of 22 000 rpm for 4 min sing a laboratory blender (Model: IKA Ultra Turrax, Janke & Kunkel GmbH, Staufen, Germany) at RT (25_1°C).

2. Emulsions Characterization:

2.1 Emulsions stability:

The stability of emulsions was determined by placing 10ml of each emulsions before freeze drying in a test tube and stored at 25°C for a period of one day. The emulsion stability was analyzed by the phase separation observed throughout 24 h after emulsion preparation.

2.2 Emulsions Size:

The droplet size distributions of feed emulsions were determined by using a laser-based static light scattering particle size analyzer Malvern Mastersizer Hydro 2000G (Malvern Instruments Ltd., Worcestershire, UK). The sample was suspended in distilled water using magnetic agitation at stir speed of 850 rpm. The refractive index of sample and dispersant is 1.5 and 1.33, respectively, with absorption of 0. The emulsion droplet size was expressed as the mean volumetric size (d43; De Brouckere mean diameter).

2.3 Emulsions Viscosity:

The viscosity of emulsions was measured using a Viscometer (M/s Rheology International Shannon Ltd., Shannon, Ireland) at 25°C fitted with a rotating spindle (no. 3) at a constant shear rate of 100 rpm.

3. Microencapsulation process:

Emulsions were prepared, frozen at -80°C, freeze-dried by a VIRTIS lyophilizer, Mod. Genesis 25 LL Hucoa-Herlos. After the lyophilization process microcapsules were grinded and transferred to double layer plastic bags.

4. Powder Analysis:

4.1 Surface oil content and microencapsulation efficiency:

Briefly, 5 g of microcapsules were precisely weighted in a beaker and 50 mL of hexane was added and shaken during 15 s at ambient temperature to extract superficial oil [20]. The solvent mixture was then filtered throughout a filter paper, and after that, unencapsulated oil was collected after vacuum hexane evaporation. To measure the encapsulated oil, oil was extracted by 4 h Soxhlet of the same powders. After that, hexane was evaporated and internal oil was scaled. Free oil content was then calculated as percentage taking into account the total oil.

4.2 Moisture Content:

The moisture content of the powders was determined using infrared moisture analyzer (XM 120; Precisa Instruments Ltd, Switzerland).

4.3 Bulk Density:

2g of powder was transferred to a 10 ml graduated cylinder. Packed bulk density was calculated from the height of powder in the cylinder after being tapped by hand.

4.4 Particle morphology:

Scanning electron microscopy (LEO 1455VP, Cambridge, UK) was used for determination of surface morphology of the fish oil microparticles. The samples were placed directly onto the SEM sample holder using double-sided sticking tape and was gold spray-coated.

III.Results & Discussions

3.1 Emulsion Preparation:

Wall material of two different ratios was evaluated (Table 1). In model 1 wall material and oil was mixed in 1:1 ratio and in model 2 wall material and core oil was mixed in 1:1.5 ratio and tween 80 was used as emulsifier. Carbohydrates such as maltodextrin, gum acacia was used extensively as encapsulating material. Combined wall material will result in higher oil yield hence maltodextrin and gum acacia was used in combination for the encapsulation of *nigella sativa* oil.

3.2 Emulsion Characteristics:

3.2.1 Emulsion Stability, viscosity, and droplet size

The emulsion were prepared (Table 2) and evaluated for mean volume diameter (d43), viscosity and phase separation. 24 h stability study revealed that emulsion was kinetically stable. Selected wall material possess high binding properties and functional properties required to encapsulate hydrophobic core materials, both

Maltodextrin and Gum Acacia has good emulsion stabilization process. Model 1 and 2 had shown no phase separation.

Viscosity of emulsion has increased in the Model 2 since the concentration of wall material in higher. Increase in wall material concentration reported similar observation for liquid emulsion prepared with maltodextrin which showed high viscosity [6].

The emulsion droplet size or volume diameters of emulsion varied from 0.8-0.5µm. Droplet size is often reported to be related with viscosity where the most viscous emulsion has a smaller droplet mean diameter. The increase in emulsion viscosity would lead to reduce the rate of particle sediment or creaming [15]. Hence, preventing droplet coalescence and emulsion with lower droplet size can be obtained. There is a relationship between the droplet size of the emulsion and the particle size, the smallest microcapsules always obtained from the emulsions with the smallest droplet size.

Table I Wall Material Composition

Formulation	**Model 1**	**Model 2**
Oil	25	20
Maltodextrin	12	14.5
Gum Acacia	12	14.5
Tween 80	01	01
Ratio	**1:1**	**1:1.5**

Table II Mean bulk density, moisture, particle size, viscosity, stability, encapsulation efficiency

Ratio of wall to core	**Emulsion Characteristics**			**Powder Analysis**				
	d 43(µm)	**Viscosity (Pa.s)**	**Phase separation (24h)**	**Bulk Density (g/cm^3)**	**Moisture (%)**	**Total Oil (%)**	**Surface Oil (%)**	**Encapsulation Efficiency (%)**
Model 1	0.8± 0.00	10.24±0.12	No	0.23±0.015	2.72±0.1	38.2 ±1.24	14.6±0.86	92.6±0.23
Model 2	0.5± 0.00	13.14±0.26	No	0.25±0.019	2.66±0.1	32.5 ±1.68	14.2±0.74	89.2±0.68

3.3 Powder Analysis:

3.3.1 Bulk Density:

The bulk density of the powder of model 1 was 0.23 and model 2 was 0.25g/cm3, model 2 having the highest bulk density. The sample with higher bulk density could compact more under various loads, and less storage space is needed when compared to samples with lower densities. In addition higher bulk densities result in less occluded air being present in the powders, thereby decreasing the possibility for oxidative degradation [14] Bulk density of food powders is mainly affected from the particle size and shape, density of the wall and core material and moisture content of the microcapsules [10]. As a general rule small, round, and irregular particles have higher bulk density [5]. According to Bae and Lee [12], higher particle size of the microcapsules results the lower bulk density.

3.3.2Moisture Content:

Moisture content is an important variable for the shelf life of powders. The moisture content of the model 1 and 2 showed 2.72% and 2.96% . Interaction of wall material content and oil concentration had a significant effect on the moisture content of model 1 and 2. The increasing wall material content with a higher ratio of oil concentration at low temperature resulted in reduction in moisture content [14]. In addition, an increase in the wall material content was exhibited when water available in a low level for evaporation, leads to a reduction in the moisture content. Fernandes [22] reported that the moisture content of microencapsulated Lippia sidoides oil was significantly reduced with increasing wall material content.

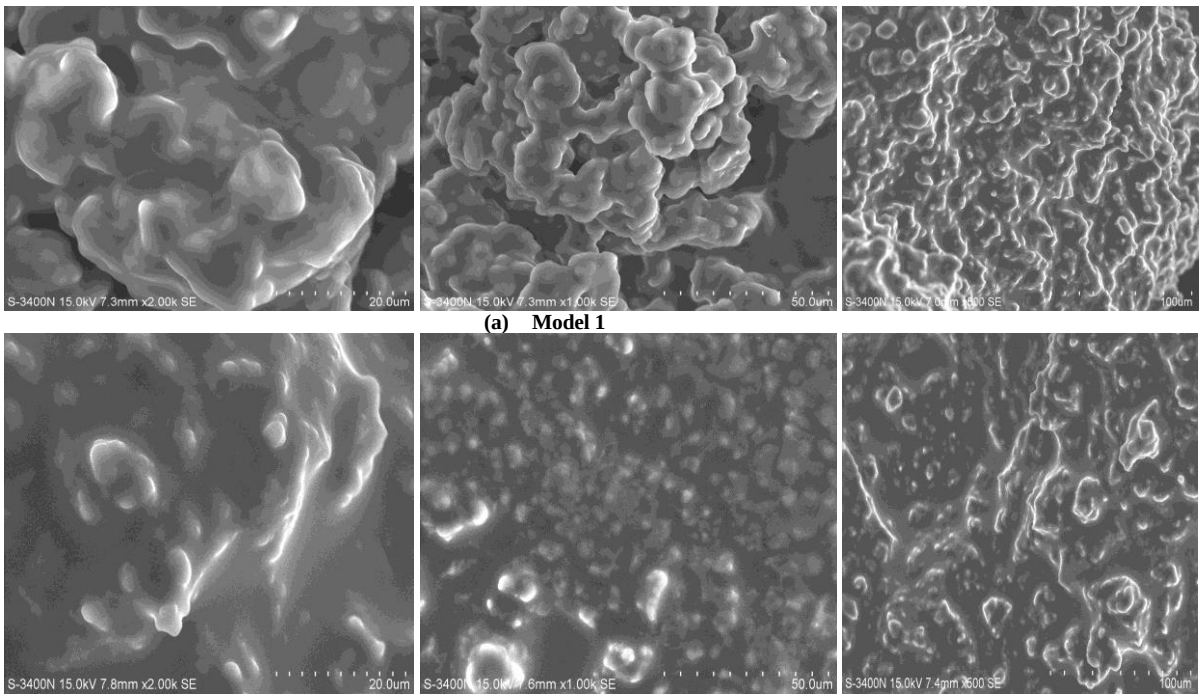
3.3.3 Encapsulation Efficiency:

To evaluate the influence of wall materials on the microencapsulation efficiency, the amounts of surface oil and total oil of the powders were determined. The surface oil is crucial for the oxidative stability of the powder as the surface oil may oxidize easier in the presence of oxygen [11]. Table 2 shows the percentage of surface oil obtained and thus the encapsulation efficiency influenced by ratio of wall materials used. The encapsulation efficiency values varied from 89.2% to 92.6%. The combination of maltodextrin and gum acacia in different ratio produced a system with the lowest surface oil and, hence, the highest encapsulation efficiency. Maltodextrin helps to improve the drying properties of the wall matrix by enhancing the formation of a dry crust around the drying droplets [21]. Results have been observed in a study of microencapsulation of flaxseed oil where a wall material combination of GA with MD had higher microencapsulation efficiency [7]. Many studies

have revealed that GA has good film forming ability leading to the formation of a macromolecular stabilizing layer around oil droplets. It is revealed that use a high gum to oil weight ratio (1:1) to produce stable droplets [33].

3.4 Powder Morphology:

Fig. 1 illlustrates the surface charateriscitcs shape and size of the freeze dried *Nigella sativa* seed oil using scanning electron microscopy SEM. In this studymorphology of freeze dried powder is irregular in structure, very light, highly porous and has a large surface area. Based on its microstructure, the microcapsules can facilitate diffusion of oxygen from the air onto the particle surface, which gets into the inner part of the particle due to the amorphous state of the matrix. Because of this accessibility, oxygen can easily decompose lipids that are not protected by matrices and those on the particle surface. Surface or non-encapsulated oil or free oil is responsible for high formation of peroxides in freeze-dried samples in many studies [16]. The literature suggests that the microstructure of free oil on the freeze-dried oil-in-water emulsions was comprised of discontinuous oil deposits, caused by emulsion destabilization during freezing and drying, rather than in the form of individual oil globules [17].



(a) Model 1

(b) Model 2

Figure 1 Scanning Electron micrographs of Freeze dried powder (a) Model 1 (b) Model 2

IV. Conclusion

Knowledge about wall composition can help the research to design appropriate encapsulating coating materials. Coating of*Nigella Sativa* oil with mixture of Maltodextrin and Gum Acacia in 1:1 ratio produced stable emulsion and found superior to 1:1.5 ratio with respect to encapsulation efficiency and surface oil seems to have a protective effect against oxidation. Freeze drying has proved to be suitable to protect the bioactive compounds from the indigenous herb oils and economical process for drying. The major disadvantages include high energy use, long processing time, and high production costs compared to other drying methods. Freeze-dried ingredients may have higher porosity, thereby exposing the core material to the surrounding environment.However, the porous structures of freeze-dried bioactive products offer a higher drug release. The findings of this study may provide information of suitable ratios for encapsulating oils by freeze drying used for future research.

Acknowledgement

The first author acknowledges Dr.M.R.Prakaash, CEO, Aarupadai Vedu Medical College & Hospital (AVMC) for supporting during the work and Dr.T. Leema Roseline for timely help. Authors acknowledge Dept of Food Science & Technology, Central Instrumentation Facility, Pondicherry University and Dept of Pharmcognosy, Mother Theresa Postgraduate & Research Institute for utilizing facilities.

References

[1] Yuqi Pang, Xu Duan, Guangyue Ren, and Wenchao Liu. Comparative Study on Different Drying Methods of Fish Oil Microcapsules. *Journal of Food Quality* Volume 2017, Article ID 1612708, 7 pages.
[2] J. Barbosa, S. Borges, M. Amorim et al., "Comparison of spray drying, freeze drying and convective hot air drying for the production of a probiotic orange powder," *Journal of Functional Foods*, vol. 17, pp. 340–351, 2015.
[3] R. Singh and J.W. Lillard, "Nanoparticle-based targeted drug delivery" *Experimental and molecular pathology*, 86(3), pp.215–23, 2009.
[4]. C. B.Wandrey and A. Harding, "Materials for encapsulation", 2010.
[5]. V. Nedovic, "An overview of encapsulation technologies for food applications*". Procedia Food Science*, 1, pp.1806–1815, 2011
[6] S. Ramakrishnan, M. Ferrando, L. Ace˜na-Mu˜noz, M. Mestres, S.De Lamo-Castellv´ı, and C.G¨uell, "Influence ofEmulsification Technique andWall Composition on Physicochemical Properties and Oxidative Stability of Fish OilMicrocapsules Produced by Spray Drying," *Food and Bioprocess Technology*, vol. 7, no. 7,
pp. 1959–1972, 2014.
[7] Carneiro HCF, Tonon RV, Grosso CRF, Hubinger MD.Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinationsof wall materials. *J Food Eng* 2013;115:443–51.
[8] Koç, M., Güngör, O., Zungur, A., Yalçın, B., Selek, _I., Ertekin, F., & Ötles, S. (2015).Microencapsulation of extra virgin olive oil by spray drying: Effect of wallmaterials composition, process conditions, and emulsification method. *Food andBioprocess Technology*, 8, 301–318
[9] Tulukcu, F. (2011). A comparative study on fatty acid composition of black cuminobtained from different regions of Turkey, Iran and Syria. *African Journal ofAgricultural Research*, 6, 892–895
[10] T.C. Kha, et al., Microencapsulation of gac oil by spray drying: optimization of wall material concentration and oil load using response surface methodology, Dry. Technol. 32 (4) (2014) 385–397
[11] Ramakrishnan S, Ferrando M, Aceña-Muñoz L, Mestres M, De Lamo-Castellví S & Güell C (2012) Fish Oil Microcapsules from O/W Emulsions Produced by Premix Membrane Emulsification. Food Bioprocess
Technology, DOI 10.1007/s11947-012-0950-2, in press
[12] Bae, E. K., & Lee, S. J. (2008). Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. Journal of Microencapsulation, 25, 549–560.
[13] Kim, Y. D., & Morr, C. V. (1996). Microencapsulation properties of gum Arabic and several food proteins: Spray dried orange oil emulsion particles. Journal of Agriculture and Food Chemistry, 44, 1314–1320.
[14] Tonon RV, Grosso CRF, Hubinger MD. Influence of emulsioncomposition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Food Res Int 2011;44:
[15]Dickinson E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*2003;17:25–39.282–9S.
[16] Sri Haryani Anwar, Benno Kunz. The influence of drying methods on the stabilization of fish oil microcapsules:Comparison of spray granulation, spray drying, and freeze drying. *Journal of Food Engineering* 105 (2011) 367–378
[17] Gejlhansen, F., Flink, J.M., 1977. Freeze-dried carbohydrate containing oil-in-wateremulsions – microstructure and fat distribution. *Journal of Food Science* 42 (4),1049–1055.
[18] Krishnan, S., Kshirsagar, A. C., Singhal, R. S., The use ofgum arabic and modified starch in the microencapsulation of a food flavoring agent. *Carbohydr. Polym*. 2005, 62, 309–315.
[19] Reineccius, G. A. (2004). Multiple-core encapsulation. The spray drying of food ingredients. In P. Vilstrup (Ed.), *Microencapsulation of Food Ingredients* (pp. 151–179). Surrey, UK: Leatherhead
[20] Calvo P, Castaño ÁL, Lozano M, González-Gómez D. Influence of the microencapsulation on the quality parameters and shelf-life of extra-virgin olive oil encapsulated in the presence of BHT and different capsule wall components. *Food Res Int* 2012;45: 256–61.
[21] Calvo P, Castaño ÁL, Lozano M, González-Gómez D. Microencapsulationof refined olive oil: influence of capsule wallcomponents and the addition of antioxidant additives on theshelf life and chemical alteration. *J Sci Food Agric*2012;92:2689–95
[22] Fernandes, R., Borges, S., Botrel, D., & Oliveira, C. (2014). Physical and chemical properties of encapsulated rosemary essential oil by spray drying using wheyprotein-inulin blends as carriers. *International Journal of Food Science &Technology*, 49, 1522–1529.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

FORMULATION OF ICE CREAM FROM PEANUT (*ARACHIS HYPOGAEA*) MILK

Sharmila R*, KalaiRajan P, Nandhini A and Shenbagam T

*Assistant Professor, Department of food technology

K .S.Rangasamy college of technology

K.S.R Kalvi nagar, Tiruchengode

kalai6101998gmail.com, nandhininainika@gmail.com

Abstract: Ice cream is a frozen sweetened product made out of heat treated mix consisting of edible fat and milk solids with or without other ingredients and permitted additives. In this study Peanut Milk (*arachishypogaea*) was used as the main source for the preparation of Ice Cream. For that Peanut Milk Ice Cream, physico-chemical, nutritional quality and sensory evaluation was carried out. Peanut milk was rich in proteins, fibers, polyphenols, antioxidants, vitamins and minerals which can be added as a functional ingredient to the ice cream. The study was divided in to 2 phases. First phase deals with the collection of raw material (whole milk, peanut milk, fresh cream & other additives) and preparation of ice-cream was done by six different trials(control, A, B, C, D & E). Pasteurization was performed to inactivate the enzymes and microbes present in peanut milk. All the above ingredients are mixed well and it was filled in air tight container. Then it is stored in freezer condition at -5˚C. Second phase deals with the analysis of physico-chemical property, nutritional quality and sensory evaluation. From the result, it has been found that the incorporated .According to the sensory analysis, the aroma, taste and flavour of peanut milk ice cream trial D was scoring higher than other trials. In future, Peanut milk ice cream will beavailable in wide range of flavors.

Key words: Ice Cream, Peanut milk, Fat, Trials, Pasteurization, Physico-chemical, nutrient analysis and Commercial value

1.Introduction

Ice cream is a liquid mixture it will turns into a paste after simultaneously shaking and cooling [1], though the definition of ice cream varies from country to country due to different composition and traditions of regulation ([2],[3]). The ice cream mix will become ice cream with so many elements of different nature as sugars, fats, dairy, stabilizer, water, among others. And it must be blended correctly and emulsified together so there will be nothing left behind that, so it may reduce the quality of the final product[1].

Smooth ice cream requires the majority of ice crystals to be smaller than about 50 µm in size. Ice crystals larger than 40–55 µm leave a coarse and sandy texture [4]. Ice crystals in ice cream must be generally range from 20 to 100 µm [5]. The Fat content influences the size of the ice crystals in fat globules and impede the ice crystal growth [4].

The United States of America, Peanuts (*Arachis hypogaea L.*) are one of the important oil crops in the world. The major production of peanut in the world are China, India, Nigeria, Argentina, Sudan, Burma, Indonesia and seeds are a rich source of edible oil (43–55%) and protein (25–28%). Peanuts are important for the diet of the Aztecs and other native Indians in South America and Mexico[6].

The peanut milk is an non dairy beverage, prepared by using water and peanuts. Ingredient variations include salt, sweeteners, and grains. It does not contain lactose and it will be suitable for people with lactose intolerance. This process is same as the production of almond milk, soy milk, and rice milk. The peanut milk is prepared by typically ground, soaked an sometimes heated, and then filtered through a fine filter, the resulting liquid is considered as peanut "milk"[7].

2. MATERIAL AND METHODS:

2.1 RAW MATERIAL:

The raw material, such as; whole milk, peanut, sugar, CMC, GMS powder, corn flour, cream, essence were purchased from local market erode Tamil nadu, India.

2.2 PREPERATION OF PEANUT MILK:

Dry matured peanuts were purchased from the open market in erode, Tamil nadu. About 100g of peanut was soaked in water for 6 to 8 hours. Then kernel was removed from peanut seeds and cleaned.Peanut seeds was washed and comminuted using an electric blender with 600 mL of warm kuwater. This was then pressed through a music cloth and strained to obtain peanut milk.

2.3 PREPERATION OF ICECREAM :

Peanut milk ice cream was prepared by composition given in the table 1 [8].

Boiling the mixture (till the quantity reduce to 1/3rd)
▼
Addition of sugar, corn flour, CMC and GMS powder
▼
Mixing the mixture thick
▼
Cooling the mixture at room temperature
▼
Blending the mixture (1 min) incorporation of cream, essence
▼
Setting the mixture in the freezer (-4 to -5°C) for 5 h
▼
Removing the mixture from the freezer and beating for 20 min
▼
Setting the mixture in the freezer (-4 to -5°C) for 5 h
▼
Freezing and storing the ice cream in the freezer (-4 to -5°C)
▼
Removing the mixture from the freezer and beating for 20 min
▼
Freezing and storing the ice-cream in the freezer (-4 to -5°C)

Fig.1 Ice-cream making process above

Table 1: Composition of peanut milk ice cream and their variants

Ingredients	Control	A	B	C	D	E
Whole milk(ml)	300	250	200	150	100	50
Peanut milk(ml)	-	50	100	150	200	250
Sugar (g)	90	90	90	90	90	90
GMS(g)	3	3	3	3	3	3
CMC(g)	1	1	1	1	1	1
Corn flour(g)	6	6	6	6	6	6
Cream(g)	100	100	100	100	100	100
Vanilla essence	3-5 drops	-	-	-	-	-

2.4 TRIAL ANALYSIS

The ice-cream sample Control, A, B, C, D, E were analyzed for their different sensory properties such as aroma, taste, colour, appearance and texture. Finally the highest scoring trial was selected for the further analysis.

2.5 PROXIMATE ANALYSIS OF PEANUT ICECREAM:

The peanut ice cream was analyzed for Total Soluble Soilds (TSS), pH, titrable acidity, ash, ascorbic acid, Moisture content, total carbohydrates and Protein. A refractometer was identified and used for testing the TSS of peanut ice cream. The pH of peanut milk was determined by using digital a digital pH meter. Acidity of peanut ice cream was determined with standard NaoH using phenolphthalein as an indicator. Muffle furnance was used for determination of ash. The ascorbic acid was determined using standard iodine using starch as an indicator. The Moisture content was determined in a digital Moisture analyser. The total carbohydrate content of the sample was determined by the phenol sulfuric acid method. Protein content was estimated by Lowry ‘s method.

2.6 MICROBIAL ANALYSIS:

Microbial count was estimated by serial dilution method. Using decimal dilution technique original suspension is serially diluted from the appropriate dilution 10^{-3} (for fungal growth) and 10^{-7} (for bacterial growth).

2.6 SENSORY QUALITY EVALUATION

The sensory analysis was performed on a 9-point Hedonic scale by a panel of 12 semi trained members including different age categories. The jelly was evaluated for color, appearance, flavor, taste and overall acceptability.

3. RESULT AND DISCUSSION

3.1 TRIAL ANALYSIS

Ice creams (Control, A, B, C, D, E) was analysised on the basic of aroma, taste, flavor, Texture & mouth feel characteristics. Then concluded that by Trial D was scoring high compared other trials. Trial D was selected as the perfect composition for the Peanut milk ice cream.

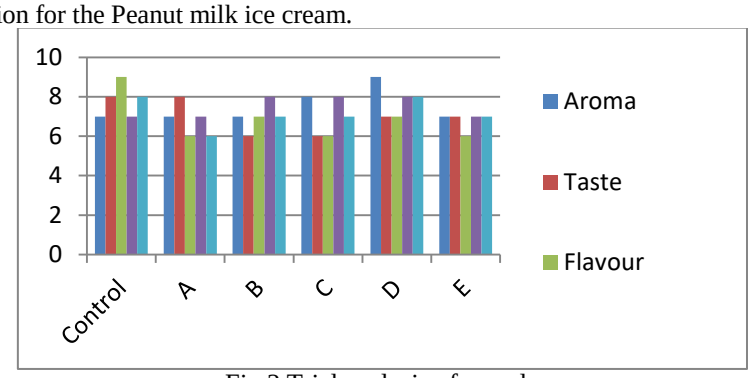


Fig.2 Trial analysis of sample

3.1 GENERAL APPEARANCE

Peanut ice-cream is generally soft ,the trial D texture is so good when compared to the other trials. So we concluded by trial D for processing such as proximate analysis and microbial analysis.

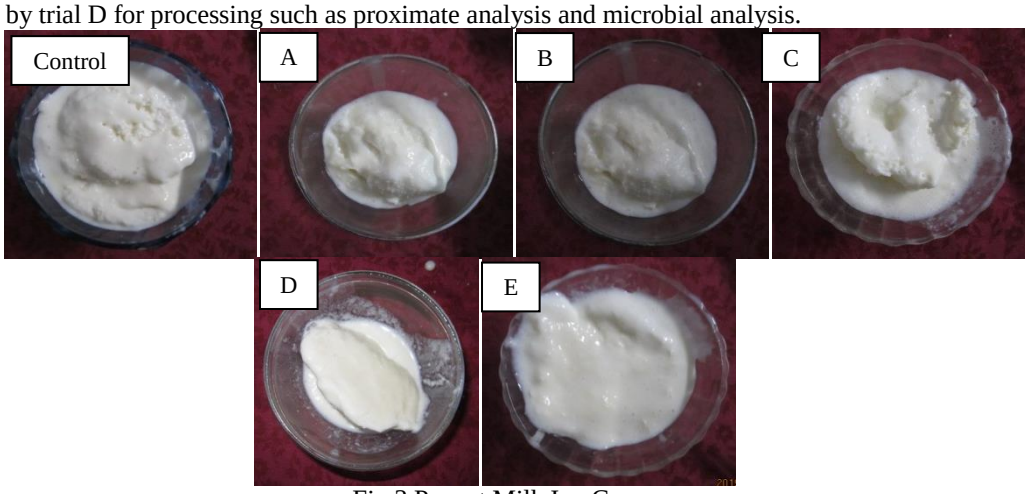


Fig.3 Peanut Milk Ice-Creams

3.2 PROXIMATE ANALYSIS

The physico-chemical characteristics of peanut ice cream It was evaluated which are shown in Table 2. The values were taken for the discussions.

Table 2: Nutritional composition of peanut milk ice cream

S. No	Nutrient	Peanut milk ice cream
1	Total soluble solids (º Brix)	35
2	pH	6.75
3	Titrable acidity (%)	0.667
4	Ash Content (%)	0.762
5	Ascorbic acid (mol/lit)	0.00028
6	Moisture content (%)	51.07
7	Total carbohydrates(mg/100g)	7.092
8	Protein (mg/100g)	6.325
9	Fat(mg/100g)	8.156

3.3 MICROBIAL ANALYSIS

Table 3 showed the enumeration of microbial load in peanut milk during storage period. Initially there was no bacterial and fungal population in the product. At the end of the storage period the bacterial count in product was 13 and fungal count for product was 0.

Table 3: Microbial Load of peanut milk ice cream

Microbial Count	**Initial day**	**Final day(15 days)**
	Product A	**Product**
Bacterial Count 10^{-7} (CFU/g)	0	13
Fungal Count 10^{-3} (CFU/g)	0	0

3.4 SENSORY EVALUATION

The result of the sensory analysis by the different panelist was calculated into a single average value and given bellow the Table 4.

Table 4: Sensory Evaluation Table

Content	**Product**
Appearance	8
Taste / Flavor	7
Texture / Consistency	8
Aroma / Smell	8
Overall Acceptability	7

4. CONCLUSION

We concluded that peanut ice-cream were highly nutritious and gives balanced diet. From the result it is concluded that, the characteristic of peanut ice-cream were analyzed by physiochemical properties, microbial analysis and sensory evaluation successfully. The peanut milk is a good source of ascorbic acid, Among all official method we used muffle furnace method for ash analysis. The acidity of the sample was determined by direct titration with 0.1 molar sodium hydroxide, using phenolphthalein as indicator. The ascorbic acid concentration was determined by titration method. Measurement of pH relates to acidity or alkalinity. The pH of peanut ice cream is alkalinity and studies the shelf life of peanut milk ice cream by microbial analysis. The shelf life of the peanut ice cream was analyzed with the help of microbial plate count. As final conclusion the peanut ice cream is good nutritive product, being natural product with important concentration of some micronutrients.

5.REFERENCES

[1] Fiol, Camila, et al. "Introduction of a new family of ice creams." *International Journal of Gastronomy and Food Science* 7 (2017): 5-10.

[2] Clarke, Chris. *The science of ice cream*. Royal Society of Chemistry, 2015.

[3] Goff, H. D., and W. K. Jordan. "Action of emulsifers in promoting fat destabilization during the manufacture of ice cream." *Journal of Dairy Science* 72.1 (1989): 18-29.

[4] Marshall, Robert T., H. Douglas Goff, and Richard W. Hartel. *Ice cream*. Springer, 2012.

[5] Buyong, Nurliza, and Owen Fennema. "Amount and size of ice crystals in frozen samples as influenced by hydrocolloids." *Journal of Dairy Science* 71.10 (1988): 2630-2639.

[6] Salve, Akshata, and Shalini Arya. "PHYSICAL, CHEMICAL AND NUTRITIONAL EVALUATION OF Arachis hypogaea L. SEEDS AND ITS OIL." *Journal of Microbiology, Biotechnology & Food Sciences* 8.2 (2018).

[7] Sobolev, Victor S., and Richard J. Cole. "Note on utilisation of peanut seed testa." *Journal of the Science of Food and Agriculture* 84.1 (2004):105-111.

[8] Patel, Hemali H., and Bijal K. Amin. "Formulation and Standardization of Different Milk Ice-Cream Fortified with Pink Guava Pulp." *InternationalJournalofDairyScience* 10(2015):219-227.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Development of Value Added Health Mix using Pumpkin Seeds and Almonds

Dr. B. Premagowri

Assistant professor Department of Nutrition and Dietetics

Dr.NGP Arts and Science College (Autonomous), Coimbatore, Tamilnadu

India, msgpremagowri@gmail.com

Abstract

Mental health includes our emotional, psychological and social well being. Globally, an estimated 322 million people were affected by depression was reported by WHO in 2015. A multivitamin supplement is never going to replace the things missing in the diet to its fullest. Pumpkin seeds and almonds have many beneficial effects as it contains antioxidants, vitamins, polyphenols and unsaturated fatty acids that becomes protective against the development of cognitive decline and depression. A number of well- controlled depression treatment studies have found therapeutic benefits following n-3 supplementation. Nuts and seed powders can be used as nutritional supplements in the production of various instant food mixes that are ready to cook and in great demand presently. Majority of the health mixes are made from different cereals and pulses. Value added health mix was developed using pumpkin seeds and almonds with three variations. They were variation-1 (pumpkin seeds-40g, almonds-20g), variation-II (pumpkin seeds-30g, almonds-30g) and variation-III (pumpkin seeds-20g, almonds-40g). The standardized best value added health mix (variation-I)was evaluated for sensory quality, physicochemical, nutritional characteristics, cost effectiveness, consumer acceptability and popularization with respect to control. The organoleptic scores for variation-1 got the highest mean score for all sensory attributes. The proximate properties and the physico-chemical and the storage stability have contributed favorable results. Free samples were provided to random customers visiting supermarkets and showed positive results. Hence the value added health mix could be a best naturally flavored health mix for all age groups.

Key words: Health mix, antioxidants, pumpkin seeds, almonds)

I. Introduction

Globally, an estimated 322 million people were affected by depression in 2015 (WHO, 2016) A sedentary lifestyle paves way for depressiveness and associated conditions .Smoking, alcohol consumption and lack of exercise conspicuous in both genders.

New researches has fixed the idea of positive mental health is responsible for good physical health and biological functioning. A review states evidence of high positive affect in terms of joy and happiness is connected with lower morbidity, increased longevity and preferably less health symptoms (Cohen, 2005).

Pumpkin seeds and almonds have many beneficial effects as it contains antioxidants, vitamins, polyphenols and unsaturated fatty acids that becomes protective against the development of cognitive decline and depression.(Maes *et al.*,2010) . Pumpkin seeds and almonds can improve mood by their role in synthesis of neurotransmitters. Nuts and seeds have omega-3 fatty acids that can act as mood boosters that modulate the magnitude of inflammatory responses to stressors (kiecolt et al., 2015)

The effect of serotonin on mood has been investigated using an acute tryptophan depletion technique where lowering dietary tryptophan levels causes a lowering of brain serotonin levels, allowing analysis of serotonin-dependent behavior (Young, 2013) Finally, in remitted depressed patients, temporarily lowering tryptophan levels can result in an acute depressive Also, low serotonin can contribute to a lowered mood state(Smith *et al.*,1997).

Approximately 200g of pumpkin seeds provides around one gram of tryptophan which helps in inducing better sleep. A study in the Journal of Research and Medical Science found that supplementation of 500mg of magnesium appears to improve insomnia in the elderly. That's about ½ cup of pumpkin seeds and 1 cup of

cooked leafy greens daily.(Mila,2013). Almonds contain B vitamins, folate, tyrosine, a precursor to dopamine .usually that are depleted by stress.

Rodent studies have shown that acute tryptophan depletion reduced tryptophan levels in the brain by up to 70% (Lieben *et al*.,2004) with associated decreases in central serotonin and reduced 5-HT receptor binding (Cahir,2017)). In humans acute tryptophan depletion inhibits serotonin synthesis (Nishizawa,1997) and also lowers cerebrospinal fluid concentrations of tryptophan (williams,1999) and 5-hydroxyindoleacetic acid (5-HIAA), the major serotonin metabolite (Moreno et al.,2010). Hence the present study was carried out with the following objectives.

- To develop a naturally flavoured health mix packed with nutrients replacing synthetic flavours
- To assess the organoleptic evaluation of the developed value added health mix
- To estimate the nutrient and microbial analysis of the value added health mix.
- To calculate the cost of the value added health mix
- To create awareness and popularize the value added health mix and benefits

II Methodology

Selection of ingredients The study was carried out to develop a good nutritious health mix prepared from locally availabale ingredients like pumpkin seeds, almonds, nutmeg, cardamom, milk powder. a. Pumpkin seeds: Appearance :- For this study the pumpkin seeds were collected from the locally available natural food sections of the grocery store.These edible seeds are a medium-dark green color (white shell has been removed)(Casio,2015) Size- The seeds were measured for its length and width Length and width of 10 seeds were measured using electronic digital calipers.the average length,width of the seeds were 16.91mm and 8.67mm respectively. Nutrient composition: In this study the particular seed variety is chosen as it comprises of beneficial fatty acids and 30% unsaturated fatty oil (linoleic and oleic fatty acids).Triterpenoids, flavonoids, coumarins, saponines, cucurbitacins, vitamins, minerals, notably zinc amino acid,tryptophan. (Fu *et al.,* 2006). b.Almonds : Appearance: For this study, Non - pareill variety is chosen, they are medium sized, flat shape,bright skin colour,fairly wrinkled.this variety is easily blanched as per almond board of California in India 1970. Size The nuts were measured for its length and width using electronic digital caliper, the average length and width of the nut were 25.49mm and 17.03mm respectively. Nutrient composition: In this study the particular nut is chosen as it is said to be rich in tryptophan,zinc,mg,and found to improve mood by their role in synthesis of neurotransmitters. c.Cardamom Appearance : For this health mix early matured ,dark green,bold capsules were selected. It belongs to idukki (kerala) variety Nutritrient composition: Since cardamom can supply melatonin to the body to start up the sleep cycle, it is included in the health mix. d.Nutmeg Appearance: Whole nutmeg is approximately the size of an apricot pit and has an indefinite shelf life. Because nutmeg is so strongly flavored, only a small amount is needed to impart a large amount of flavor as per Indian Institute of Spices Research kozhikode ,so whole nutmegs were chosen to retain its flavour. Nutrient composition : Researchers published a study in the *Avicenna Journal of Phytomedicine* that stated that nutmeg or "myristica fragrans" has the ability to boost mood and exhibit antidepressant properties comparable to that of imipramine, which is medication used to treat depression.So nutmeg is included in the health mix for flavour . Nutmeg is a rich source of myristicin, a natural compound that acts to inhibit the release of certain enzymes associated with stress.

Processing and development of health mix

In this study the value added health mix was processed using the following steps like soaking, blanching, drying, dehydrating, grinding and mixing. The application of heat blanching to fruits and vegetables before air-drying is, therefore, aimed at stopping enzymatic activity and avoiding undesirable changes to the sensory and nutritional properties during drying and storage, enhancing product quality (Chantaro *et al*., 2003).

Standardization of the health Mix

The Three different variations in three different proportions were standardized. The base ingredients like almonds and pumpkin seeds were mainly altered at different proportions

TABLE – I
Standardization of Value Added Health Mix

S.No.	criteria	Pumpkin seeds (g)	Almonds (g)	Milk powder (g)
1	Variation-1	40	20	30
2	Variation-2	30	30	30
3	Variation-3	20	40	30

Organoleptic evaluation of health mix

Sensory quality is a combination of different senses of perception coming into play in choosing eating a food. Appearance, flavor, taste ,texture and aroma decide the acceptance of the food. The prepared health mix drink were given to 25 semi – trained panel members with care to evaluate the products for various sensory parameters such as colour, taste, texture, flavour and overall acceptability depending upon the type of the product using a five point scorecard that was developed on the basis of numerical rating scale(5-like moderately to 1- dislike extremely). Efforts were made to keep the same panel for sensory evaluation throughout the entire period of study. The samples were presented to the judges the way they are normally consumed. Plain water was given to the judges to rinse their mouth in between the evaluation of samples for evaluating the sensory characteristics of the product.

Nutrient Analysis of health mix

The variant which was selected to be the most acceptable among the three variations was considered and it is used for further analysis of nutrients content. Proximate composition such as Energy, Protein ,carbohydrate, fat, moisture ,total ash and omega 3 fatty acid were analysed.

Storage stability of health mix

Product durability is assessed by storage stability tests. This is to investigate the character of the product, its properties and the quantitative composition of its contents under storage conditions. This type of investigation can also be extended to cover the container. The prepared health mix was stored in two different packaging materials (Plastic container and ALP.) and both are stored at refrigerated condition for a period of 30 days and the microbial contamination were tested during the initial and final day. Packaging plays a visible and catalytic role in a modern economy, with the widespread and adoption of branding and development of products according to consumer preferences. It enables to preserve the quality and increase the shelf life of innumerable products (Soroka,2002).

Microbial Analysis of health mix

Microbial analysis is a term used to identify bacteria and other microbial growth .microbial testing is very essential in food processing to ensure food safety and quality product to the consumer. Microbial testing at various points of products is essential quality control fact. (Jay,2005) Microbial analysis of the standardized product was done after 30 days after initial packaging stored at room temperature

Consumer acceptability

The value added health mix developed was given free samples to the supermarkets near Thondamuthur. 50 random customers who visited the supermarkets were given free samples of the developed product and commercially available product to taste to find the consumer acceptability.

Packaging of health mix

The health mix is packed in ALP (Aluminium Laminated pouches).In a study of development of instant mixes using dehydrated pumpkin powder used ALP. This packaging creates less waste and more efficient, which ultimately conserves resources .It have moisture resistence, good chemical resistance, readily processed

III Results and Discussion

Sensory evaluation of the health mix The mean values scores for colour, flavour ,taste, appearance, overall acceptability are presented in table

TABLE – II

Mean Sensory Scores of Value Added Health Mix

S.No	Criteria	Control	Variation I	Variation II	Variation III
1	Appearance	4.52±0.5	5 ±0	4.32 ±0.4	3.96± 0.5
2	Colour	4.48± 0.5	4.76± 0.4	4.2± 0.4	4.12± 0.3
3	Consistency	4.76± 0.4	4.84± 0.3	4.24± 0.4	4.24± 0.4
4	Taste	5± 0	5± 0	4.52± 0.5	4.28± 0.4
5	Aroma	4.56 ±0.50	4.88 ±0.3	4.76± 0.4	4.08± 0.2
6	Overall acceptability	4.8± 0.4	5± 0	4.3± 0.4	4.2± 0.4

From the below table it is clear that that the mean score of variation-I was found to be high when compared to that of variation-II and variation-III. Hence it is clear that the presence of highest amount of pumpkin seeds is highly acceptable. In a study they concluded that instant porridge mix incorporated with granulated pumpkin seeds powder seem to be highly acceptable (Vidhya,2015). It was found that appearance, colour, consistency, taste, aroma And overall acceptability of health mix were influenced by the incorporation of pumpkin seeds and nutmeg.

TABLE III

Nutrient Content of Value Added Health Mix

Parameters	Result
Energy	381.31kcal
Carbohydrates	77.35gm
Fat	3.11gm
Protein	9.64gm
Moisture	2.86%
Total Ash	2.04%
Omega 3 Fatty Acid	3.2gm

The health mix contained 3.11 g and 9.64g of fat and protein respectively. It yields about 381.31kcal. It contains about 3.2g of omega 3 fatty acid since the health mix is rich in pumpkin seeds and almonds.

Microbial analysis

The packed health mixes were enumerated for microbial analysis after 30 days of packing kept in room temperature .since it was found to have zero microbial count by the end of the analysis it is proved to have stable shelf life. The reason for the no bacterial count is that the health mix preparation was done at the utmost hygiene conditions. Gloves were used for each and every step during the preparation. All vessels and storage containers were sterilized before use and made sure that they were devoid of moisture.

Only airtight containers were used for storing the ground spices to retain its flavour and aroma. Almonds and pumpkin seeds were blanched and used and they were dried using tissue towel after peeling their skin to remove the excess moisture on them. Even they were defatted during dehydration so it prevents from getting spoiled as a result of rancidity due to the oils present in the almonds and pumpkin seeds

Packaging of health mix

In a study of development of instant mixes it was found that the instant mix packed in ALP(Aluminium laminated pouch) retained more crude protein as compared to(PolyethylenePouch) during storage(PEP)This might be due to better conditions inside ALP as compared to PEP (**vidhya,2015**). The health mix was packed in aluminium laminated pouches and immediately sealed using heat sealing machine.and this is the reason why it had more stability during storage.

Consumer Acceptability

Consumer acceptance was analyzed by providing the product to 50 customers. The mean score was 4.8 ± 0.8 for like extremely. Consumers needed a variety in taste in usually available health mixes and found the developed value added health mix satisfying their needs. The developed value added health mix scored high in aroma since pumpkin seeds in hot milk gives a unique aroma. Additionally no artificial flavours were added to the value added health mix that helped in scoring more

V. Conclusion

Pumpkin seeds and almonds have many beneficial effects as it contains antioxidants, vitamins, polyphenols and unsaturated fatty acids that becomes protective against the development of cognitive decline and depression. Pumpkin seeds and almonds can improve mood by their role in synthesis of neurotransmitters. Nuts and seeds have omega-3 fatty acids that can act as mood boosters that modulate the magnitude of inflammatory responses to stressors . Nuts and seed powders can be used as nutritional supplements in the production of various instant food mixes that are ready to cook and in great demand presently.

IV References

[1]. Cahir, M.; Ardis, T.; Reynolds, G.P.; Cooper, S.J. Acute and chronic tryptophan depletion differentially regulate central 5-HT 1A and 5-HT 2A receptor binding in the rat. Psychopharmacology 2007, 190, 497–506.

[2] Feder, A.; Skipper, J.; Blair, J.R.; Buchholz, K.; Mathew, S.J.; Schwarz, M.; Doucette, J.T.; Alonso, A.; Collins, K.A.; Neumeister, A.; et al. Tryptophan depletion and emotional processing in healthy volunteers at high risk for depression. Biol. Psychiatry 2011, 69, 804–807.

[3 Hamilton L, Greiner R, Salem N Jr, Kim HY. n-3 fatty acid deficiency decreases phosphatidylserine accumulation selectively in neuronal tissues. *Lipids* 2000;35:863-869.

[4] Jay. Storage of food products. Packaging techniques. Nostrand publishers; 2005; p. 87 – 92.

[5] Kiecolt-Glaser JK, Belury MA, Porter K, Beversdorf D, Lemeshow S, Glaser R. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. Psychosom Med 2007;69:217–24.

[6] Maes M, Christophe A, Bosmans E, Lin AH, Neels H. In humans, serum polyunsaturated fatty acid levels predict the response of proinflammatory cytokines to psychologic stress. Biol Psychiatry 2000;47:910–20.

[7] Mila Wood ; http://www.weekendnotes.com; Seeds and nuts to boost your moods; June 14th, 2013

[8] Report of the World Health Organization.,Department of Mental Health and Substance Abuse in Collaboration with the Victorian Health Promotion Foundation and University of Melbourne. Report 2016. WHO,Geneva

[9] Smith, K.A.; Fairburn, C.G.; Cowen, P.J. Relapse of depression after rapid depletion of tryptophan. Lancet 1997, 349, 915–919.

[10] Soroka (2002) *Fundamentals of Packaging Technology*, Institute of Packaging Professionals ISBN 1-930268-25-4

[11] Vidhya Devi,Development of instant food mixes from dehydrated pumpkin 2015-Krishikosh.egranth.ac.in

[12] Young, S.N. Acute tryptophan depletion in humans: A review of theoretical, practical and ethical aspects. J. Psychiatry Neurosci. JPN 2013, 38, 294–305

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Effect of Hydrocooling and application of Anti-Microbial Extract on Quality attributes of Selected Tropical Fruits

R.Renu [1], Dr Kavita Waghray [2], Dr P.Dinesh Sankar Reddy [3]

1,2 Department of Food Technology, University College of Technology, Osmania University, Hyderabad, Telangana

3 Department of Chemical Engineering, College of Engineering, JNTU, Anantapur, Andhra Pradesh

1 renu.pillai96@gmail.com

Abstract: *Tropical fruits such as Mangoes and Sapota are highly perishable which makes it mandatory to subject them to cooling. On the other hand, Edible antimicrobial coating has emerged as a major criterion to reduce Post-Harvest losses. The present work was aimed to develop Hydrocooling technique and to determine the effect of use of antimicrobial extract on Mangoes and Sapota stored at ambient and Low Temperature storage. Fruits such as mango and Sapota with and without neem leaf extract were cooled using Hydrocooling system. The Core Temperature of Fruits during cooling was recorded and it was found that for mangoes the core temperature was reaching to 20-25˚C and that for Sapota it was 14-16˚C. Cooling time for Mango and Sapota was 40 and 20 mins respectively. Physical analysis which includes Percentage loss in weight (PLW), Chemical analysis which includes Total Soluble Solids (TSS), pH and Microbial analysis were carried out at regular intervals for fruits stored at recommended storage temperature. Hydrocooling of fruit Mango and Sapota at 14˚C and 20 ˚C using anti-microbial extract, wrapped in LDPE pouches stored at low temperature and attained a maximum shelf life of 25days and 16 days compared to control (without extract). There was no much significant effect of coating on quality attributes of fruits but significant effect on microbial quality of stored fruits.*

KEYWORDS: Hydrocooling, Anti-microbial extract, cooling time, Percentage loss in weight (PLW), Total Soluble Solids (TSS).

I. Introduction

Hydrocooling is a form of precooling in which the product is sprayed with or immersed in an agitated bath of chilled water. The use of systems utilizing cool water (hydrocooling) is an interesting technology, allowing high heat-transfer rates, which can result into three times-shorter cooling times in comparison with products cooled by forced air, or ten times, when products are placed in conventional or storage room [7,14,4,5,16].Fruits with different geometries and sizes are cooled differently when submitted to similar conditions (Baird & Gaffney, 1976). Oranges (r= 3.75 cm) hydrocooled at 1ºC reach cooling temperature in 57.5min, while limes (r = 3.01 cm) are cooled in 30 min [15,16]. Sapota being climacteric fruit, has very short shelf life and marketing of fresh fruits to distant places is very difficult [3]. Also, the fruit exhibits large postharvest losses because of its high respiration rate [6]. Optimum storage temperature for sapodillas is either 0 or 20°C for fruit at the turning or ripe stages, respectively [13] while for sapote mamey it is between 10and 15°C [2]. Mango (Mangifera indica L.) is an important tropical fruit with a rich nutritional composition (fiber, minerals, organic acids, vitamins and polyphenolics) and high marketing value but a relatively short shelf life at ambient temperatures. [12]. Cold storage is used extensively to extend the shelf life of crops; however, mangoes are susceptible to chilling injury (CI) when stored below 10–13℃ [18,19]. Various chemicals and waxing material are used to enhance the shelf life of fruits which are believed to be ecologically unsafe and economically not viable beside leaving their residue on the fruit surface, which may have the direct effect on human health [10]. Hence, there is a huge requirement to replace these chemical origin or wax coating material with natural material. A number of post-harvest treatments like waxing, fungicidal dip, polyethylene film, etc. have been used to extend the shelf life of fruits. The extracts of neem (Azadirachta indica L.) have antifungal and antimicrobial properties [11]. The objectives of the work were to develop Hydrocooling system, with the application of antimicrobial (neem leaf) extract on tropical fruits such as Mango and Sapota. A study on physio-chemical properties of cooled fruits was also carried out.

II. Collection of samples and Neem (Azadirachta indica) Leaves:

Uniform ripe and free from damage fruits like Mangoes, and Sapota were procured from local market. Fresh leaves of Azadirachta indica were obtained from the campus of the Osmania university. The leaves were ground using mixer to get a fine paste.

III. Hydrocooling unit construction:

In this study, two thermocol boxes were mounted one over the other. Top thermocol box acts as water reservoir to which showers were fixed at the base and second box acts as a precooling chamber in which commodity placed for cooling. First box with showers was placed on the supporting stands and precooling chamber placed exactly below the reservoir. Fruits were weighed on digital scale. water reservoir was filled with chilled water ($7^{O}C$) and neem leaf extract added along with chilled water. The fruits were placed in the Hydrocooling chamber and were subjected to cooling. Retention time of commodity in the hydrocooler varied according to fruit core temperature. Internal temperature of fruits was measured by using thermocouple into the fruit center and holding for about 1min.The water temperature was held between 4 to 7°C. Thermocouples were inserted into the fruits according to the r/4 (spherical products) or r/3 (cylindrical products) relation. Fruits were taken out and wiped with clean cloth after attaining required temperature and then wrapped in LDPE pouches. precooled fruits were kept for storage under ambient and Low storage temperature. Control was without coating and hydrocooling.



Fig 1: Hydrocooling unit

IV. Cooling time

Cooling time is a parameter that can be used to evaluate the efficiency of fast-cooling systems for commercial and/or research purposes. The term related to cooling time are considered, namely half cooling time (TAT 1/2). This parameter (time) can be determined by the Dimensionless Temperature Rate (TAT½) :

$$\text{TAT}(½) = \frac{Tp-Ta}{Ti-Ta} = 0.5$$

where Tp is the temperature measured in the product during cooling, Ti is the initial temperature of the fruit, and Ta is the temperature in the cooling medium (water at 7ºC).

V. Per Cent Loss in Weight (PLW)

Per cent loss in weight (PLW) was determined by weighing the fruits after 4 days interval during storage with the equation used

$$PWL = \frac{W1 - W2}{W1} x100$$

Where W1: Weight of sample before storage, W2: Weight of sample after storage

VI. Total Soluble Solids

The TSS was determined by a Hand Refractometer of 0-32ºBrix.Results were expressed in ºBrix .

VII. Determination of pH

To determine the pH, the pH paper was dipped in a fruit pulp and the colour developed in the paper is compared with the colour chart and the approximate pH of the solution can be identified.

VIII. Microbial Analysis

(a)Total Plate Count Method:

The nutrient agar medium is taken and dissolved in distilled water according to formulation requirement. The sa mple is prepared by making solution of 5gms of coated fruit pulp in 45m1 of distilled water. Under the sterilized conditions conduct the analysis. Take l ml of sample in a petri plate and pour the agar media in it. After solidify ing. the petri plates are kept in incubator for 24hrs at 30±1°C. After 24hrs the total plate count is done by usin g colony counter and results are noted.

IX. Results and Conclusions:

Cooling of fruits: Intial temperature of Mangoes and Sapota was 35˚C and 25˚C. Cooling time of fruits and vegetables varied proportionally with volume, Temperatures dropping to 14˚C and 20 ˚C in time span of 40min and 20min (Fig 2,3).

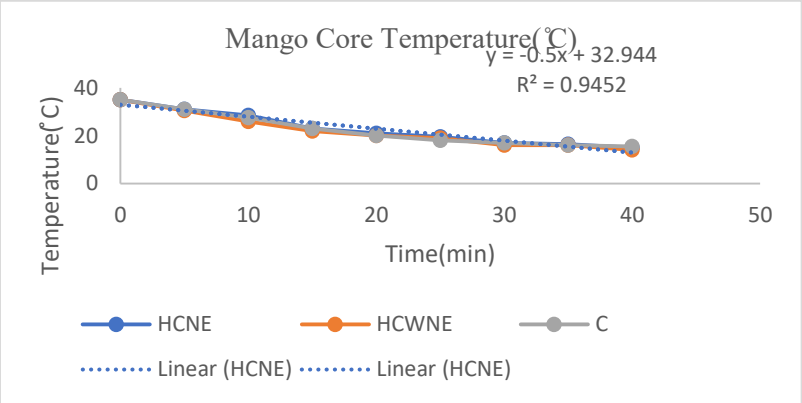


Fig 2: Graphical representation of time versus Fruit core temperature

Weight Loss was measured for regular intervals. Hydrocooled and wrapped (with LDPE pouches) fruits with and without neem leaf extract were found to have less weight loss (Fig 5,6) compared to control and unwrapped fruits (without neem leaf extract) in low temperature storage [9]. There was no much significant change in TSS and pH.

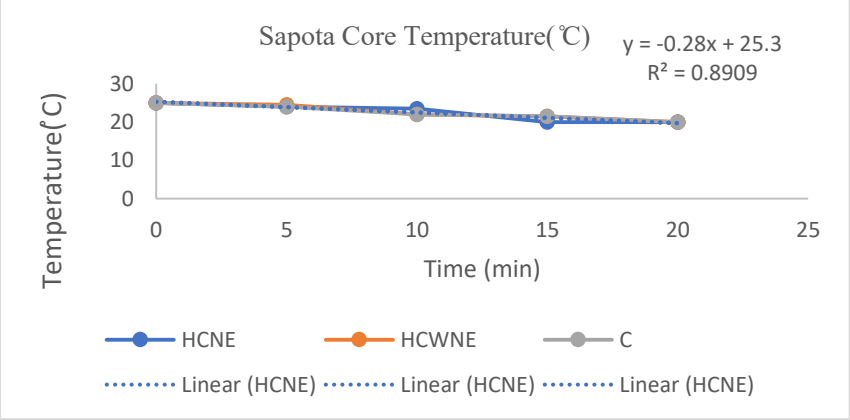


Fig 3: Graphical representation of time versus Fruit core temperature

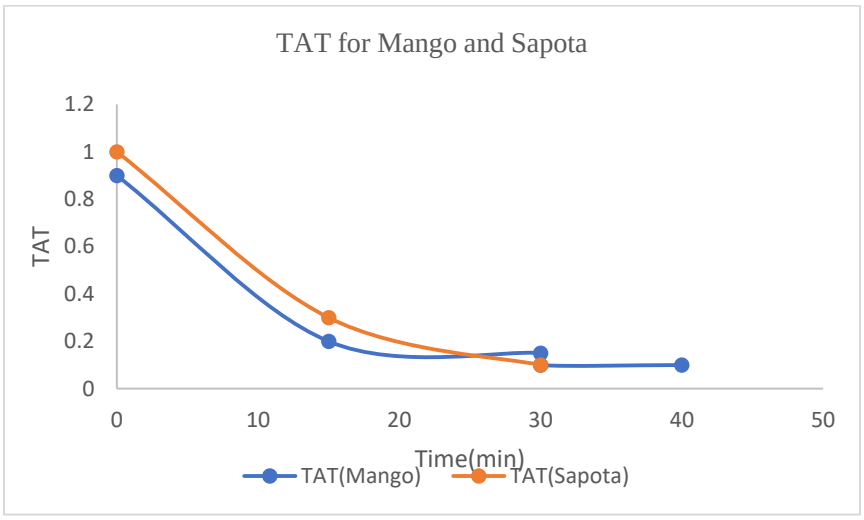


Fig 4: Graphical representation of TAT of fruits versus Time

The values of total soluble solids were high for control while the total solids values were low in case of neem leaf extract coated hydrocooled wrapped and low temperature stored fruits (Fig 7,8) due to slowing down of ripening rate with the removal of heat at the harvest time [8]. There was no much difference in pH value for the fruits stored at low temperature wrapped with LDPE pouches (Table 1). The results for decay and quality attributes showed that fruits were best stored at 14℃ and 20℃ for Sapota and Mango. This approach requires the application of both hydro-cooling and LDPE wrap to ensure good quality attributes. Hydrocooled, Neem leaf extract coated and wrapped Mango and Sapota were stored best for 25 and 16 days. Decay for both fruits was reduced significantly by applying neem leaf extract coating along with hydrocooling (Table 2) [17]. From the present study, it can be concluded that hydrocooling of fruit Mango and Sapota at 14˚C and 20 ˚C using anti-microbial extract, stored at low temperature and attained a maximum shelf life compared to control (without extract). There was no much significant effect of coating on quality attributes of fruits but significant effect on microbial quality of stored fruits.

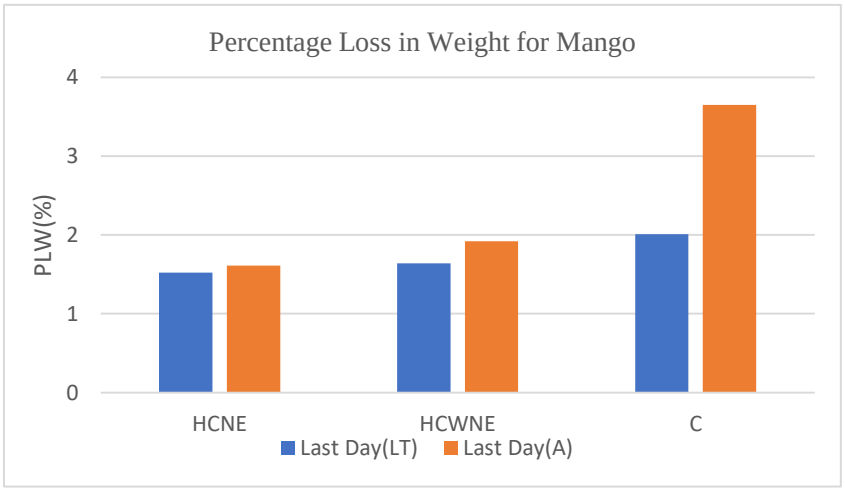


Fig 5: Graphical representation of Percentage Loss in Weight for Mango (at low temperature storage)

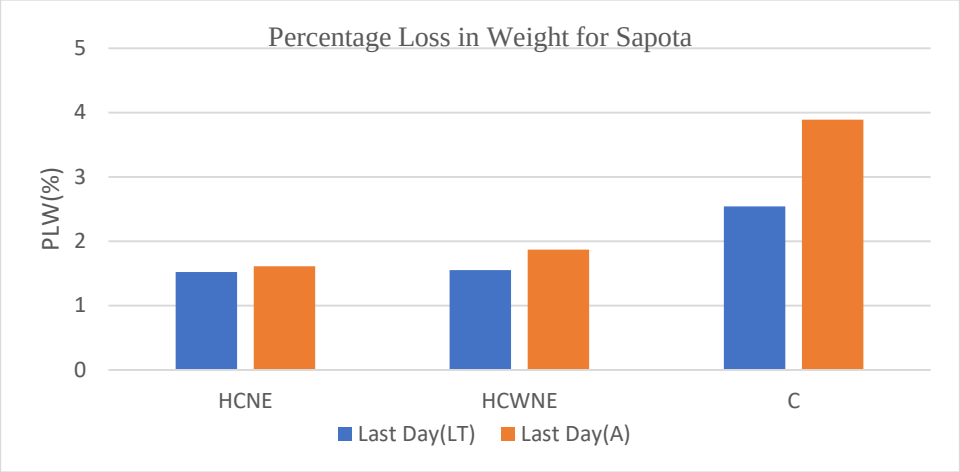


Fig 6: Graphical representation of Percentage Loss in Weight for Sapota (at low temperature storage)
*LT- Low Temperature, A- Ambient

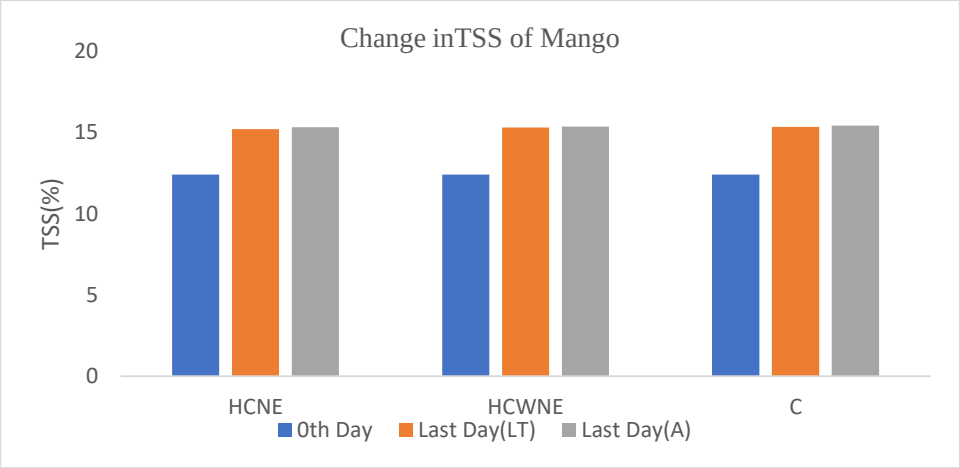


Fig 7: Graphical representation of Change in TSS for Mango (at low temperature storage)

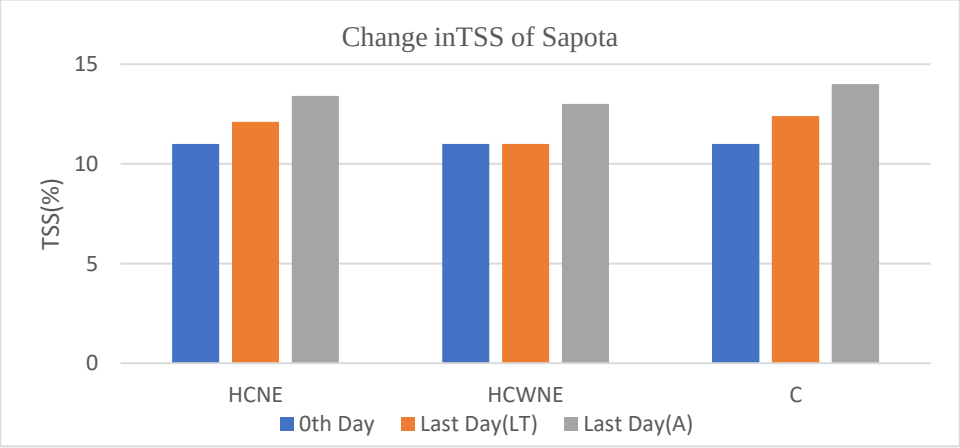


Fig 8: Graphical representation of Change in TSS for Sapota (at low temperature storage)
*HCNE- Hydrocooling with Neem Leaf Extract, HCWNE- Hydrocooling without Neem Leaf Extract, c-Control

Treatment	Mango		Sapota	
	0th day	Last day	0th day	Last day
C	5.59	5.19	6.20	6.00
HCNE	5.59	5.34	6.19	6.20
HCWNE	5.12	5.10	6.36	6.10

Table 1: Change in pH for Mango and Sapota

Treatment	Mango		Sapota		Mango		Sapota	
	TPC (cfu/g)				Fungal Growth			
	0th day	Last day	0th day	Last day	0th day	Last day	0th day	Last day
C	Nil	TFTC*	Nil	TFTC*	Nil	Nil	Nil	Nil
HCNE	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
HCWNE	Nil	Nil	Nil	TFTC*	Nil	Nil	Nil	Nil

Table 2: Microbial Quality of neem leaf extract coated Mango and Sapota
*TFTC: Too Few To Count

X. Conclusion

Hydrocooling of fruit Mango and Sapota at 14˚C and 20 ˚C using anti-microbial extract, wrapped in LDPE pouches stored at low temperature and attained a maximum shelf life of 25days and 16 days compared to control (without extract). There was no much significant effect of coating on quality attributes of fruits but significant effect on microbial quality of stored fruits.

REFERENCES

[1] Baird, C.D.; Gaffney, J.J. An numerical procedure for calculating heat transfer in bulk loads of fruits or vegetables. ASHRAE Transactions, v.82, p.525-535, 1976.

[2] Balerdi, C.F., Crane, J.H., Campbell, C.W., The Sapote Mamey. Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Document FC-30, 1996.

[3] D. K. Antala, R. M. Satasiya , P. D. Akabari, J. V. Bhuva, R. A. Gupta and P. M. Chauhan., Effect of Modified Atmosphere Packaging on Shelf Life of Sapota Fruit, International Journal of Agricultural Science and Technology (IJAST) Volume 2 Issue 1, 2014. doi: 10.14355/ijast.2014.0301.05

[4] Delgado, A.E.; Wen Sun, D., Heat and mass transfer models for predicting freezing process – a review. Journal of Food Engineering, v.47, p.157-174, 2001.

[5] Fraser, H.; Otten, L. Predicting 7/8 cooling times for peaches by comparing heat transfer modelling and field measurement methods St Joseph: ASAE, 1992. 10p. (Paper, 92-6016).

[6] Kader, A.A., Postharvest biology and technology: an overview, In: Kader, A.A. (Ed.), Postharvest Technology of Horticultural Crops. University of California, Oakland, CA, pp. 15–20 Publication 3311, 1992.
[7] Mitchell, F.G. The need for cooling. In: KADER, A.A. (Ed.) Postharvest technology of horticultural crops. 2ed. Berkley: Division of Agriculture and Natural Resources, University of California, 1992. p.53-56. (Publication, 3311).

[8] Musa Kaleem Baloch, Farzana Bibi , Muhammad Saleem Jilani ,Quality and shelf life of mango (Mangifera indica L.) fruit: As affected by cooling at harvest time. Scientia Horticulturae 130 ,2011, 642–646. doi:10.1016/j.scienta.2011.08.022.

[9] Peter M.A. Toivonen . The effects of storage temperature, storage duration, hydro-cooling, and micro-perforated wrap on shelf life of broccoli ***(Brassica oleracea*** L., Italica Group). Postharvest Biology and Technology ,1997/59-65. Elsevier Science B.V. ***PII*** SO925-5214(96)00064-6.

[10] Sandeep K. Chauhan, Thakur K. S, Jawa N. K and Thakur K. P., Botanical formulation and extracts based on plant leaves and flower, a substitute for toxic chemical and waxes for shelf life extension and quality retention of apple cv Starking Delicious in India, Journal of Horticulture and Forestry Vol. 4(12) pp. 190-100, December 2012. DOI: 10.5897/JHF09.040.

[11] Shweta Chauhan, K.C Gupta1 and Mukesh Agrawal, Efficacy of natural extracts on the storage quality of Apple, International .Journl .Curr.Microbiol.App.Sci , 3(3): 706-711, 2014. ISSN: 2319-7706.

[12] Singh, Z., Singh, R. K., Sane, V. A., & Nath, P., Mango-postharvest biology and Biotechnology, Critical Review in Plant Sciences, 32, 217–236, 2013.

[13] Snowdon, A.L., A Color Atlas of Post-harvest Diseases of Fruits and Vegetables: General Introduction and Fruits, vol. 1. CRC Press, Boca Raton, FL, 1990.
[14] Talbot, M.T.; Chau, K.V. Precooling strawberries, Gainesville: Institute of Food and Agricultural Sciences, University of Florida, 1991. 8p. (Circular, 942).

[15] Teruel M.B.J.; Cortez, L.A.B.; Leal, P.M.; Lima, G.A.B. Estudo teórico do resfriamento com ar forçado de frutas de geometrias diferentes. Revista Ciência e Tecnologia dos Alimentos, v.21, p.228-235, 2001.

[16] Teruel, B.; Cortez, L.; Neves, L. Estudo comparativo do resfriamento de laranja Valência em três sistemas de resfriamento. Revista Brasileira de Engenharia Agrícola e Ambiental, v.5, p.481-486, 2002.

[17] Yan Wang , Xingbin Xie , Lynn E. Long, The effect of postharvest calcium application in hydro-cooling water on tissue calcium content, biochemical changes, and quality attributes of sweet cherry fruit, Food Chemistry 160, 2014, 22–30. http://dx.doi.org/10.1016/j.foodchem.2014.03.073

[18] Zaharah, S. S., & Singh, Z., Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold-stored 'Kensington Pride' mango, Postharvest Biology and Technology, 60, 202–210, 2011.

[19] Zhang, Z., Gao, Z., Li, M., Hu, M., Gao, H., Yang, et al., Hot water treatment maintains normal ripening and cell wall metabolism in mango (Mangifera indica L.) fruit. HortScience, 47, 1466–1471, 2012.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Study on Drying Characteristics of Guava Leaves

[1] K Shravya ,[2] V Mounika ,[3] R Renu

[1,2,3] Department of Food Technology, University College of Technology Osmania University, Tarnaka, Hyderabad-500007, Telangana, INDIA.

kommidisravya@gmail.com , vangapallimounika@gmail.com , renu318@yahoo.co.in

Abstract*: Psidium guajava L., popularly known as Guava belonging to Myrtle family is grown in tropical and subtropical climatic regions. Guava leaves has been used as medicine for various health benefits in several countries. In the present investigation, the efforts have been made to prepare the powder of Guava Leaves by applying different drying technique viz. Tray drying, Vacuum drying and Hot air oven drying. The drying was done at different temperatures 50°C, 60°C and 70°C. At a temperature, the Vacuum dryer took maximum time for complete drying of Guava leaves followed by Hot air oven and Tray dryer. Maximum moisture content was removed from fresh Guava leaves using Tray dryer whereas for the other dryers it varied with temperature. As temperature increased from 50°C to 70°C, the drying rate increased and hence the removal of moisture. The studies indicated that Tray drying was faster and effective in the removal of moisture. Through all the techniques, at various temperatures the chlorophyll content of the leaves were noted and is observed that, the highest value in Vacuum Oven and Tray dryer is at 60^{0}C.*

Keywords: *guava, Tray dryer, Vacuum dryer, Hot air oven.*

I. Introduction

Studying about plant parts and foods are of great interest nowadays due to their high nutritional content and various health benefits [1]. Guava leaves are known as *Psidium guajava* L., are small evergreen tree belonging to the myrtle family [Myrtaceae] [2]. The Guava leaves are aromatic when crushed and has dull-green color with pronounced veins. These are native to tropical and subtropical climates, allowing production around the world [3]. The consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders, such as rheumatism, diarrhea, diabetes mellitus, mouth ulcers, and cough in countries in South Asia and Nigeria. For skin and wound applications, poultice is externally used in Mexico, Brazil and Philippines. This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity [4,5,6]. The Phenolic compounds present in Guava leaves helps in the regulation of blood-sugar level which is widely popular in Japan [7]. Guava leaf extract was found to possess properties like anticestodal, analgesic, anti-inflammatory, antimicrobial, hepatoprotective and antioxidant activities. The leaf extract also contains flavonoids like quercetin derivatives, which are hydrolyzed in the body to give the aglycone quercetin which is responsible for the spasmolytic activity [8]. Drying is an important and often necessary unit operation for many processes. It can determine the quality of the final product, establish its shelf life, or expose defectiveness. It is also extremely energy intensive and time consuming, so a lot of attention should be paid to optimizing the drying step to make the total process competitive. So, an optimal temperature and the drier must be identified for the specific products to be dried [9,10]. Drying of Methi Leaves using Open sun, Tray dryer, Microwave and Vacuum oven were studied. It was found that the drying time using Tray dryer at 35^{0}C was 11hrs when compared to microwave drying and Vacuum oven drying [11]. In the present study, the drying characteristics of Guava leaves using different drying techniques at different temperatures was done.

II. Materials and Methods

In accordance with the objectives of the study, experiments on drying of Guava leaves were conducted. Using the data, the drying behavior of Guava leaves from different drying techniques and different temperature and time taken were studied.

(a) Materials

Raw Material: The Guava leaves were collected from the Osmania University, Hyderabad. The freshly collected Guava leaves were sorted, washed then kept for drying processes.

Equipment: Electronic balance, Tray dryer, Hot air Oven and Vacuum dryer.

III Preparation of Guava leaves for drying:

a) *Sorting*: The stems and other unwanted parts of the collected fresh Guava leaves were removed.
b) *Washing*: The leaves were washed with slightly warm water to remove the dirt particles and the excess water was drained out from leaves. The Guava leaves were then cleaned by a dry cloth to remove the water particles.

After the complete removal of water particles, the leaves were then kept in thin layers in the trays for actual drying process. Initial Guava leaves samples of 20g were weighed accurately for Oven, Tray and Vacuum drying process. The driers were pre-heated to corresponding temperature before the drying process.

a) **Tray drying**: The dryer was pre-heated to required temperature and then the Guava leaves of 20g were spread in thin layers on the trays. Once the drying process started, the weights of the sample were collected at every 20 min until a constant weight was reached. The process was done by taking three samples of 20g each at three different temperatures 50°C, 60°C & 70°C.
b) **Vacuum drying**: The vacuum chamber was pre-heated to required temperature and then Guava leaves of 20g were placed inside the chamber. The drying process was started by creating the vacuum inside the chamber. The weights of the sample were collected at every 20 min until a constant weight was reached. The process was done by taking three samples of 20g each at three different temperatures 50°C, 60°C & 70°C.
c) **Hot air Oven drying**: The Oven was pre-heated to required temperature and then the Guava leaves of 20g were spread in single layers on the trays. The weights of the sample were collected at every 20 min until a constant weight was reached. The process was done by taking three samples of 20g each at three different temperatures 50°C, 60°C & 70°C.

IV Results and Discussion

Drying rates: The effect of drying at various temperatures using different dryers [Tray, Vacuum & Hot air Oven] is presented below. The model of the drying graphs is referenced from [12]. **Drying at 50^{0}C**: The drying process was started by taking an initial sample weight of 20g. At 50^{0}C, Vacuum dryer took 4hr20min to reach a constant weight of 8.3g. Hot Air Oven took 3hrs to reach the weight of 8.2g and the tray dryer took only 2hr40min to reach the constant weight of 8g.

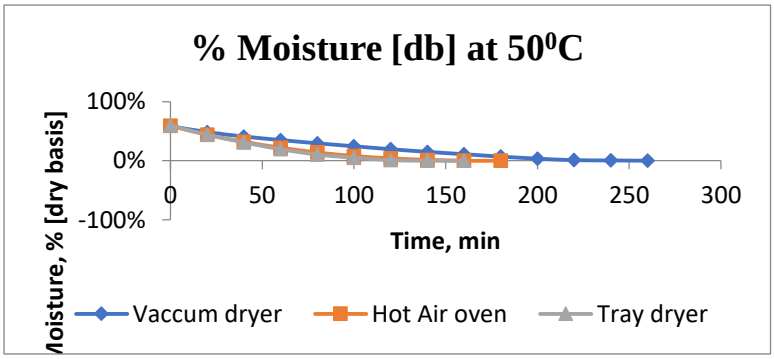


Fig 1: Drying behavior of Guava leaves at 50^{0}C using Tray, Vacuum and Hot air oven

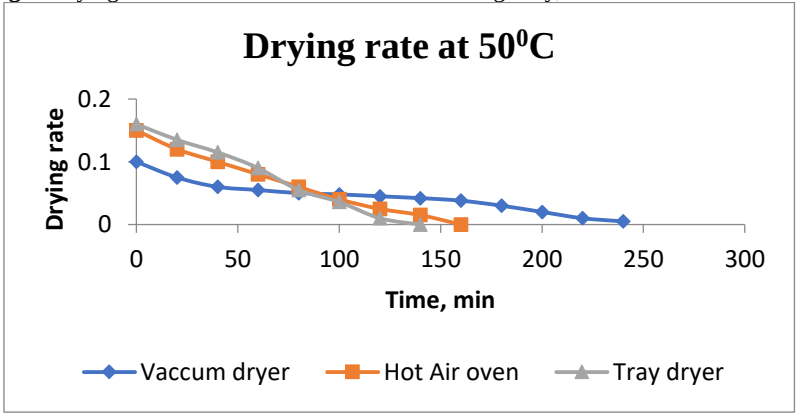


Fig 2: Drying rate of Guava leaves at 50^0C using Tray, Vacuum and Hot air oven

The graph is plotted for the Moisture content [% dry basis] vs time taken [min]. The graph shows that the % moisture loss is approximately same for all the dryers i.e., 60%. But the time taken to reach the constant value is different for dryers. The Vacuum dryer took more time to dry the leaves when compared to Hot Air Oven and Tray dryer. Of all the dryers, Tray dryer took a minimum time for drying and was also effective in the removal of the moisture.
The Drying curves were plotted for the Drying rate vs drying time [min]. The curves show that the drying rate for all the dryers decreased with increase in time. All the dryers have approximately linear curves i.e., most of the curves were under falling rate period. No early transient stage was found [during which the product heats up]. The curve for the vacuum dryer shows there were two falling rate periods.

Drying at 60^0C:
The drying process was started by taking an initial sample weight of 20g. At 60^0C, Vacuum dryer took 3hr40min to reach a constant weight of 8.3g. Hot Air Oven took 2hr20min to reach the constant weight of 8.2g and the tray dryer took only 2hrs to reach the constant weight of 8g.
Fig 3: Drying behavior of Guava leaves at 60^0C using Tray, Vacuum and Hot air oven.

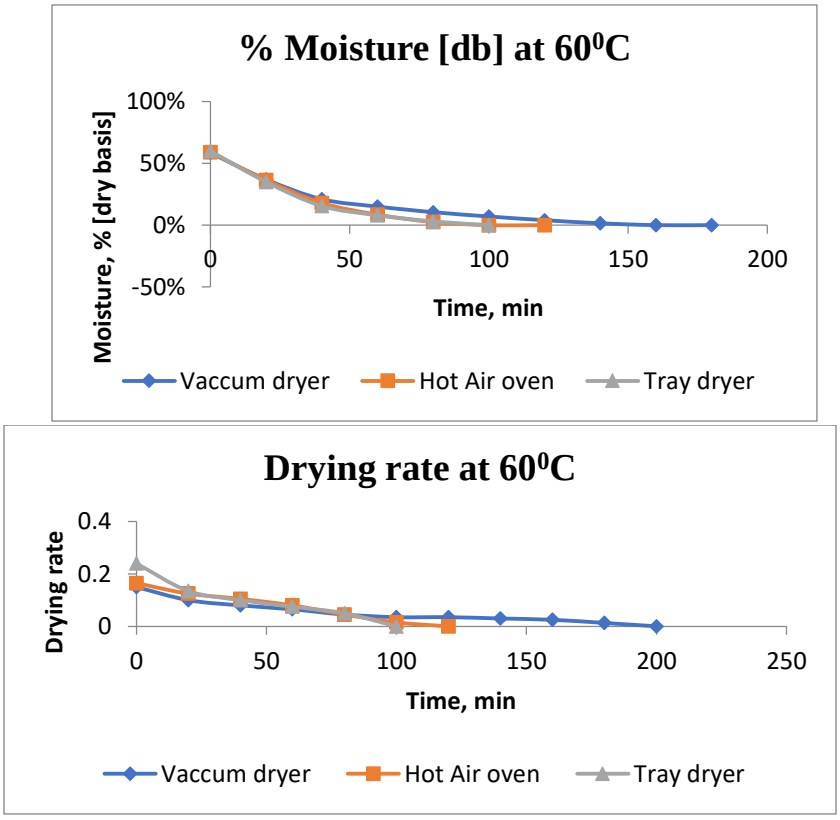


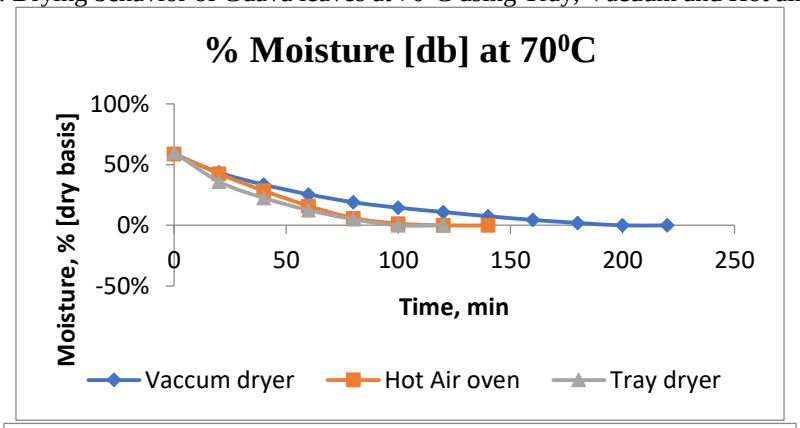
Fig 4: Drying rate of Guava leaves at 60^0C using Tray, Vacuum and Hot air oven

The graph is plotted for the Moisture content [% dry basis] vs time taken [min]. The graph shows that the % moisture loss is approximately same for all the dryers i.e., 60%. But the time taken to reach the constant value is different for dryers. The Vacuum dryer took more time to dry the leaves when compared to Hot Air Oven and Tray dryer. Of all the dryers, Tray dryer took a minimum time for drying. Also, it is noted that as the temperature increased from 50^0 to 60^0C, the moisture loss was same but the drying time reduced.
The Drying curves were plotted for the Drying rate vs drying time [min]. The curves show that the drying rate for all the dryers decreased with increase in time. Tray dryer and the Vacuum dryer has two falling rate periods where as Hot air Oven has the similar curve that of 50^0C. No early transient stage was found [during which the product heats up].

Drying at 70^0C:

The drying process is started by taking an initial sample weight of 20g. At 70^0C, Vacuum dryer took 3hrs to reach a constant weight of 8.3g. Hot Air Oven took 2hrs to reach the constant weight of 8.2g and the tray dryer took only 1hr40min to reach the constant weight of 8g.

Fig 5: Drying behavior of Guava leaves at 70^0C using Tray, Vacuum and Hot air oven



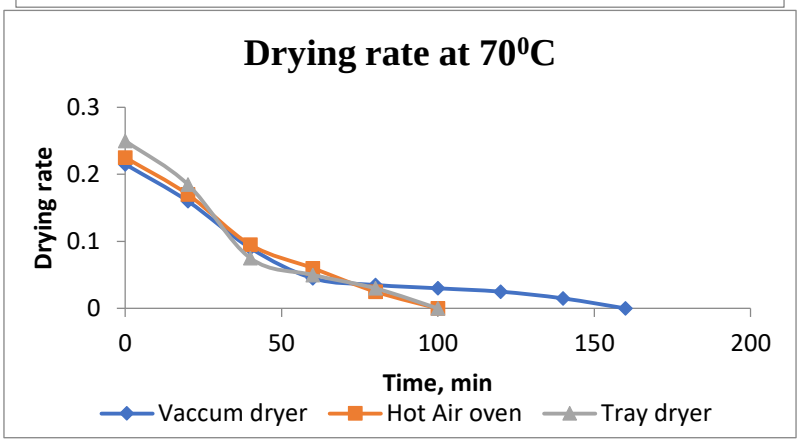


Fig 6: Drying rate of Guava leaves at 70^0C using Tray, Vacuum and Hot air oven

The graph is plotted for the Moisture content [% dry basis] vs time taken [min]. The graph shows that the % moisture loss is approximately same for all the dryers i.e., 60%. But the time taken to reach the constant value is different for dryers. The Vacuum dryer took more time to dry the leaves when compared to Hot Air Oven and Tray dryer. Of all the dryers, Tray dryer took a minimum time for drying. Also, it is noted that as the temperature increased from 60^0 to 70^0C, the moisture loss was same but the drying time reduced. The Drying curves were plotted for the Drying rate vs drying time [min]. The curves show that the drying rate for all the dryers decreased with increase in time. All the dryers have two falling rate periods. No early transient stage was found [during which the product heats up].

Chlorophyll content of Guava leaves powder

The Chlorophyll content of the powders obtained from different dryers at different temperatures for fresh powder and stored powder (120 days) is presented below.

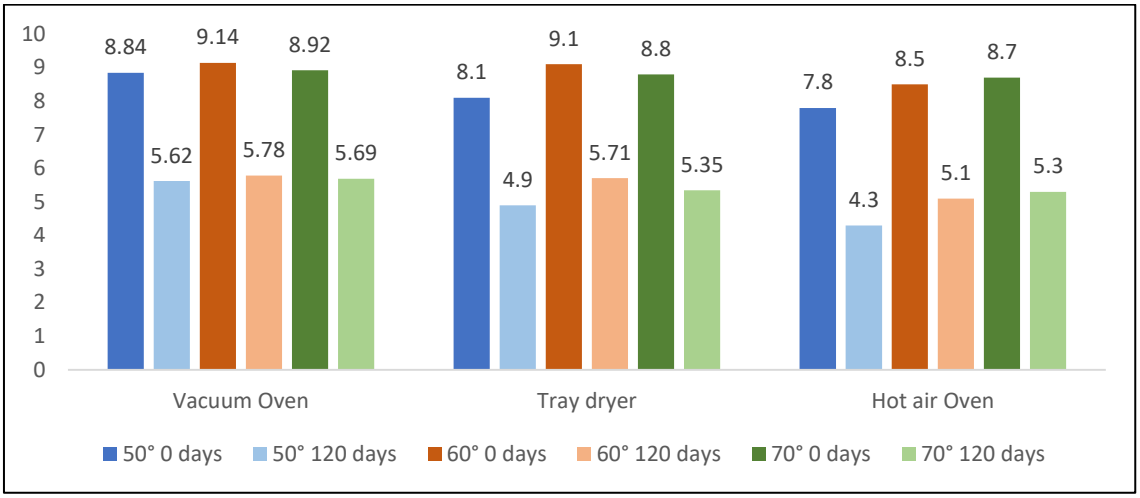


Figure 7 Chlorophyll (mg/Liter) content of Guava leaves powder at 50^0C, 60^0C and 70^0C

The Chlorophyll content for the Guava leaf powder obtained from Vacuum Oven and Tray dryer at 60^0C has the highest value of ~9.1mg/L when compared with other dryers and temperatures. The shelf life study indicated that the Chlorophyll content reduced drastically by ~4% for 120 days. The powders obtained at 50^0C has less Chlorophyll because of the longer duration of the drying time.

Table 1 Total Chlorophyll (mg/Liter) content of Guava leaves powder

	50°C at 0 days	50°C at 120 days	60°C at 0 days	60°C at 120 days	70°C at 0 days	70°C at 120 days
Vacuum Oven	8.84	5.62	9.14	5.78	8.92	5.69
Tray dryer	8.1	4.9	9.1	5.71	8.8	5.35
Hot air Oven	7.8	4.3	8.5	5.1	8.7	5.3

V. Conclusion

It can be concluded that the Tray drying method was the best method of dehydration of Guava leaves. At all the temperatures (50°C, 60°C and 70°C), Tray dryer took minimum time for the removal of the moisture content when compared to Hot Air Oven and Vacuum Oven. As the temperature increased from 50°C to 70°C, the drying rate increased and hence the removal of moisture for each dryer. [11]

References

[1] Bernal J., Mendiola J.A., Ibáñez E., Cifuentes A. Advanced analysis of nutraceuticals. J. Pharm. Biomed. Anal. 2011; 55:758–774. doi: 10.1016/j.jpba.2010.11.033.

[2] Morton J.F. Fruits of Warm Climates. Creative Resource Systems, Inc.; Winterville, NC, USA:1987.

[3] Salazar D.M., Melgarejo P., Martínez R., Martínez J.J., Hernández F., Burguera M. Phenological stages of the guava tree [Psidium guajava L.] Sci. Hortic. 2006; 108:157–161, doi:10.1016/j.scienta.2006.01.022.

[4] Gutiérrez R.M.P., Mitchell S., Solis R.V. Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 2008;117:1–27. doi: 10.1016/j.jep.2008.01.025.

[5] Shruthi S.D., Roshan A., Sharma S., Sunita S. A review on the medicinal plant Psidium guajava Linn. [Myrtaceae] J. Drug Deliv. Ther. 2013;3:162–168.

[6] Morais-Braga M.F.B., Carneiro J.N.P., Machado A.J.T., dos Santos A.T.L., Sales D.L., Lima L.F., Figueredo F.G., Coutinho H.D.M. Psidium guajava L., from ethnobiology to scientific evaluation: Elucidating bioactivity against pathogenic microorganisms. J. Ethnopharmacol. 2016;194:1140–1152. doi: 10.1016/j.jep.2016.11.017.

[7] Matsuda K., Nishimura Y., Kurata N., Iwase M., Yasuhara H. Effects of continuous ingestion of herbal teas on intestinal CYP3A in the rat. J. Pharmacol. Sci. 2007; 103:214–221. doi: 10.1254/jphs. FP0061311.

[8] Elixabet Diaz-de-Cerio et al : Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int J Mol Sci. 2017 Apr; 18[4]: 897

[9] Dilip M Parikh, 2014, Dpharma Group Inc: Solids drying basics and applications.

[10] Joachim Muller and Albert Heindl , University of Hohenheim, Germany: Drying of Medicinal Plants.

[11] Shobha H 2009 University of Agricultural sciences, Banglore: Studies on drying of Methi leaves

[12] Satwase AN, Pandhre GR, Sirsat PG and Wade YR: Studies on Drying characteristics and Nutritional composition of Drumstick leaves by Using Sun, Shadow, Cabinet, Oven drying Methods. Open Access: http://dx.doi.org/10.4172/scientificreports.584

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

QUALITY EVALUATION OF COATING ON SAPOTA

[1.]Sruthi Josyula (1008-16-804-015), [2.] Chalamalasetti Hema Sai Samhitha (1008-16-804-002)
B. Tech Food Processing Technology,
Under the guidance of R. Renu, Assistant Professor, University College of Technology (Autonomous), Osmania University, Taranaka, Hyderabad-500007, INDIA
srooti747@gmail.com, samhithach20@gmail.com

***Abstract**: To study the effect of coating and cooling on storage quality of Sapota. The physical and chemical characteristics of the fruit have immense significance as they ultimately affect the quality of processed prod ucts prepared from them. Over ripening of Sapota (Manilkarazapota) fruits at the post-harvest stage usually results in dramatic decline in quality. In the present study, chemical analysis which includes Total Soluble Solids (TSS), Sugars and microbial analysis includes Total Plate Count (TPC) of Aloe Vera coated Sapota f ruits. The precoat was prepared using Aloe Vera gel. Aloe Vera gel is an edible coating. Edible coatings ar e environmentally friendly that is applied on many products to control moisture transfer. gas exchange or o xidation processes. Edible coatings can provide additional protective coating to produce and can also give t he same effect as modified atmosphere storage in modifying internal gas composition. One major advantage of using edible films and coatings is that several active ingredients can be incorporated into the polymer m atrix and consumed with the food. Thus, enhancing safety or even nutritional and sensory attributes. The us e of different edible coatings (polysaccharides, proteins, lipids and composite) as carriers' functional ingre dients on fresh fruits and Vegetables is to maximize their quality and shelf life. The fruits were dipped in pre coat solution for 5 minutes and is air dried. The coated Sapotas were stored in two different conditions/equi pment and observed. Here, one bunch of coated Sapotas were packed in LDPE bags and stored at refrigerat ed temperature and another bunch of coated Sapotas were stored in fruit preserver equipment without pack aging and these were studied at regular intervals on every 2nd day. The dip treatment of Aloe Vera coating was useful in retaining the chemical and microbial characteristics when compared to other treatment metho ds. This treatment was found to be the best and most effective treatment in maintaining the fruit quality attri butes and providing shelf life extension for about one month.*

***Keywords:** Sapotas, Shelf life extension, Aloe Vera gel.*

Introduction

To study the effect of coating and cooling on storage quality of Sapota. The physical and chemical characteristics of the fruit have immense significance as they ultimately affect the quality of processed products prepared from them. Over ripening of Sapota (Manilkarazapota) fruits at the post-harvest stage usually results in dramatic decline in quality.

1.1 Sapota:

Sapota fruit is a good source of sugar which ranges between 12 and 14 per cent. A 100g of edible portion of fruit contains moisture (73.7 g), carbohydrates (21.49 g), protein (0.7 g), fat (1.1 g), calcium (28 mg), phosphorus (27 mg), Iron (2 mg) and ascorbic acid (6 mg) [12]. Sapota fruits are used for making jams, jellies. osmodehydrated slices and squash[13]. Products like sweet chutney, dried Sapota pieces, Sapota milk shake, nectar blended Sapota drinks, pickle, preserve and candy can also be prepared with good sensory quality[14]. Even wine can be prepared from Sapota fruit[15].

1.1.1 Physical and Chemical Parameters of Sapota

Ripening stage of fruit affects Physio-chemical parameters of fruit which ultimately affects the quality of processed products prepared from them.

The physical parameters are Weight, Diameter, Length, Length/Diameter ratio.

The Physio-chemical parameters are pH, TSS, Titrable acidity.

The chemical parameters are Moisture, Reducing sugars, Total sugars, Ash, Ascorbic acid, Tannins [6].

1.1.2 Cooling techniques

Fruits were maintained in two different cooling techniques:

Cooling technique1: A Fruit preserver was used where fruits were successfully stored by maintaining its chamber temperature at 13-17◦c with water as medium for cooling.

Cooling technique 2: Fruits are stored under refrigerated conditions, where Chilling injury was observed in controlled fruits but not in modified atmosphere packaged fruits[2]. Fruit texture and weights were maintained best in low-density polyethylene (LDPE) packaging and these fruits had the highest sensory scores for taste, color, texture, and overall acceptability.

1.2 Edible Coating:

Edible coatings are environmentally friendly technology that is applied on many products to control moisture tra nsfer, gas exchange or oxidation processes. Edible coatings can provide additional protective coating to the prod uce and can also give the same effect as modified atmosphere storage in modifying internal gas composition. On e major advantage of using edible films and coatings is that several active ingredients can be incorporated into t he polymer matrix and they can be consumed along with the food thus enhancing safety or even nutritional and s ensory attributes. The use of different edible coatings (polysaccharides, proteins, lipids and composite) as carrier s' functional ingredients on fresh fruits and Vegetables is to maximize their quality and shelf life[5].

1.2.1 Edible coating with Aloe Vera gel

Recently, interest has increased in using Aloe Vera gel-based edible coating material for fruits and vegetables. A loe Vera gel has been proven one of the best edibles and biologically safe preservative coatings for different typ es of foods because of its film-forming properties, antimicrobial actions, biodegradability and biochemical prope rties. It is composed mainly of polysaccharides and acts as a natural barrier to moisture and oxygen, which are th e main agents of deterioration of fruits and vegetables. Aloe Vera gel has the ability to prolong shelf life of the f ruits and vegetables by minimizing the rate of respiration and maintaining quality attributes (like colour, flavour etc.). It has antifungal and antibacterial property which provides a defensive barrier against microbial contamina tion of fruits and vegetables.

II. Preservater Chamber working

The Fruit Preservater was designed for storage of fresh fruits. This portable structure is made of fiber and consists of two compartments with circular holes all around the periphery they are kept offset by 1-inch thick pine grass mat. Water is dripped on to the mats continuously through a circular flexible pipe placed above the mats. This will decrease the temperature and increases humidity. A thermocol sheet was placed in the fruit preservater to avoid contact and to increase area of contact to air. Fresh ripen Sapota were placed on the thermocol sheet. Cool water is poured into the circular pipe, then water starts dripping on the mat. Evaporative cooling takes place which decreases the chamber temperature and maintains relative humidity.

The ambient and cabinet temperature was measured using digital thermometer and relative humidity by digital humidity temperature meter. Products weight (preserved and unpreserved) was determined by digital weight balance. The evaporative cooling system was tested over a period of 11days using Sapota fruit. The chamber was tested for its suitability to reduce the temperature while maintaining the increased relative humidity. The experiment was carried out using the developed evaporating cooling system. During the testing period, the thermometer was suspended in the chamber ascertain the variation of temperature in the chamber, while a control sample, Sapota fruit was exposed to the open air.

III. Materials and Methods

3.1 Materials Required:

1. *Fruit Material*:

Fresh mature, ripen Sapota fruits of the local popular variety were obtained from the local market directly. The Sapota were selected, based on uniformity of size, absence of physical damage and fungal infection.

2. Aloe Vera gel:

The inner gel is collected and separated from the outer leaf, grinded in mixer.

3. LDPE bags:

LDPE bags are available in local markets and super markets.

3.2 Process Of Coating For Sapota:
The Aloe Vera gel extract grinded in the mixer and 10 ml of that extract is used for coating each Sapota. The Sa pota were dipped in the coating medium for about 5-7 minutes and air dried. They are Stored in preservater cha mber and the fruits stored under refrigerating conditions are packed LDPE bags.

3.2.1 Chemical Analysis

Total Soluble Solids:
The TSS was determined by a Hand Refractometer of 0-32°Brix.Results were expressed in ^{o}Brix[9].

Total sugars:
Pipette out 50 ml of prepared sample solution into a 250ml of conical flask. Add 5gms of citric acid and 50 ml o f H_2O. boil gently for 10 min to complete the inversion of sucrose and then cool transfer to a 250ml volumetric f lask and neutralize with 1N NaoH using phenolphthalein as indicator till pale pink appears .and now titrating ag ainst 10ml of mixed Fehling solution adding two drops of methylene blue with 50ml of distilled water till brick r ed color appears as end point.

Determination of pH:
To *determine* the *pH*, the *pH paper* was dipped in a Sapota solution and the color developed in the *paper* is com pared with the color chart and the approximate *pH* of the solution can be identified.

3.2.2 Microbial Analysis

Total Plate Count Method:
The nutrient agar medium was taken and dissolved in distilled water according to formulation requirement. The sample prepared by making solution of 5gms of coated Sapota pulp in 45m1 of distilled water. Under the steriliz ed conditions conduct the analysis. Take l µml of sample in a Petri plate and pour the agar media in it. After Soli difying the Petri plates are kept in incubator for 24hrs at 30±1°C. After 24hrs the total plate count is done by usi ng colony counter and results are noted. The above tests were done for coated Sapota and control Sapota on thei r final day stored both at preservater temperature and refrigerating temperature.

IV. RESULTS AND DISCUSSIONS

Table 1: Results For Chamber Temperature

DAY	*TSS (^{0}B)*		*PH*		*TOTAL SUGARS%*	
	CONTROL	SAMPLE	CONTROL	SAMPLE	CONTROL	SAMPLE
1	11	-	6	6	12.5	-
3	12.4	8.5	6	6	-	-
6	9.4	9.4	6	6	20.5	18.6
9	13	9.3	6	6	-	-
12	-	13	-	6	-	24.4
15	-	12.6	-	6	-	-
18	-	16	-	6	-	25.2

The above tests are done for every 3 days interval and total sugars for every 6 days.
Control fruit sustained about 11 days with PH - 6
TSS -13.4^{0}B
TOTAL SUGARS -22.5%
Coated Sapota sustained about 18 days and results are tabulated above.

Table 2: Results For Refrigerating Temperature

DAY	*TSS^{0}B*		*PH*		*TOTAL SUGAR (%)*	
	CONTROL	SAMPLE	CONTROL	SA6MPLE	CONTROL	SAMPLE
1	11	-	6	-	13.4	-
3	10.4	9.3	6	6	-	-
6	8	10.2	6	6	-	-
9	9.4	10.4	6	6	12.5	13.4
12	11	11	6	6	-	-
15	13.4	8.2	6	6	-	-
18	13	9.4	6	6	11.7	18.5
21	-	10.5	-	6	-	-
24	-	14.2	-	6	-	-
27	-	14	-	6	-	23.5
30	-	14.4	-	6	-	23.7

Control fruit sustained about 18 days and coated fruit sustained about 30 days and the results are tabulated abov e.

Table 3: Microbial Evaluation:

DAY	*TOTAL PLATE COUNT (TPC)*			
	CHAMBER TEMP		REFRIGERATING TEMP	
	CONTROL	SAMPLE	CONTROL	SAMPLE
FINAL DAY	26	2	0	1

TOTAL PLATE COUNT:

over range of 30-300 CFU

<30 -TOO FEW TO COUNT

>300 –TOO LARGE TO COUNT

As per the results of uncoated Sapota fruit and coated Sapota fruit there is a comparison of results.
The coated Sapota stored at refrigerating temperature sustained for one-month shelf life with the good chemical properties stored at refrigerated temperatures. The coated Sapota free from microbial load. The taste and flavour of coated Sapota is good. The coated Sapota has the weight loss and shrinkage texture also.
The coated Sapota at preservater temperature is sustained for one 18 days with good chemical properties stored at refrigerated temperatures. The coated Sapota is free from microbial load. The taste and flavour of coated Sapo ta is good and odour of coated Sapota is acceptable. The coating layer was good enough, but the coated Sapota h as the weight loss and shrinkage in texture.

V. CONCLUSIONS

Aloe Vera coating found to be most effective treatment in maintaining the fruit quality attributes. It has retained the chemical properties and organoleptic properties. the coated Sapota at temperature 13-17^{0}C stored in preserva

ter has sustained about 18 says while control fruit sustained 11 days. At refrigerating temperature, the coated fru it sustained about 30 days while the control fruit about 18 days with acceptable condition.

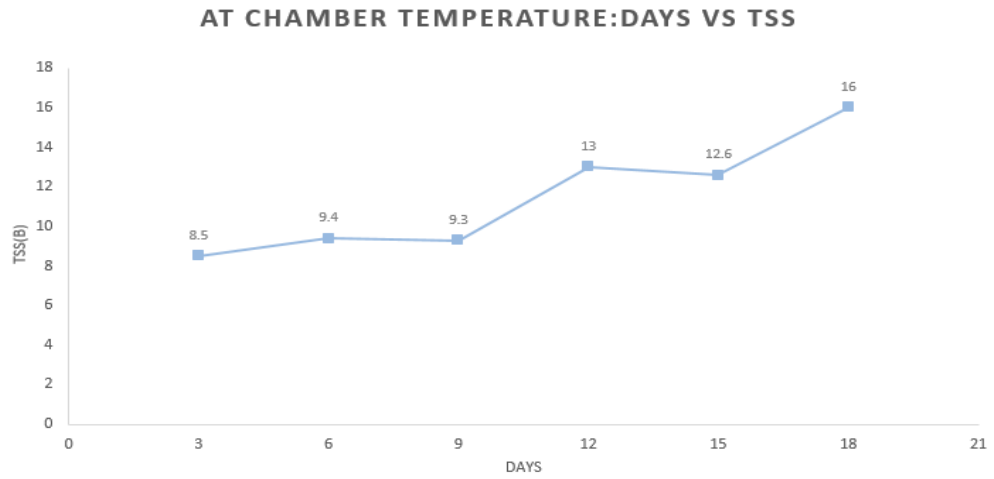


Fig-1: This is a graph which represents TSS values with respect to days at chamber temperature.

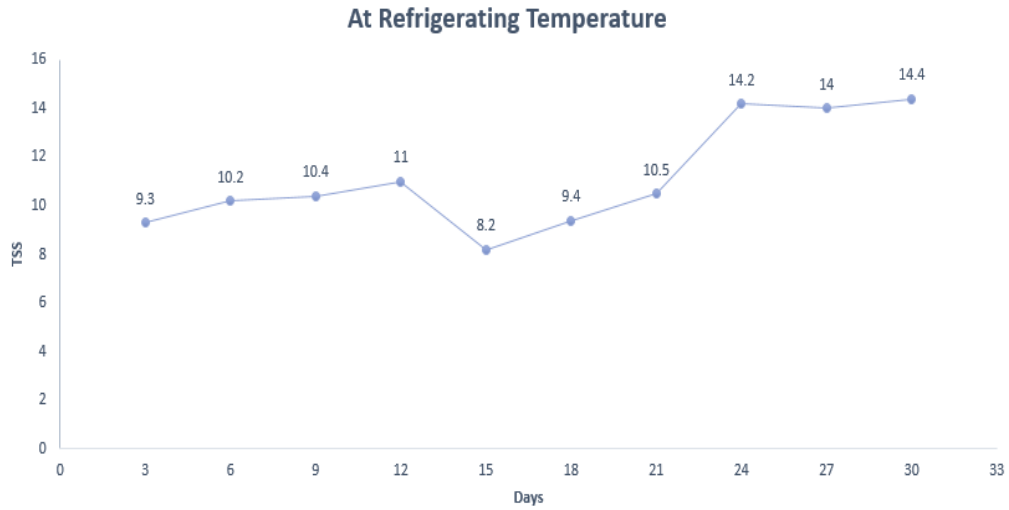


Fig-2 : This is a graph which represents TSS values with respect to days at refrigerating temperature.

References

[1] AnuAhiawat, AnuradhaSaha, Yogesh K. Tyagi and Rajinder K. Gupta. Development of chitosan based edible coatings to study S apota (Manilkarazapota) fruit shelf life. journal ot-Chemical and Pharmaceutical Research, 2015

[2] Asrar.Y.1. Mohamed, H.E. Aboul-Anean and Amal, M. Hassan. Utilization of edible coating in extending the shelf life of minim ally processed prickly pear. journal of Applied Sciences Research,

[3] Y. K.A. Athmaselvi, Pandian Jenney, C. Pavithra, and Ishita Roy, 2013 on physical and biochemical properties of selected tropic al fruits

[4] K. Dey, A. Ghosh, N. Bhowmick conducted on Physio-chemical properties of sapota (Manilkaraachras (Mill) Fosb.) fruits coated with corn starch. Journal of Crop and Weed, 2014

[5] R. K. Dhall, 2013 article on Advances in Edible Coatings for Fresh Fruits and Vegetables.

[6] Edy Sousa de Brito and Narendra Narain, 2002 done study on Physical and chemical characteristics of sapota fruit at different sta ges of maturation.

[7] Gajanana T. M.; Sudha.; Dakshin Moorthy. study on Marketing and post-harvest loss assessment in sapota. Journal of Horticultur al Sciences 2006

[8] R. M. Nilanthi AnuruddikaWijewardane and S. P. S. Guleria.On Effect of pre-cooling, fruit coating and packaging on postharvest quality of apple. J Food Sci Technol. M11 article.

[9] N. Padmaja,, S. John Don Bosco and J. Sudhakara Rao 2015. A research article on Physico Chemical. Analysis of Sapota (Manil karaZapota) Coated by Edible Aloe Vera Gel.
[10] R.G. Patel, B.R. Parmar and S.J. Pati.study on biochemical changes of sapota varieties in cold storage with application of GA3.A sian Journal of Horticulture, 2010
[11] Rodge AB a technical article on Cultivation, Processing and Value Addition of Guar Chun in 2015.
[12] A 100g of edible portion of fruit contains moisture as reported by Bose and Mitra,1990
[13] Uses of sapota by by Reddy,1959
[14] Candy preparation from sapota by Sawant,1989
[15] Wine preparation from sapotas by Gautam and Chundawat,1998
[16] Sirichit Lerdthanangkul And John M. Krochta. Edible Coating Effects on Postharvest Quality of Green Bell Peppers. Journal of F ood science 1996

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

Physico-chemical composition, Sensory and Shelf-life quality of value added pizza base

[1]Dr.M. Sivasakthi, [2] Najma.P.V

[1]Assistant Professor, [2]PG student, Department of Nutrition and Dietetics, Dr.N.G.P. Arts and Science College, Coimbatore-641 048, India.

Email ID: sivasakthiphd2015@gmail.com

Abstract: *The prevalence of degenerative diseases could be in check and control by including dynamic composition of ingredients can considerably enhance the therapeutic value of these foods. Value added Pizza base was enriched with arrow root powder, chia seed powder and subject it to acceptability in comparision to control. In the present study, variation 1 (V1) made with the addition of functional ingredients like arrowroot flour, wheat flour and flax seed flour (25, 15, 20g), variation 2 (V2) made (15, 25,25g), each 5 g of chia seed and milk powder was added to variation 1 &2, and various parameters like sensory, physico-chemical, nutritional and shelf-life were investigated. A considerable improvement in sensory characteristics was observed upon addition of the functional ingredients and based on overall acceptability, V1 was selected as the best. Physical parameters and physico-chemical traits and nutritional parameters like energy, protein, fat, carbohydrate, fibre, calcium showed significant difference ($p \leq 0.05$) between control and value added pizza base. The total microbial load of the control and V1 pizza base revealed that no count was observed at 0th day, whereas at 15th day, there observed 1×10^3(cfu/gm) which was considered negligible and didn't affect the product quality. The value added pizza which was incorporated with functional ingredients like flaxseed and chia Seeds are high in omega-3 fatty acids and antioxidants. Value added pizza base was much better than the standard pizza base as it is rich in nutrients like energy, carbohydrate, protein, fibre, calcium. It is highly nutritious and acceptable and it was formulated into various recipes by using the new food processing techniques.The value added pizza could be endorsed as a best snack food for all age group of people from childhood to elderly.*

Keywords: *pizza base, flax seeds, chia seeds, sensory and physico-chemical quality*

I. Introduction

The food industry has gone through many changes in the past two decades, mostly due to the development and implementation of new technology to meet growing consumer demands for convenience products. Current consumer trends bode well for companies able to develop healthy foods that taste good, are good for people, and are also good for on-the-go eating. Only 60 percent of all meals today are prepared and eaten in the home, largely because the increasing number of two working parent and single-parent households means less time to cook. Pizza, one of the most commonly purchased items in retail food stores, has maintained its market share through the changing nature of the processed foods industry and even grown in popularity [1].

Pizza was introduced in the middle of 20th century. Gradually it gained huge popularity and now a day's it ranks among the world's most widespread fast foods. Pizzas are known for their wide variety and attractive appearance. It is liked by all aged groups especially in youth. Pizza is a type of flat bread, leavened chemically or by yeast and it contains different types of toppings, essentially comprising of cheese, chicken and tomato sauce with some other variables depending on the choice of the consumers. About 40% of the weight of the pizza is dough base or shell and remaining 60% consists of topping [2].

Pizza is a flatbread generally topped with tomato sauce and cheese and baked in an oven. It commonly topped with a selected of meats, vegetables, and condiments. Pizza is a value added item made from flour amongst processed cereal products in India. Pizza as a, fast food is unhealthy, hunger satisfying food, which is easy to make and easy to consume. Pizza contains a high level of refined sugar, white flour, polyunsaturated fats, salts and numerous food additives but lacking in protein, vitamin, and fibres. Pizza is popular snake's food because of their simplicity of manufacture, consumption and good taste [3].

The basic formulation of the dough includes flour, water, salt, sugar, and bakers' yeast. The pizza dough is a significant fraction of the product and its appearance, texture, and taste are important attributes for its acceptance by consumers and identification [4].

Bakery products have become popular in India as evidenced by two fold increase in their production in recent years. Mushrooming growth of fat food joints serving fancy bakery products like bread-butter, pizza, burger, puff, hotdog, pastries etc. along with traditional bakery products like biscuits, cookies, cakes etc. is the indication that bakery industry has developed its own remarkable position in the industrial map of India. Baking can be defined as the process which transforms dough, basically made of flour, water and leavening agents, in a food with unique sensory features by application of heat inside an oven [5].

Flax (*Linum usitatisimum* L.) is grown as either an oil crop or as a fiber crop, with fiber (for linen) derived from the stem of fiber varieties and oil from the seed of linseed varieties (Diederichsen and Richards 2003; Vaisey-Genser and Morris 2003). Flax is making its mark in the world's food supply as a functional food. It delivers a health boost beyond what might be expected from their traditional nutrient content. Flax fits this description perfectly, being rich in alpha-linolenic acid (ALA), the essential omega-3 fatty acid, and phytochemicals such as lignans [5].

Flax is making its mark in the world's food supply as a functional food. It delivers a health boost beyond what might be expected from their traditional nutrient content. Flax fits this description perfectly, being rich in alpha-linolenic acid (ALA), the essential omega 3 fatty acid, and phytochemicals such as lignans [6].

Chia (*Salvia hispanica L.*) is an edible seed that comes from an annual herbaceous plant, *Salvia hispanica L*. of mint family (Lamiaceae). The shape of Chia seed is oval with 1 to 2mm in size, which may be black, grey or black spotted to white in colour. Chia means strength and the tiny seeds have been used as energy booster. Chia seeds can be used in the form of whole seeds, mucilage, flour, and oil seed Chia seeds are highly valued for their nutritional properties and medicinal value. It contains healthy omega-3 fatty acids, polyunsaturated fatty acids, dietary fiber, and protein-including all essential amino acids, vitamins, calcium and other important minerals. In water, Chia seeds produce a gel, which have a great potential in the development of food products as thickener, emulsifier and stabilizer [7].

Maranda arundinaceaL. commonly referred to as arrowroot, is an herbaceous, perennial plant that grows to a height of 0.9-1.5 m (Raymond and Squires, 1959). Arrowroot is cultivated primarily as a source of food starch, which is found in cylindrical rhizomes. Arrowroot (Maranta arundinacea. L) is a locally grown tuber crop in Indonesia. The arrowroot starch has high digestibility and is commonly used as a thickener in many foods such as puddings and sauces, cookies and other baked goods. Arrowroot is bland, making it suitable for neutral diets, especially for people who are feeling nauseous. The arrowroot tuber contains plenty of starch and other compounds. The starch from arrowroot flour has a nutrition composition of 11.9% water, 0.58% ash, 25.9% amylose, 0.14% protein, 0.84% fat, 8.7% insoluble dietary fiber, and 5.0%soluble dietary fiber. Recent study suggested that the arrowroot flour is a potential source of prebiotics (Harmayani *et a*l. 2011).

OBJECTIVES OF THE STUDY:

The present study entitled "Development, Characterization And Shelf Life Optimization Of Value Added Pizza Base" has been carried out with the following objectives:-

- To formulate and standardize the value added pizza.
- To evaluate the acceptability of the value added pizza base.
- To analyze the physical characteristics of value added pizza base
- To analyze the nutrient content of the value added pizza.
- To analyze the physio chemical properties of value added pizza base.
- To determine its storage stability & microbial count of the value added pizza base.
- To examine the antioxidant activity of the formulated pizza base.

The food industry has gone through many changes in the past two decades, mostly due to the development and implementation of new technology to meet growing consumer demands for convenience products. Current consumer trends bode well for companies able to develop healthy foods that taste good, are good for people, and are also good for on-the-go eating. Only 60 percent of all meals today are prepared and eaten in the home, largely because the increasing number of two working parent and single-parent households means less time to cook. Pizza, one of the most commonly purchased items in retail food stores, has maintained its market share through the changing nature of the processed foods industry and even grown in popularity [2].

Pizza was introduced in the middle of 20th century. Gradually it gained huge popularity and now a day's it ranks among the world's most widespread fast foods. Pizzas are known for their wide variety and attractive appearance. It is liked by all aged groups especially in youth. Pizza is a type of flat bread, leavened chemically or by yeast and it contains different types of toppings, essentially comprising of cheese, chicken and tomato sauce with some other variables depending on the choice of the consumers. About 40% of the weight of the pizza is dough base or shell and remaining 60% consists of topping [3].

Pizza is a flatbread generally topped with tomato sauce and cheese and baked in an oven. It commonly topped with a selected of meats, vegetables, and condiments. Pizza is a value added item made from flour amongst processed cereal products in India. Pizza as a, fast food is unhealthy, hunger satisfying food, which is easy to make and easy to consume. Pizza contains a high level of refined sugar, white flour, polyunsaturated fats, salts and numerous food additives but lacking in protein, vitamin, and fibres. Pizza is popular snake's food because of their simplicity of manufacture, consumption and good taste [4].

The basic formulation of the dough includes flour, water, salt, sugar, and bakers' yeast. The pizza dough is a significant fraction of the product and its appearance, texture, and taste are important attributes for its acceptance by consumers and identification [5].

Bakery products have become popular in India as evidenced by two fold increase in their production in recent years. Mushrooming growth of fat food joints serving fancy bakery products like bread-butter, pizza, burger, puff, hotdog, pastries etc. along with traditional bakery products like biscuits, cookies, cakes etc. is the indication that bakery industry has developed its own remarkable position in the industrial map of India. Baking can be defined as the process which transforms dough, basically made of flour, water and leavening agents, in a food with unique sensory features by application of heat inside an oven. The process steps responsible for gas retention and increased dough volume are: mixing and forming the dough, dividing and molding, fermenting, rolling, and baking. These steps are designed to develop the dough, forming a sponge-like structure, and stabilize the porous structure during baking [6].

Flax (*Linum usitatisimum* L.) is grown as either an oil crop or as a fiber crop, with fiber (for linen) derived from the stem of fiber varieties and oil from the seed of linseed varieties. Flax is making its mark in the world's food supply as a functional food. It delivers a health boost beyond what might be expected from their traditional nutrient content. Flax fits this description perfectly, being rich in alpha-linolenic acid (ALA), the essential omega-3 fatty acid, and phytochemicals such as lignans [7].

Flax is making its mark in the world's food supply as a functional food. It delivers a health boost beyond what might be expected from their traditional nutrient content. Flax fits this description perfectly, being rich in alpha-linolenic acid (ALA), the essential omega 3 fatty acid, and phytochemicals such as lignans [7].

Chia (*Salvia hispanica L.*) is an edible seed that comes from an annual herbaceous plant, *Salvia hispanica L*. of mint family (Lamiaceae). The shape of Chia seed is oval with 1 to 2mm in size, which may be black, grey or black spotted to white in colour. Chia means strength and the tiny seeds have been used as energy booster. Chia seeds can be used in the form of whole seeds, mucilage, flour, and oil seed Chia seeds are highly valued for their nutritional properties and medicinal value. It contains healthy omega-3 fatty acids, polyunsaturated fatty acids, dietary fiber, and protein-including all essential amino acids, vitamins, calcium and other important minerals. In water, Chia seeds produce a gel, which have a great potential in the development of food products as thickener, emulsifier and stabilizer [8].

Maranda arundinaceaL. commonly referred to as arrowroot, is an herbaceous, perennial plant that grows to a height of 0.9-1.5 m. Arrowroot is cultivated primarily as a source of food starch, which is found in cylindrical rhizomes [9].

Arrowroot (Maranta arundinacea. L) is a locally grown tuber crop in Indonesia. The arrowroot starch has high digestibility and is commonly used as a thickener in many foods such as puddings and sauces, cookies and other baked goods. Arrowroot is bland, making it suitable for neutral diets, especially for people who are feeling nauseous. The arrowroot tuber contains plenty of starch and other compounds. The starch from arrowroot flour has a nutrition composition of 11.9% water, 0.58% ash, 25.9% amylose, 0.14% protein, 0.84% fat, 8.7% insoluble dietary fiber, and 5.0%soluble dietary fiber. Recent study suggested that the arrowroot flour is a potential source of prebiotics [10].

OBJECTIVES OF THE STUDY:

The present study entitled "Development, Characterization And Shelf Life Optimization Of Value Added Pizza Base" has been carried out with the following objectives:-

- To formulate and standardize the value added pizza.
- To evaluate the acceptability of the value added pizza base.
- To analyze the physical characteristics of value added pizza base
- To analyze the nutrient content of the value added pizza.
- To analyze the physio chemical properties of value added pizza base.
- To determine its storage stability & microbial count of the value added pizza base.
- To examine the antioxidant activity of the formulated pizza base.

II.Methodology

The methodology pertaining to the study **"Development, characterization and shelf life optimization of value added pizza base"** is discusses under the following headings:-

PHASE 1: Selection and Processing
3.1) Selection of ingredients
3.2) Processing of ingredients
PHASE 2: Standardization of Pizza Base
3.3) preparation of different variation of pizza base
PHASE 3: Quality Evaluation of Formulated Pizza Base
3.4) preparation of pizza base
3.5) preparation of value added pizza base
3.6) sensory evaluation of the value added pizza base
3.7) physical characteristics of pizza base
3.8) nutrient analysis of standard and value added pizza base
3.9) storage stability of value added pizza base
3.10) microbial analysis of value added pizza base
PHASE 4: Statistical Analysis of Mean Score of Pizza Base

PHASE 1

3.1) Selection & procurement of ingredients

The food items selected for preparation of value added pizza base are flaxseed, chia seed, sesame seed, arrowroot flour, wheat flour, and maida were procured from the departmental stores. These ingredients were selected for its nutrient content and traditional aspects. Flaxseed, chia seed, wheat flour, sesme seed, maida were procured from local super market in sundharapuram, Coimbatore. Arrow root flour was purchased online from amazon.

3.2) Processing of Ingredients



Figure I: a) flax seeds processing



Figure II: Arrowroot Flour Processing

PHASE 2

PREPARATION OF DIFFERENT VARIATION OF PIZZA BASE

Table 1

Standardization of value added pizza base

Products	Maida	Arrowroot flour	Wheat flour	Flaxseed flour	Chia seed	Milk powder
CONTROL	100	-	-	-	-	-
Variation 1	30	25	15	20	5	5
Variation 2	25	15	25	25	5	5

The value added pizza base is developed using the above proportions, permutation and combinations comprised for both control and experimental pizza base.

PHASE 3

3.4) Preparation of Pizza Base

Pizza crust is the major component of the finished pizza product. Typically, the crust is leavened by the addition of baker's yeast, added at levels of 0.5 to 3% based on the flour weight. (Lehmann & Director,1997). The standard pizza base was prepared using straight dough process. The ingredients consists of maida, water, yeast, salt, olive oil and sugar are mixed. Kneaded the flour by adding water and made in to dough. It was allowed to ferment for 1 hour. After rising process, the dough has been shortly kneaded. This second kneading phase takes one minute. The dough was shaped into a round and baked at a temperature of 230^0 c for 8 minutes. The bottom has risen and is lightly cooked. The alcohol this formed by the yeast evaporates at a temperature of 67^0 c. the procedure is depicted in figure 3.

3.5) Preparation of Value Added Pizza Base

The same procedure was followed to prepare value added pizza base. Along with maida, the processed flours such as flaxseed flour, chia seed, arrowroot flour, wheat flour was added. The procedure is depicted in **figure 4.**



Figure 3

Preparation Of Value Added Pizza Base



Figure 4

Preparation Of Value Added Pizza Base

3.6) Sensory Evaluation of Pizza Base

For the sensory evaluation of the product under study, the method of Quantitative Descriptive Analysis (QDA) was applied, as described by (Stone et al., 1974), and, to identify, describe, and measure the intensity of overall appearance, aroma, taste, and texture perceived in the samples (Battochio et al., 2006). All the two variation of value added pizza base were subjected to organoleptic evaluation for colour, flavour, appearance, taste and texture to find out the overall acceptability. It was evaluated by twenty panel members by using five point hedonic scale. Based on overall acceptability the variation 2 was selected among two variations.

Plate 1
Sensory Evaluation of Pizza Base



3.7) Physical Characteristics of pizza base

The value added pizza base was analyzed for Bulk density, Water holding capacity, Foam capacity and foam stability and Swelling index. The methods used for physical properties are given in **Table 2**.

Table 2
Analysis of physical characteristics of value added pizza base

Physical properties	Reference
Swelling power	Leach*et al.*,1959
Bulk density	Okka and Potter,1979
Foam capacity and stability	Narayana and Narasinga rao,1982

Height:-Height of 2 pizza bases measured by placing the pizza base edge to edge and by staking one above the other respectively. Measurements by rearranging and restacking were made and average values were taken and expressed as cm, **[1].**

Diameter:- The diameter of pizza base was measured by laying 2 pizza base edge to edge & measuring to the nearest mm **[1].** The pizza bases were rotated to 90o and their diameter was re-measured as a check determination. The average diameter of pizza base was reported in cm.

Weight:-Two pizza bases of uniform size and thickness were taken and weighed. The average weight was expressed as gram.

Water absorption capacity (WAC)

Water absorption capacity was determined according to the method of **[1].**

3.8) Nutrient Analysis of Standard and Value Added Pizza Base

Value added pizza base are subjected to nutrient analysis to find out the composition of energy, carbohydrate, protein, omega 3, fat, dietary fiber, moisture, ash, (i.e,) its initial and final after 15 days.

Table 3
Nutrient Analysis of Standard and Value Added Pizza Base

Nutrient analysis	Method
Energy	Para Oxygen Calorimeter
Carbohydrate	Anthrone method
Protein	Lowry's method
Fat	Soxhlet method
Calcium	AOAC Method

3.9) Storage Stability of the Value Added Pizza Base

Value added pizza is stored in a plastic foil and its storage stability was checked by organoleptic evaluation in initial and its final i.e., after 15 days. the plastic foil that is used is a cling wrap. The cling wrap is loosely put around the pizza and sealed and stored at -20^0 c for 15 days.

3.10) Microbial Analysis

Value added pizza base is analysed for the microbial growth of aspergillus and lactic acid bacteria at initial day (1st day) and final stage i.e. after 15 days by using serial dilution – agar plate method [1]. method.

PHASE 4: STATISTICAL ANALYSIS OF MEAN SCORE OF PIZZA BASE

Results obtained from different parameters were subjected to statistical analysis using t test and it is interpreted. The statistical formula is given in appendix

PHASE: 5

COST CALCULATION AND NUTRITIONAL LABELLING OF VALUE ADDED PIZZA BASE

The cost of value added pizza base and normal pizza of per 100 gm and cost calculation of the value added pizza base recipe are done for per serving. Food labels are designed to help consumers make healthy food choices. Food labels must follow the standard formats. All labels contain basic product and information such as the name of the products, the nutrient content or weight, the date by which the product should be sold, and the name and place of business of the manufacturer, packager, or distributer.

Nutrition labelling is one example of a population-based approach aimed at helping to make the food selection environment more conducive to healthy choices by providing information to consumers about the nutrient content of a food. This information, along with a knowledge of basic nutrition principles, interest and confidence in adopting a healthy diet, is intended to contribute to informed food purchase decisions. **(Cowburn & Stockley, 2005).** Nutrition labels ware designed specifying the nutritional facts, ingredients, storage period, cost price, manufacture date and benefits of the value added pizza base. Nutritional labelling is given in the plate 3.

III. Results and Discussion

4.1 ORGANOLEPTIC EVALUATION OF THE VALUE ADDED PIZZA BASE

In the evaluation, the qualities of the product were asked to judge by the twenty panel members with respect to appearance, taste, texture, flavour and overall acceptability. The individual scores was presented in **appendix-1** and the mean sensory score for 0 days and 15 days are presented in the **Table 4.**

Table 4

Mean Sensory Score of the Value Added Pizza Base

S.No	Criteria	Control	Variation 1	p value
1	Appearance	4.08±0.57	4.8±0.40	**0.000898***
2	Taste	3.64±0.56	4.88±0.33	
3	Texture	3.96±0.45	4.8±0.40	
4	Flavor	4.36±0.48	4.92±0.27	
5	Overall acceptability	4.12±0.33	4.92±0.27	

From the above **TABLE 4** it is clear the organoleptic scores for the variation 1 has got highest mean scores for all the sensory attributes Appearance **(4.8±0.40)**, Taste **(4.88±0.33)**, Texture **(4.8±0.40)**, Flavour **(4.92±0.27)**, Overall acceptability**(4.92±0.27)**, in comparison with other one variation.

Table 5

Mean Sensory Score Comparison Between Control And Variation 1 (15th day)

S.No	Criteria	Control	Variation 1	p value
1	Appearance	3.6±0.57	4.52±0.50	**0.008509***
2	Taste	3.84±0.62	4.6±0.5	
3	Texture	4.16±0.47	4.28±0.54	
4	Flavor	3.76±0.43	4.12±0.33	
5	Overall acceptability	3.92±0.27	4.68±0.47	

The values are expressed in mean ±standard deviation, *significant difference

The statistical attributes like appearance, taste, texture, flavor, overall acceptability showed significant difference ($p \leq 0.05$) between control and variation 1 at 5%.

Above **Table 5** depicts that the mean sensory score of value added pizza base and standard pizza base which are kept in plastic foil and its sensory evaluation was done initial and 15 days. The t test were calculated and compared between standard pizza base and value added pizza base in which it shows significant difference at 5% level at refrigeration temperature.

4.2 NUTRIENT ANALYSIS OF VALUE ADDED PIZZA BASE

Standard and experimental pizza base were stored in plastic foil at room temperature and it was subjected to energy, carbohydrate, fat, protein, fiber, calcium, omega3 value to carry out using standard procedures. The analysis of nutrient of stored value added pizza base and standard pizza base were done initial & 15 days were presented in **Table 6.**

STORAGE QUALITY OF VALUE ADDED PIZZA BASE AND STANDARD PIZZA BASE IN PLASTIC FOIL (PER 100G) DURING STORAGE PERIOD

Table 6

Nutritive Value Comparison Between Standard And Value Added Pizza Base for Initial Day

S.No	Parameters	Standard	Experimental	p value
1	Calories (Kcal)	215±1	321±1	**0.123514***
2	Carbohydrate (g)	33±1	56±1	
3	Fat (g)	7.0±1	8.2±1	
4	Protein (g)	5.0±1	9.5±1	
5	Fibre (g)	1.0±1	4.8±1	
6	Calcium (mg)	1.0±1	113±1	
7	Omega 3	-	5.56±1	

The values are expressed in mean ±standard deviation, *significant difference

The nutritive value parameters like energy, protein, fat, carbohydrate, fibre, calcium showed slight significant difference **(p≥0.05)** between control and value added pizza base at 5%.

Table 7

Nutritive Value Comparison Between Standard And Value Added Pizza Base for Final day (15th Day)

S.No	Parameters	Standard	Experimental	p value
1	Calories (Kcal)	215±1	321±1	**0.058667***
2	Carbohydrate (g)	33±1	56±1	
3	Fat (g)	7.0±1	8.2±1	
4	Protein (g)	5.0±1	9.5±1	
5	Fibre (g)	1.0±1	4.8±1	
6	Calcium (mg)	1.0±1	113±1	
7	Omega 3	-	5.12±1	

The values are expressed in mean ±standard deviation, *significant difference

The nutritive value parameters like energy, protein, fat, carbohydrate, fibre, calcium showed significant difference (p≤0.05) between control and value added pizza base at 5%.

4.3 PHYSICO-CHEMICAL CHARACTERISTICS OF VALUE ADDED PIZZA BASE

Table 8

Physico-chemical characteristics of pizza base

S.No	parameters	standard	Value added pizza base
1	Height (cm)	1.70	1.85
2	Diameter (m)	12.80	13.52
3	Weight (g)	60.60	62.7
4	Water absorption capacity (WAC %)	28.16	293
5	Bulk Density	0.960	0.84
6	Swelling index	9.78	8.56
7	Foam capacity	10	11.3
8	Moisture (%)	25.53	28.86
9	Ash content (%)	0.98	1.44

At refrigeration temperature the moisture content of the standard pizza base was 25.53% initially and at the end of the period (15 days) it was increased to 26.67%. at refrigeration temperature the study moisture of the value added pizza base was 28.86% initially and at the end of the period (15 days) it was increased to 19.16%. The ash content of the standard pizza base was 0.98% initially and at end of the period (15 days) it was decreased to 0.90%. At refrigeration temperature, the ash content of the value added pizza base was 1.44% initially and at end of the period (15 days) it was decreased to 1.40%.

4.4. MICROBIAL ANALYSIS

The storage stability of the value added pizza base was done by microbial analysis. In the pizza base, the total bacterial, yeast and mould counts has analysed. This test is done initial and 15th day.

a) Total Bacterial Count

Bacillus species (spp) are the major contributor to spoilage of bakery products, which cause ropes spoilage **(bailey, 1993).** The total bacterial count of formulated pizza base is shown in **table 18**

Table 18

Total Bacterial Count (Cfu/gm) of value added pizza base

Storage period	Standard	Experimental
Initial	nil	Nil
15 days	1×10^5	1×10^3

The microbial population of total viable bacteria was determined for formulated pizza base and compared with control and experimental. In initial stage there were no total viable bacteria present in control and experimental. At 15th day, it was noted that the bacterial count for standard pizza base was 1×10^5 and for the value added pizza base was 1×10^3.

Total Mould Count

Moulds are the main cause of spoilage of bakery products and may not be the shelf life determinant **(guynot et al., 2003).** The total mould count of formulated pizza base is shown in **Table 9**

Table 9

Total Mould Count of value added pizza base

Storage period	Control	Experimental
Initial	Nil	Nil
15 days	1×10^3	1×10^3

In initial stage there was no total count present in control and experimental. At 15th day, it was noted that the mould count for standard pizza base was 1×10^3 and for the value added pizza base was absent $\times10^3$.

B) Total Yeast Count

Yeast normally spoils intermediate- moisture to high moisture bakery products **(smith et al., 2004).** The total yeast count of formulated pizza basis shown in **Table 10**

Table 10

Total Yeast Count of value added pizza base

Storage period	Control	Experimental
Initial	Nil	Nil
15 days	1×10^3	Absent $\times10^3$

In initial stage there was no total yeast count present in control and experimental. At 15th day, it was noted that the yeast count for standard pizza base was 1×10^3 and for the value added pizza base was Absent $\times10^3$.

IV.CONCLUSION

Flax seeds and chia seeds are very special. Benefits of flaxseed include helping improve digestion, giving you clearer skin, lowering cholesterol, reducing sugar cravings, balancing hormones and even helping fight cancer Chia Seeds are High in Omega-3 Fatty Acids. Like flax seeds, chia seeds are very high in Omega-3 fatty acids. They are an excellent source of omega-3 fatty acids, rich in antioxidants. From the study it was concluded that out of the two variation, the first variation was selected and highly acceptable. Value added pizza base was much better than the standard pizza base as it is rich in nutrients like energy, carbohydrate, protein, fibre, calcium. It is highly nutritious and acceptable and it was formulated into various recipes by the new processing technology and we can lead a healthy nutritious life. The value added pizza could be endorsed as a best snack food for all age group of people categories rised from childhood to elderly.

V. REFERENCES

[1] A.A.C.C. (1967). Approved methods of American cereal chemists cereal laboratory methods St. Puul. Minnesots USA.
[2] Ahvenainen, R. (Ed.). (2003). *Novel food packaging techniques*. Elsevier.
[3] Autio, K., & Laurikainen, T. (1997). Relationships between flour/dough microstructure and dough handling and baking properties. *Trends in Food Science & Technology*, *6*(8), 181-185.
[4] BattOchiO, J. R., Cardoso, J. M. P., KiKuchi, M., Macchione, M., Modolo, J. S., Paixão, A. L., ... & Bolini, H. M. A. (2006). Sensorial Profile Of Wholewheat Bread [perfil Sensorial De Pão De Forma Integral]. *Ciência e Tecnologia de Alimentos*.
[5] Brown, A. C. (2018). *Understanding food: principles and preparation*. Cengage learning.

[6] Brown, J. (1993). Advances in breadmaking technology. In *Advances in baking technology* (pp. 38-87). Springer, Boston, MA.
[7] Bush, S. (1989). *The Book of Pizzas and Italian Breads*. Penguin.
[8] Cauvain, S. P. (Ed.). (2012). *Breadmaking: improving quality*. Elsevier.
[9] Ciacco, C. F., & D'appolonia, B. L. (1977). Characterization of starches from various tubers and their use in bread-baking. *Cereal Chem*, *54*(5), 1096-1107.
[10] Clarke, C. I., & Farrell, G. M. (2000). The effects of recipe formulation on the textural characteristics of microwave-reheated pizza bases. *Journal of the Science of Food and Agriculture*, *80*(8), 1237-1244.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

DESIGN AND FABRICATION OF THREE-DIMENSIONAL PRINTER FOR FOOD PRINTING

DINESH.E[1], Revathi.R[*]

[1] Department of Food Technology, K. S. Rangasamy College of Technology

[*]Assistant professor, Department of Food Technology, K. S. Rangasamy College of Technology

nasdinesh200@gmail.com

Abstract: The upcoming technology of rapid prototyping in the field of food technology has led to the origin of 3D technology for food printing in the convenient and hygienic manner moreover the idea of tailored made foods has paved way for further development and adaptation in food industries to save time and resources. The ability of 3D printing technology to add different ingredients prooves it's unique feature to customize food as per one's nutrient requirement. The system design consists of a user interface, 3D printing software to design the food and subsequent machines to dispense and print. Different techniques are available to print in food products as per the raw materials and ingredients used. However, it promises a great potential to produce nutrient specific and cost effective food products in future. This review article discusses the basic principle, materials used and various techniques used for 3D printing of food.

Keywords: *rapid prototyping, food printing, tailored foods, interface 3D printing.*

I. Introduction

3D printing could be a sort of additive producing technology wherever a 3 dimensional object is made by giving ordered layers of fabric. It is also known as rapid prototyping,a mechanized method whereby 3D objects are quickly made on a reasonably sized machine connected to a computer containing blueprints of the object. The 3D printing conception of custom producing is exciting feature].

The need for creating 3D models with the use of inkjet technology saves time and cost by eliminating the need to design; print, glue and separate model parts. This technology has overcome these complications as you can create a complete model in a single process using 3D printing. The basic principles include materials cartridges, flexibility of output, and translation of code into a visible pattern.

3D Printers are machines that produce physical 3D models from digital data by printing layer by layer. It can make physical models of objects either designed with a CAD program or scanned with a 3D Scanner. It is also used in a various fields which includes jewelry, footwear, industrial design, architecture, engineering and construction, automotive, aerospace, dental and medical industries, education and consumer products.

II. challenges

2.1. cost: Feb 2017-The current cost of a printer is $ one lakh. The cheapest commercial printer can be commissioned at $ 35,000

2.2. Inability to easily print in multiple materials on the same machine: particularly plastic, metal and conductive materials. The colors are a touch dull and matt finished work.

2.3.A decent standard for model Design: The current standard STL files has some limitations. Feb 2017-A new standard has been proposed the AMF file format. But it is yet to be known.

2.4. speed: printing is slow: It takes a long time to build even smaller pieces. Most videos you see are speeded up or cut while you build a tap washer, oct2017- the ultimaker is able to print much faster than makerbot now.

III. Expermental Procedure

3.1.SOFTWARE

When we run the Gcode through Micro Controller, certain code is needed to confirm that it works in comply with the 3D printer. Opening the file that was saved from Microcontroller during a text editor like pad their area unit bunch of various lines of letters and numbers. These area units the Gcode coordinates the area unit sent to the machine that commands the printer.

Finnaly, take the food nozzle on the Z-axis and move it all the way down so that it barely touches the Y-axis platform[5]. Making sure everything is turned on and hot, and making sure that Arduino is connected to the computer, open the microcontroller software and open up the link to the Arduino.

After everything is checked, click the start button from the Repeater host.

3.1.1.REPEATER HOST

The Repeater-Host is a tool to host software system, which is compatible with most firmware's around. You can add and position your STL files on the stimulated print and slice all of them along. For slicing you'll be able to use the constitutional Slic3r slicer or use the well-known Skein forge.

3.1.2. ARDUNIO CODING

The Arduino is an versatile open source platform. Unlike other programmable circuit boards, the Arduino does not need a separate piece of hardware (called a programmer) in order to load new code onto the board –you can simply use a USB cable. Additionally, the Arduino IDE uses a simplified version of C++, making it easier to understand the programs. Finally, Arduino provides a typical type issue that breaks out the functions of the micro-controller into a lot of accessible package.

3.2.HARDWARE

The 3D printers primarily rely upon the development and principle of CNC machine. Most of the 3D printer's are costly, but there are some recent trends in 3D printer which causes reduction in their price. Companies have conjointly accomplished the potential of a shopper marketplace for 3D printers and intrinsically are sharply courtship enthusiasts with cheaper and higher models.

3.2.1.HARDWARE MATERIALS

The raw materials required for a printer consists of the following parts:

- ❖ 3D Printer parts
- ❖ Features of the selected 3D printer kit
- ❖ Chassis
- ❖ Axis X, Y and Z including carriage
- ❖ Printing Bed
- ❖ Arduino board and accessories

3.2.2.HARDWARE ASSEMBLY

ASSEMBLY → X, Y, Z axis designing and assembly for three Dimensional printing

↓

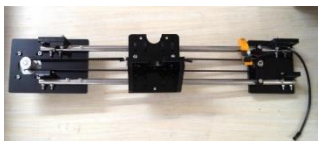
NOOZLE DESIGNING → Nozzle designing for the printing of specific food product printing

↓

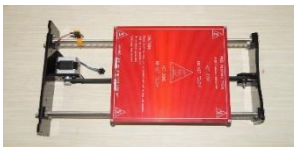
RAW MATERIAL PREPERATION → Formulation of ingredients and mixing with fluids.

↓

CODING FOR THREE DIMENSIONAL PRINTING → Arduino board coding for nozzle injection of desired food products



a)



b)



c)

a) X-Axis assembly
b) Y-Axis assembly
c) Z-Motors with threaded rods.

3.3. FOOD NOZZLE

Food Printer nozzle is one amongst the foremost necessary parts of FDM 3D Printers. This is the last part of printer where the fabric touches before it gets extruded onto the build plate. Previously there was only one kind of nozzle – Brass Nozzle (0.4mm diameter). Due to excellent thermal conductivity properties of brass and low melting point temperature & non-abrasive nature of ABS, PLA material, this nozzle was more than sufficient for FDM 3D Printing needs.

IV. Configuration and Testing

To become functional, this equipment needed new set of configurations. These new configurations had to be created from scratch. The goal was to work around the parameters like part filling, layer height, working temperatures, number of runs per layer and speeds. These parameters define the kind of print that is being done. To make these configurations possible, the software Slic3r was used which allows all kinds of modifications to fit in values.

Later, these parameters were brought in and before using a paste to be extruded, the component's functionality had to be tested. These simple tests granted the proper operation of the equipment (Table 1).

Table 1. Bio plotter parameter settings

Parameter	Chocolate	Bakery product	cream
Plastic tip size	500 μm	500 μm	500 μm
Speed	20 mm/s	40.2 mm/s	25 mm/s
Pressure	0.3 bar	0.3 bar	0.2 bar
temperature	20°C	20°C	20°C
Pre-flow delay	0.04 s	0.04 s	0.04 s
Post-flow delay	-0.09 s	-0.09 s	-0.09 s

This equipment's functionality was fixed through the amount of chocolate, which were systematically changed to find the best deposition pattern. Each feature was not tested instead merged together in a way that the printed part itself analyzes the faults,this improves the print quality. In this stage the temperature remains high. Using Nutella chocolate gave affordable results as its extrusion was easy to the extruder engine and gears. In addition, the chocolate consistency allowed the layer overlap, maintaining its geometry until the fourth passage. Iterations were made until the filling percentage was raised to 30% and the layer height increased to 1.6 mm, which is 80% of de extrusion diameter (maximum value). The newly developed configurations were used in the subsequent operation with chocolate (Table 2).

The chocolate tests allowed a data gathering, leading to a new configuration upgrade started with the Nutella chocolate, introducing new features, such as raising the temperature in both nozzle and reservoir. The usage of different kinds of chocolate revealed that the temperature values had to be changed.

Table 2. chocolate Testing

Types of Tests	Functions of Test
Essential parameters configuration	Relation between velocity and displacement in deposition Relation between velocity and displacement in deposition
Temperature insertion	chocolate extrusion
Previous parameter adaptation	Filling, layer heights, velocities
Nozzle temperature iteration	chocolate heating in this area
Reservoir temperature iteration	chocolate heating in this area
Geometry tests	Ability to draw simple shapes
Overlapping tests	Overlapping with several layers
Angle tests	Ability to draw closed angles

V. RESULT AND DISSCSSION

The taste of the printed food material was compared to a dish including three scoops of chocolate, bakery products and cream assembled by a skilled cook. Three folks together with the arch cook tasted each dishes and concluded that the tastes the dishes were equivalent. At First, different printing surfaces were trialed to determine which would support a successful print. It was found that the traditional sur-faces of greaseproof paper and aluminum foil had too little surface friction to allow a successful attachment of the first printed layer.

This surface was found to be extremely appropriate for printing and change of state was a transparency film of cellulose ester. Secondly, the body of the purees has to be set in such a way that the residual pressure from the printing barrel doesn't squeeze out the fabric. Minimal level of deposit materials (0.2 to 0.3 bar).

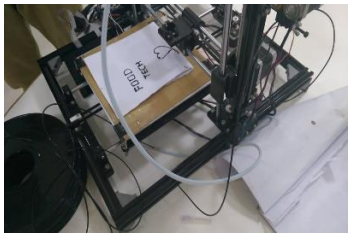


Fig 5.1.3D printed shapes

VI. Conclusions

Focusing on the biggest barriers that new 3D printing users will face, since the purchase of a kit to the end. The open source models allow the users to modify them to implement personal projects; however, this demands specific knowledge. The selected equipment, RepRap Prusa I3 3D printer, is an Open source model, normally used in activities that imply modifications and improvements. These changes are only accessible for consumers predisposed to learn by themselves about equipment's working.

The configurations developed were the toughest part, which evolved a lot of iterative testing. The development of an extruder equipment to be used in chocolate pastes demanded an in-depth study of the equipment programming. The conception of the new extruder was done in different steps, presenting several challenges. The chocolate has a more solid consistency, even with high temperatures, and it is more granny. These properties made the piston displacement harder, causing mechanical troubles. However, the deposition of this material presented a more solid structure after cooling than the previous test, which led to a better overlapping layers and better geometries definition.

Despite these, this work has produced functional equipment, printing with adequate quality and high accuracy level, further research is still demanded to develop a device with commercial interest. With this extruder, movement control, heating and cooling during and after extrusion, opened up a new area of exploration for printing with new food materials that have consistencies similar to chocolate.

VII. References

[1] J. Kietzmann, L. Pitt, P. Berthon, Bus. Horiz. 209 (2015) 15.
[2] I.A. Ferreira, MSc thesis, Faculdade de Engenharia da Universidade do Porto, Porto, Portugal, 2015.
[3] I. Gibson, D.W. Rosen, B. Stucker (Ed.), Additive Manufacturing Technologies, Springer, 2010.
[4] Biggest ever growth for 3D printing, Met. Powder Rep. 69 (2014) 38.
[5] T.T. Wohlers, T. Caffrey, Wohlers Report, 2013.
[6] H. Lipson, M. Kurman. Fabricated, The New World of 3D Printing, John Wiley Sons, Inc., 2013.
[7] [7] B. Berman, Bus. Horiz. 55 (2012) 155.
[8] [8] M.J. Barros, MSc thesis, Faculdade de Engenharia da Universidade do Porto, Porto, Portugal, 2014.
[9] http://www.mechanicalhero.com/2011/11/cnc-computer-numeric-control.html
[10] http://www.toptenreviews.com/computers/articles/history-of-3D-printing
[11] https://redshift.autodesk.com/history-of-3D-printing
[12] https://3Dprintingindustry.com/3D-printing-basics-free-beginners-guide/history
[13] https://www.allthat3D.com/3D-printing-history
[14] Siddharth Bhandari, B Regina "International Journal of Computer Science and Information Technology Research" Vol. 2, Issue 2, pp: (378-380), Month: April-June 2014
[15] Dipayan Dey,Souvik Mondal2,Arijit Kumar Barik "3-Axis CNC Router Modifiable to 3D Printer" International Journal of Innovative Research in Science, Engineering and Technology Vol. 5, Issue 9, September 2016

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Production of extruded products using millets and *Amarphophallus paeoniifolius* (elephant foot yam) flour

Anita S*, Vigneshwaran S, Srinivasan R, Karthikeyan k, Nanda kishor MV

* Professor, Department of Food Technology

K.S.Rangasamy College of Technology

K.S.R Kalvi nagar ,Tiruchengode

hodft@ksrct.ac.in, Vigneshwaranfd99@gmail.com

Abstract: The study was performed to evaluate the drying characteristics of *Amarphophallus paeoniifolius* (elephant foot yam) and fortification of the flour in millet based noodles. Noodles were developed from finger millet, pearl millet and wheat. The cooking quality like water absorption capacity, cooking loss and volume increase were studied. Proximate analysis like carbohydrate, protein, fat, mineral and moisture were studied. The noodles developed from the combination of above flours possess good organoleptic property. The noodles developed from elephant foot yam can be consumed by all aged peoples including diabetic patients because of very low glycemic content of elephant foot yam. The present research highlights that, composition of noodles with *A. paeoniifolius* flour, millet flour, and wheat flour was succeeded in cooking properties such as blending with taste maker, leaching of ingredients, water absorption index and textural properties. Sensory evaluation results from semi-trained panelists provide that *A.paeoniifolius* flour, finger millet and wheat flour has given higher acceptance over other compositions.

Keywords: Drying, Elephant foot yam, Noodles, Glycemic, Finger millet, Wheat

I.Introduction

Today's lifestyles are vastly different from the past and food preparation and consumption habits. i.e., modernization and globalization made peoples to hasten cooking process. Through optimistic outcome of rapid advances in food technology, increasing in demand for nutritionally rich food products with therapeutic benefits has become needed choice for consumers. Noodles a type of pasta consumed across worldwide of about 100,100 million servings in 2017 according to World Instant Noodle Association. Noodles are developed from wheat as raw material combing process of sheeting and extrusion to thin and long strips often served with sauce and salt [1]. Alteration in ingredients and raw materials of noodles were made with combination of *Amarphophallus paeoniifolius* (elephant foot yam) flour, finger millet, pearl millet were rich in nutritional value [3]. Roots and tubers are important food among that elephant foot yam a staple food in south Asian countries belongs to family Araceae and genus Amarphophallus. Yam tubes are rich repositories of starch which makes up 85% of the total dry weight of the yam[2]. Elephant foot yam is a functional food due to numerous nutritional health benefits on human[3]. The corm of elephant foot yam used preparation of delicious cuisines. It is used a therupatic dietary source to minimize diabetics[5]. It act as a good alternative source to reduce hunger a major problem in developing countries

II. MATERIAL AND METHODS

2.1 RAW MATERIAL

The collection of raw materials like elephant foot yam , millets and wheat were purchased from the local market Erode Tamil nadu, India.

2.2 PRE-TREATMENT PROCESS

The corms of elephant foot yam were thoroughly washed with water ,hand peeled and trimmed to remove the defective parts .The corms were sliced manually .The batch of yams were blanched with tamarind water at 70 ˚C for 5minutes.Blanched samples were cooled in tap water, drained and blotted with a towel.

2.3 Drying

Yam slices were dried in a hot air oven for 5 hours .The temperature range in which oven drying was carried out at 70 ˚C. Drying of yam by two methods ,method 1 yam were cut in to slices and dried .Method 2 yam were

grounded into paste and dried.Dried yam slices were grounded finely and sieved through a 60 mesh screen to obtain yam flour . Both the finger and pearl millets were grounded separately and the samples were then stored in a dry container.

2.4 FORMULATION OF NOODLES

Flours from elephant foot yam, pearl millet, finger millet and wheat were taken, blended with different ratios to check the suitable composition for formulation of noodles. Trial 1, Trial 2 and Trial 3 were blended without wheat flour .Trial 4 and Trial 5 were blended with wheat flour

FIG.1 FLOWCHART PROCESS

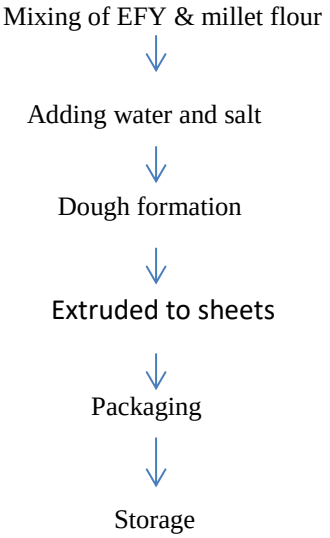


Table 1: Different combinations of flour

Trials	Weight of EFY flour(gm)	Weight of pearl millet flour(gm)	Weight of Finger millet flour(gm)	Wheat flour (gm)
1	50	25	25	-
2	25	37.5	37.5	-
3	75	12.5	12.5	-
4	40	40	-	20
5	40	-	40	20

.

2.5 EVALUATION OF COOKING QUALITY OF NOODLES

To evaluate the cooking quality of noodles by determining the water absorption capacity and volume increase .The water absorption capacity (WAC) and volume increase were determined by using formula

$$\text{Water absorption capacity (\%)} = \frac{\text{wt. of cooked noodles} - \text{wt. of fresh noodles}}{\text{Wt. Of fresh noodles}} \times 100$$

$$\text{Volume increase (\%)} = \frac{\text{Volume of fresh noodles} - \text{Volume of cooked noodles}}{\text{Volume of fresh noodles}} \times 100$$

2.6 PROXIMATE ANALYSIS AND SENSORY EVALUATION

Proximate analysis like carbohydrate, protein and mineral content by the methods, estimation of carbohydrate by phenol sulfuric acid method is a simple and rapid colorimetric method to pipette out different concentration of working standard solution. By adding of 5ml of sulphuric acid to each test tube and shake well. Read the colour at 490 nm, the amount of total carbohydrate present estimated. Estimation of protein by Lowry's method is a colorimetric method, by adding different concentration of working standard solute. Ion followed by addition of folin-cioculant reagent measured at 480nm. Amount of protein present in the sample solution were estimated. Mineral content by muffle furnace method by drying a representative sample for pretreatment with sodium hydroxide and sulphuric acid method, ignite the sample at 550˚Amount of mineral present is estimated by the below formula. Moisture analysis by moisture analyzer the sample is placed in the aluminum pan at temperature of 120˚C for 5mins. The loss of weight were represented as loss of moisture of the sample. The sensory evaluation of the developed noodles by 9-point hedonic scale by the untrained panel members and semi-trained panel members.

$$\text{Mineral content (\%)} = \frac{\text{Wt. after washing} - \text{Wt. of crucible}}{\text{Initial weight} \times \text{dry matter coefficient}} \times 100\%$$

III. RESULTS AND DISCUSSION

3.1 DRYING

The better method for the drying of the elephant foot yam was found from the two methods experimented. The method with less nutritional loss and shorter time period of time is chosen. The major drawback of the first method was indistinct formation of lumps during the process of drying. The method used to dry the elephant foot yam was cubicle chopped tray drying process. The drying characteristics of the elephant foot yam in both method 1 and method 2 were shown below

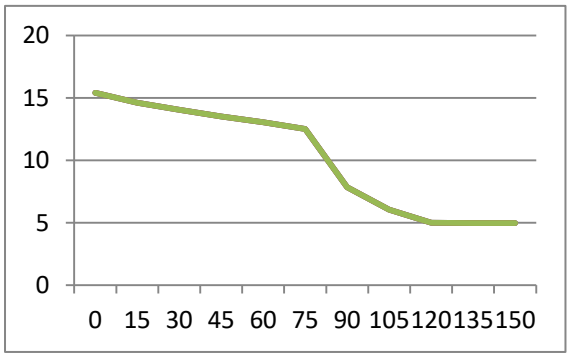


Fig.2 drying characteristics of method 2) (Selected)

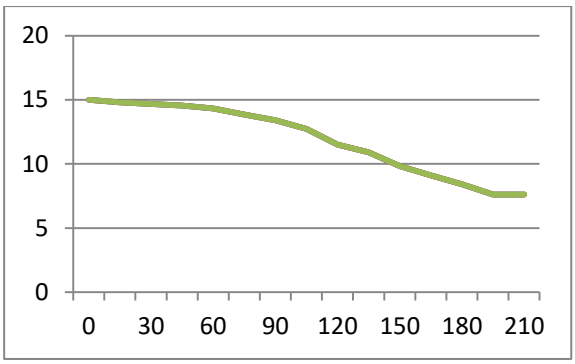


Fig.3 Drying characteristics of method 1 (Not selected)

3.2 FORMULATION OF NOODLES

Evaluation of the suitable ratio of the elephant foot yam flour, pearl millet and finger millet were analyzed. The ratio of the elephant foot yam flour; pearl millet; finger millet; tried are trial 1 (2; 1; 1), trial 2 (1; 1.5; 1.5), trial 3 (3; 0.5; 0.5). All the three trial were failed in the cooking quality test of the product, the millet flour gets leached out from the product when cooked and their textural attributes were also deprived.

Another two trials were conducted with incorporation of wheat flour as a stabilizing agent to overcome the leaching of the millet flour on cooking. The ratio of the elephant yam flour; pearl millet flour; finger millet flour; wheat flour are trial 4 (2; 2; 0; 1) and trial 5 (2; 0; 2; 1). Both trial 4 and 5 has succeeded in the cooking quality test and wheat improved the texture and stabilizing property of the noodles.

3.3 EVALUATION OF COOKING QUALITY OF NOODLES

The cooking quality like water absorption capacity and volume increase of the developed noodles were studied .water absorption capacity of noodles developed from finger millet(sample 1) is high compared to pearl millet (sample 2).volume increase of noodles from combination of two millets were equal.

.

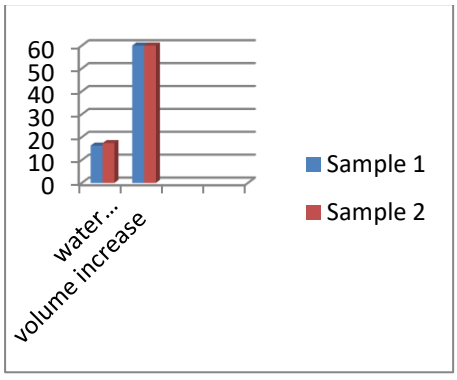


Fig .4 cooking quality of noodles

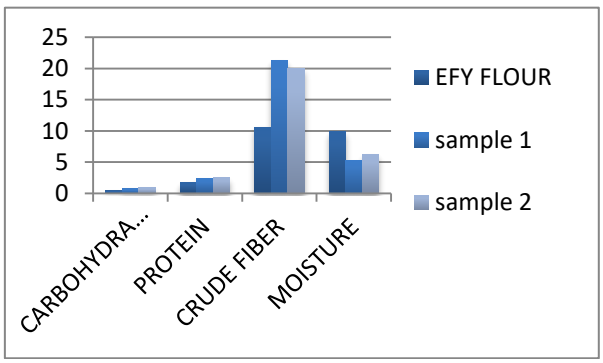


Fig .5 Proximate analysis of noodles

3.4: PROXIMATE ANALYSIS AND SENSORY EVALUATION

The proximate analysis of the EFY flour and the noodles developed from the combination of EFY flour, millet (pearl, finger), and wheat were analyzed. The sensory evaluation of the developed noodles by 9-point hedonic scale by the untrained panel members.

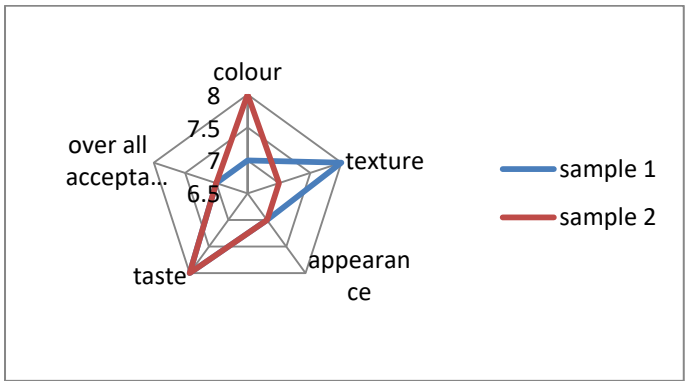


Fig .6 Sensory evaluation of developed noodles

IV. CONCLUSION

Formulation of healthy food products is one of need and necessity for improving the health status of the nation as well as individual one. The noodles developed from the combination of Elephant foot yam, pearl millet, finger millet and wheat possess less stability due to variation in starch content and gluten content compared to commercialized products available in market .Elephant foot yam is tuber crop called "power house of nutrient "contain a numerous health benefits, consumption of yam were low due to the itchiness which is removed by blanching with tamarind water. In order to increase the yield and to produce the value added products as to increase market demand. Since the noodles based on millet and *A.paeoniifolius* flour formulation, it can be easy digested and will have low glycemic index. Hence, it can give for kids, toddlers, aged people and diabetic patients.

REFERENCES

[1] Purwandari, et al, "Textural ,cooking quality and sensory evaluation of gluten-free noodle made from bread fruit , konjac, or pumpkin flour" International Food Research Journal 21(4):1623-1627(2014).

[2] Suriya , et al ,"Influence of blanching and drying methods on molecular structure and functional properties of *Amarphophallus paeoniifolius* (elephant foot yam)" LWT-Food science and Technology(2016).

[3] Dissanayake , jayawaredena "Development of a method for manufacturing noodles from finger millet ",International Conference of sabaragamuwa University of Srilanka 2015(ICSUSL 2015).

[4] Parvathi, et al ,"Development of a method for manufacturing noodles from finger millet" , International journal Food fermentation technology 6(1):67-74,june 2016

[5] Adesoji Adedipe fasanmade, et al, "Glycemic indices of selected Nigerian flour meal products in male type 2 diabetic subjects" , university of Ibadan ,Nigeria.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Identification of Food Adulterants in Some Selected Food Items using Chemical Qualitative Analysis

R. Ashokmithran[1], G. Saravanakumar[1], S. Lavanya[1], S. Nithya[1], K. Krishnaveni[2*]

[1]*Under graduate student, Department of Chemistry, Dr.N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India*

[2]*Associate Professor, Department of Chemistry, Dr.N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India*

*Corresponding Author E-mail: Primary – ravikrishna2004@gmail.com

Secondary - ashokmithran9791@gmail.com

Abstract:

Food is one of the basic needs for every living being and very important for life. Now a day's foods are affected by different adulterants. Adulterants are the foreign substance which is added to the food in order to get profit, those adulterants reduce the quality of food. Some of the common adulterants are mineral oils, castor oils in edible oils; Vanaspati, mashed potatoes in ghee; inverted sugar or jaggery in honey, kesari dhal, lead chromate in pulses etc. which can lead to harmful health effects such as Cardiac arrest, Anemia, Paralysis, brain damage, Cancer etc. Food adulteration is a major global issue due to which the developing countries are at higher risk since there is a lack of monitoring policies.

The present work is the attempt of adulterant based investigation instead of technique-based study, where the qualitative detection for the presence of adulterants from daily used food materials like Milk, Ghee, Milk powder, Turmeric powder, and Maida were carried out. The food samples were collected from different local grocery stores around Coimbatore and assessed for the presence of adulterants by chemical qualitative analysis. The chemical analysis was carried out by following standard procedure given by food safety (FSSAI).

Keywords: Food adulterants, chemical analysis, FSSAI, daily food products.

I. Introduction

Food is more basic need for man rather than shelter and clothing. It provides adequate for the body's growth, maintenance, repair and reproduction. Food furnishes the body with the energy required for all human activities. It provides material required for building and renewal of body tissues also the substance that act to regulate body process. A general classes or kinds of nutrients found in all foods are carbohydrates, lipids, proteins, vitamins, minerals and water.

Adulteration of food items is common phenomenon in India it includes both willful adulteration to improve texture and quality of food items. Foods were often purposely contaminated with additives to increase bulk, attractiveness, disguise spoilage and increase profit. Adulteration of foods was practiced even in ancient times. It was not a serious problem because business was on a small scale and the transactions involved a large measure of personal accountability. According to Indian prevention of food adulteration (PFA) act – 1954, any ingredient which when present in food is injurious to health is adulterants.

Some of the common adulterants are starch, water, urea and formalin in Milk, Brick powder in chilly powder. Lead chromate, coloured saw dust in turmeric powder. Sugar syrup, jaggery in honey,Vanaspati and mashed potatoes in ghee, boric Acid in maida [1, 2].

II. Materials and methods

The present work was carried out to analyse food adulteration in selected food items by following solutions qualitative analysis.

Chemicals and Reagents:

Resorcinol solution, Potassium Iodide solution, Starch solution, Turmeric Paper, Silver nitrate ($AgNo_3$), potassium dichromate($K_2Cr_2O_7$), Hydrochloric acid (HCl), Sulphuric acid (H_2SO_4), Neutral ferric chloride ($FeCl_3$), Nessler's Reagent. All the solutions were prepared under standard procedure and added for analysis to detect food adulteration in food samples.

Food Items Tested:

Milk, Milk Powder, Turmeric Powder, ghee and maida were purchased from local market and tested against food adulteration. We performed the analysis as per the standard procedure given by FSSAI.

Tabulation of consolidated results of analysis

Table I: Results of Milk Samples

S. No	Samples	Test for NaCl	Test for Formalin	Test for Hydrogen peroxide	Test for Boric acid	Test for sucrose	Test for starch	Test for ammonium salts
1.	Sample M_1	+	+	-	-	-	-	-
2.	Sample M_2	_	+	-	-	-	-	-
3.	Sample M_3	-	-	-	-	-	-	-
4.	Sample M_{std}	-	-	-	-	-	-	-

\+ Presence of adulterants,
\- Absence of adulterants.

Table II: Results of Ghee Samples

S.No	Sample	Test for Vanaspati
1.	Sample G_1	Sample contains pure ghee
2.	Sample G_2	Sample contains Vanaspati
3.	Sample G_3	Sample contains Vanaspati

Table III: Results of Milk Powder Sample

S.No	Samples	Test for coal tar dye
1.	Sample MP_1	Sample contains coal tar dye
2.	Sample MP_2	Sample contains coal tar dye
3.	Sample MP_3	Sample contains coal tar dye

Table IV: Results of Turmeric Powder Sample

S.No	Samples	Test for Metanil yellow
1.	Sample T_1	Sample contains metanil yellow
2.	Sample T_2	Sample contains metanil yellow
3.	Sample T_3	Sample contains metanil yellow

Table V: Results of Maida Sample

S.No	Samples	Test for Boric acid
1.	Sample MD_1	Sample contain Boric acid
2.	Sample MD_2	Sample contain Boric acid

III. Results and Disscussion

Analysis of Milk [2,6]

Test for NaCl:

On adding silver nitrate ($AgNO_3$) and potassium chromate, the yellow colour precipitate is obtained in milk sample M_1 shows the presence of dissolved chloride which indicates that the sample was adulterated. The standard sample (cow milk) gives chocolate brown precipitate indicates absence of dissolved chloride advocates that the sample was not adulterated.

Test for Formalin Milk:

Addition of Sulphuric acid (H_2SO_4) with trace amount of ferric chloride, formation of the purple ring in milk sample M_1 and M_2 indicates the presence of formaldehyde. The other milk samples did not give positive result for adulteration.

Test for Hydrogen Peroxide:

Potassium iodide - starch reagent was added to the sample and no blue colour development concludes that the milk samples M_1, M_2, M_3, M_4 were not adulterated.

Test for Boric Acid or Borates:

The samples were tested for borates and boric acid by adding hydrochloric acid (HCl) the turmeric paper did not change into red colour which shows the samples M_1, M_2, M_3, M_4 were not adulterated.

Test for Sucrose in Milk:

On adding resorcinol solution to the test samples exhibits no deep red colour on boiling which reveals the samples M_1, M_2, M_3, M_4 were not adulterated.

Detection of Starch in Milk:

On adding iodine solution, no blue colour was observed which indicates the samples M_1, M_2, M_3, M_4 were not adulterated.

Detection of Ammonium salts in Milk:

The addition of Nessler's reagent produced light grey colour instead of orange colour in the samples M_1, M_2, M_3, M_4 which shows reveals the controlled content of ammonia.

Analysis for Ghee [2,4]

Vanaspati in ghee:

Presence of vanaspati in ghee was detected by addition of HCl. The appearance of crimson colour in the sample, indicates that the ghee was adulterated with vanaspati.

Analysis of Milk Powder [2,4]

Coal tar dye in Milk Powder:

Addition of resorcinol to milk powder sample MP_1, MP_2, MP_3 produces pink colour shows that the samples were adulterated by coal tar dye.

Analysis of Turmeric powder [4]

Metanil yellow in Turmeric powder:

Detection of metanil yellow in turmeric powder identified by the addition of hydrochloric acid (HCl), appearance of instant pink colour which on dilution with water regenerates the turmeric powder which indicates that the sample T_1, T_2, T_3 were adulterated by coloured saw dust like metanil yellow.

Analysis of Maida [4]

Boric acid in Maida:

Little amount of maida was taken and added with hydrochloric acid (HCl). Solution was tested with turmeric paper produces red colour and turmeric paper changed to green colour on dipping in ammonium hydroxide (NH_4 OH) and gave red colour again re acidification with hydrochloric acid (HCl) show that the sample M_1, M_2,M_3 were adulterated by adding boric acid.



Fig- A – pink colour indicates the presence of coal tar dye in milk powder samples

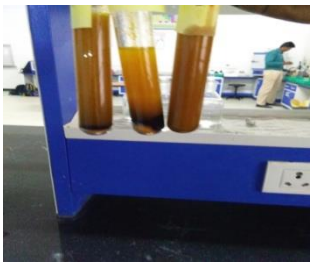


Fig- B - shows that the turmeric samples adulterated.

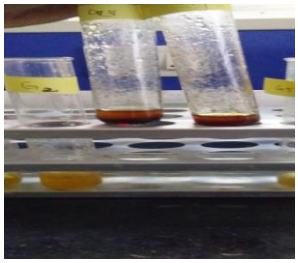


Fig- C – indicates the ghee samples were adulterated.



Fig- D – concludes with addition of boric acid in maida.



Fig- E - shows the adulterated milk sample.

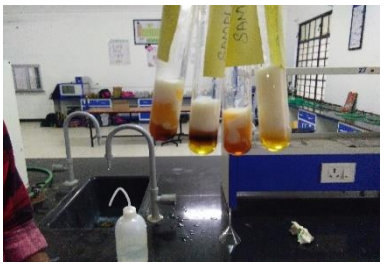


Fig- F – shows the adulterated samples of milk by formalin



Fig- G – shows the results of ammonium test.



Fig- H – shows the results of maida sample before re-acidification with HCl.

Fig- A – pink colour indicates the presence of coal tar dye in milk powder samples.
Fig- B - shows that the turmeric samples adulterated.
Fig- C – indicates the ghee samples were adulterated.

Fig- D – concludes with addition of boric acid in maida.
Fig- E - shows the adulterated milk sample.
Fig- F – shows the results of adulterated milk samples by formalin.
Fig- G – shows the results of ammonium test.
Fig- H – shows the results of maida sample before re-acidification with HCl.

IV. Conclusion

Adulteration of foods is commonly performed in both branded and unbranded foods in daily life. Adulteration is widely practiced from local market to the hypermarket, almost everywhere. But the percentage of using adulterants has reduced compared to the earlier times. In India, intentional addition of adulterants is majorly done which affects the people's health. Many of the people in rural areas are unaware of the practice of food adulteration and its harmful effects. The unawareness and carelessness of the consumers makes the producers to add unwanted materials to the food. This research work is to bring awareness in the rural area people.

References

1. Shruthi Awasthi, Kirti Jain, Anwesha Das, Raza Alam, Ganesh Sruti, Krishnan N, (**2014**), Analysis of Food quality and Food Adultrants from Different Departmental & Local Grocery Stores by Qualitative Analysis for Food safety, Journal of Enviornmental Science, Toxicology And Food Technology, 8(2)
2. Tanzina Azad, Shoeb Ahmed, (**2016**), Common milk adulteration and their detection techniques. International Journal of Food Contamination, 3(22).
3. http://old.fssai.gov.in/portal/o/pdf/draft/MILKANDMILKPRODUCTS.PDF
4. http://vikaspedia.in/health/health-campaigns/beware-of-adulteration/adulteration-in-milk-and-milk-products
5. https://fssai.gov.in/dart/
6. Manual for detection of adulteration in milk products.
   www.updairydevelopment.gov.in/.../FSSAI-Testing-Methods..

Acknowledgments

With the grace of Almighty our heartfelt thanks to our management, college, department, family and soulful friends for supporting us throughout the work.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

SENSORY AND TEXTURAL PROPERTIES OF GLUTEN BASED CHEWING GUM

Suresh kumar.J[1*], G.Nagamaniammai[2] and Shwetha.H.S[3]
[1,2]Department of Food Processing Technology, Amet Deemed to be University
East Coastal Road, Kanathur, Chennai – 603112
[3] Department of Food Process Engineering, SRM Deemed to be University
Kattangulathur. Chennai – 603203, INDIA
sureshjp590@gmail.com http://www.ametuniv.ac.in/

Abstract*: The wheat gluten was used and the developed chewing gum shows satisfactory result. The texture of the chewing gum was less hard than the commercial synthetic gums with value of 209.691 N and329.843 N respectively. The chewiness and springiness of gluten chewing gum was 0.135 and 2.035 respectively which was higher on comparing with market chewing gum. The gluten chewing gum was also found that it is rich in protein and less in fat and sugar level of 25.30%, 0.83%, and 58.81% respectively. The protein was higher, fat and sugar was found to be lower on comparing with synthetic chewing gum. The chewiness of 0.135 and 0.005 for gluten chewing gum and synthetic gluten gum respectively says gluten gum was good to chew. Hence, the final product suits best for consumption on comparing with synthetic gums.*

Keywords*: gluten, natural gum, gluten chewing gum, texture profile analysis, protein rich.*

I. Introduction

Chewing gum, a non-digestible confectionary is a combination of a water-insoluble phase, known as gum base (insoluble gum base resin), elastomers, emulsifiers, fillers, waxes, antioxidants, softeners, sweeteners, food colourings, flavouring agents, and in case of medical chewing gum, active substances[2]. Recently chewing gums along with their properties it has a pleasant way to take vitamins and medicine and recreation. Wheat gluten is the principal protein component of wheat and consists mainly of gliadin and glutenin obtained by hydrating wheat flour and mechanically working the sticky mass to separate the wheat gluten from the starch and other flour components. Vital gluten is dried gluten that has retained its elastic properties. [3]Vital wheat gluten is a sticky, paste-like substance, roughly 75% of which is protein. The primary use of vital wheat gluten is to increase the protein content of flour. The addition of wheat gluten increases the protein level in dough, thereby adding strength and elasticity needed to endure the commercial mixing and kneading processes. This is treated as gum base in this product development. Therefore, according to current FSSA its permissible concentration range in chewing gum is 12.5-15% by weight of the chewing gum.

Commercial lecithin is a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol, with smaller amounts of other lipids. Important applications as emulsifier and stabilizing agent are in food industries [1]. Lecithin in chewing gums and bubble gums also plays role in properties including Softens Gum Base and Chew Regulates Adhesive Properties, Reduces Tackiness/Stickiness, Improves Flavor Perception and Longevity, Enhances Resiliency and Stretch, Improves Shelf Life – Flexibility and Moistness, Reduces Toughness and Elasticity [3]. Willem van Nieuwenhuyzen and Michael Sponholtz in 2003 conducted a study on Quality Improvements in Chewing Gums Using Lecithin (Flexibility, Hardness and Taste). Guar gum, the natural substance obtained from the maceration of the seed of the guar plant, *Cyamopsis tetragonoloba* (Linne) Taub., or *Cyamopsis psoraloides* (Lam.) D.C.[4] used as a Natural thickener Stabiliser Bonding agent Hydrocolloid Emulsifier [31]. As it is thickener, it is not used more than 20% by weight of the wheat chewing gum in the present work being discussed. Sorbitol, a polyol (sugar alcohol), is a bulk sweetener found in numerous food products. Sorbitol is approximately 60 % as sweet as sucrose with one-third of the calories. It has a smooth mouth feel with a sweet, cool and pleasant taste. Sorbitol occurs naturally in a wide variety of fruits and berries. Sorbitol can be used as sweetener, humectants, sequestrant, texturizer, stabilizer and bulking agent in food industries. The peppermint flavour used here is a mixture of both synthetic and natural peppermint flavor. It's free from spirit. The objectives of the work were to optimize the ingredients for preparing wheat based chewing gum and to compare the sensory, textural and proximate properties with synthetic chewing gum.

II. Materials and Methods

The main ingredients chosen for wheat chewing gum are wheat gluten flour, guar gum,sorbital peppermint and liquid lecithin which has been purchased from Chennai market and also utilized from department laboratory. Intitialy the wheat gluten flour, lecithin and distilled water was standardized and guar gum was optimized. Optimization software by name The Unscrambler X 10.2 was used to optimize the hypothetical range obtained in phase 1. The intial optimization was done and optimized with design expert and the final composition was choosen for proximate and sensory evaluation. The process flow for preparation of gluten chewing is as follows

Wheat gluten flour
⇩
Addition of lecithin
⇩
Addition of guar gum
⇩
Addition of water
⇩
Addition of guar gum
⇩
Addition of sorbitol
⇩
Addition of peppermint
⇩
Mixing
⇩
Sheeting
⇩
Cutting (2mmx1mm)
⇩
Coating of 2% Methyl Cellulose Solution
⇩
Dusting with fine sugar powder

Preparation for sensory evaluation

Raw material was optimized with the design expert and 5 samples were chosen for further studies which shows a sum of 100 and subjected to sensory evaluation of 9 point Hedonic scale. The best sample was prepared and rolled into thin sheet of 5mm thickness and scored into 2mmx1mm rectangles.

Proximate Analysis

Proximate Analysis including moisture (IS: 11623-1986), Protein (IS: 7219-1973), Fat (IS: 7874 (Part I) – 1975), Total Ash (IS 14433 (Part I): 1997), Metabolizable Energy were analyzed by following AOAC methodology[5].

Texture Profile Analysis

Texture Profile Analysis (TPA) was done by Universal Texture Measurer at Central Food Technology and Research Institute, Mysore. Its model is LR-5K. Load cell was 1kN. Cylindrical plunger/probe was of 35mm diameter. Speed was 50mm/minute. TPA covers parameters like Hardness, Area, Cohesiveness, Springiness, Gumminess, Chewiness and Adhesiveness.

III. Result and Discussion

Samples were optimized with design expert and trails have been made. Out of the trail the sample shown in table shows the best output and chosen for sensory analysis. The sensory says sample no 77 was having good profile and it was chosen for further proximate and texture profile analysis and was shown in figure 2. The following pictures show the various sensory attributes of the selected samples. It has been found that sample 77 was dominating in overall acceptability, texture and equals the dominancy in chewability. Sample 74 which stands high in flavor could be because the mint and sorbital are at right ration, which enhances the mouth feel of the sample. Sample 73 doesn't prefer by sensory people other than appearance. The chewability of the product was almost same for the entire sample, it might be due to the wheat gluten that been added in same proportion for the entire sample. On considering the taste of the product, sample 77 found to be satisfactory

Table 1 – Selected samples for sensory evaluation based on the result of Design expert

Sl. No.	Lecithin	Wheat Gluten	Guar Gum	Sorbitol	Peppermint Flavour	Sum
73	2.3	46.5	9.8	6.25	35.25	100
74	2.3	46.5	9.8	11.25	30.25	100
75	2.3	46.5	9.8	16.25	25.25	100
76	2.3	46.5	9.8	21.25	20.25	100
77	2.3	46.5	9.8	26.25	15.25	100

On comparing the whole attributes and overall acceptability, it was found that sample 77 been suggested by the sensory panel. The proximate analysis was done for sample 77 and the values are shown in table.. The proximate was compared with the synthetic chewing gum that been available in local market. From the analysis, we can declare that the sample 77 was rich in protein. The result also says that there is lower level of fat and carbohydrates. This could be because of the wheat gluten as wheat is rich of protein. The moisture percent was found to be higher in sample 77 which could play role in microbial growth. The texture profile analysis was done and the pictorial representation was shown in figure 3 and 4. Figure 3 shows the hardness value for sample 77 and the figure 4 shows for commercial chewing gum.. Table 3 shows the various parameters of TPA. The hardness was found to be 209.691 and 329.843 for gluten gum and market gum respectively for the first bite which says that gluten chewing gum shows less hardness with market sample. The TPA also shows that Cohesiveness,Springiness,Gumminess,Chewiness,Adhesiveness was higher on comparing with synthetic chewing gum. This implies that the gluten chewing gum was good to consume with less hardness and good chewability. On comparing the hardness of the gum, it was found that sample 77 was less hard which implies the good chewability of the product as shown in sensory attributes that clearly explained in the figure 1.

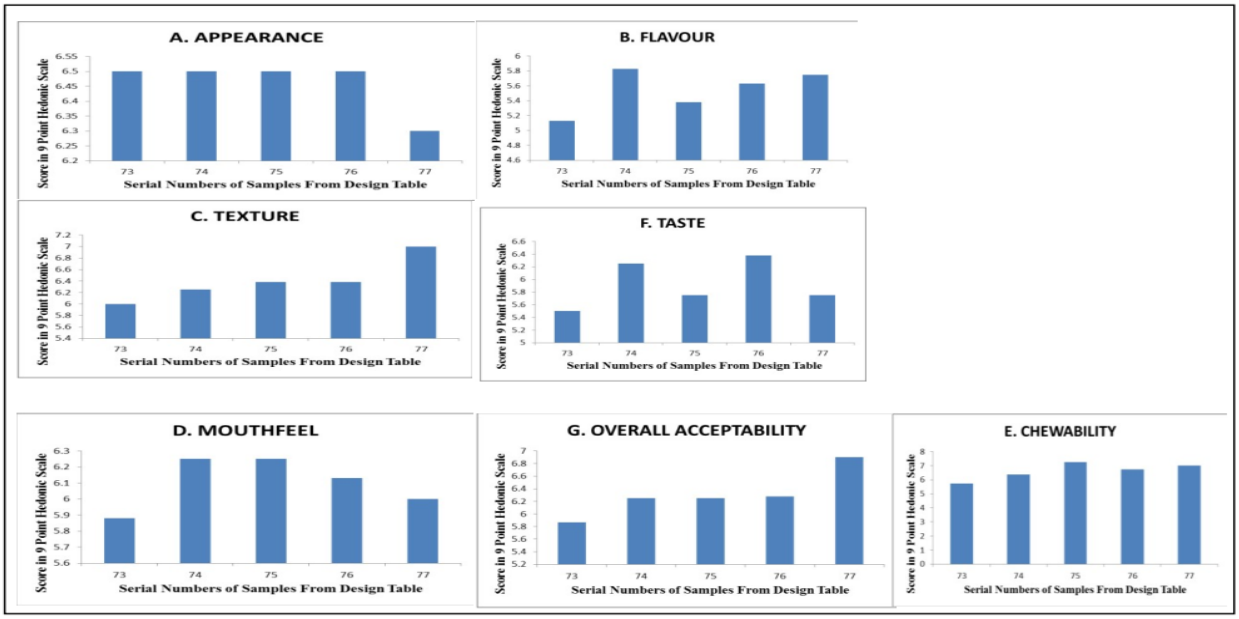


Figure 1- Sensory evaluation of various attributes for the selected sample



Figure 2 – Sample 77 – Final Wheat gluten chewing gum

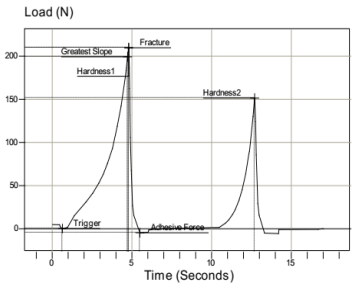


Figure 3 – Texture profile analysis of wheat chewing gum

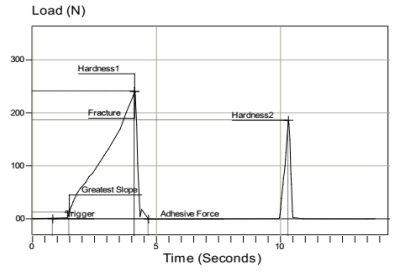


Figure 4 – Texture profile analysis of Synthetic chewing gum

Table 2 – Proximate composition of Sample 77 and synthetic chewing gum

Sl. No.	Parameters	Wheat Chewing Gum	Synthetic Chewing Gum Available in Market
1	Moisture	14.69%	1.83%
2	Protein	25.30%	0.47%
3	Fat	0.83%	11.08%
4	Total Ash	0.37%	4.72%
5	Carbohydrates	58.81%	81.90%
6	Metabolizable Energy	343.9kcal/100g	429.2kcal/100g

Table 3 – Texture Profile Analysis of Sample 77 and Synthetic chewing gum

Sl. No.	Sample	Sample Height (mm)	Hardness1 (N)	Hardness2 (N)	Area1 (kgf.mm)	Area2 (kgf.mm)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (Nm)	Adhesiveness (Nmm)
1	Wheat Chewing Gum	6.5	20.9.691	151.411	19.876	6.276	0.316	2.035	66.212	0.135	0.182
2	Market Chewing Gum	5	329.843	222.244	37.543	1.773	0.047	0.314	15.579	0.005	0.004

IV. Conclusion

The results of Gluten chewing gum was discussed earlier. This product was developed with an idea to replace the synthetic additives. In such manner the product proves that the gluten gum was found to be satisfactory on comparing the commercial gum both in texture and proximate. The rich protein content of 25% helps the growers to attain the body structure. The higher moisture that present in the gluten gum could act as a source for the microbes to develop; hence the shelf life of the final product would be of low on comparing the synthetic gums. The lower fat and carbohydrate level aids the people with CVD and diabetics to consume the gums during their leisure hours, teeth strengthener, freshener, mind relief, etc. Therefore the product made as a chewing gum from wheat gluten and other additives make as nutritious supplement and also as a replacer of synthetic gums.

References

[1] Surana SA. 2010. *Chewing gum: A friendly oral mucosal drug delivery system*. International Journal of Pharmaceutical Science; Vol.4, Issue 2, Page no.68-71.

[2] Farhad Mehta, Piyush Trivedi. 2012. *Formulation and Texture Characterization of Zein Chewing Gum.* Archives of Applied Science Research; Vol.4, Issue 2, Page no.781-791.

[3] Naik Heema, Gupta Stuti. 2010. *Medicated Chewing Gums- Updated Review.* International Journal of Pharmaceutical Research & Development. Vol. 2, Issue 8, Page no.66-76.

[4] Willem van Nieuwenhuyzen, Michael Sponholtz. 2003. *Quality Improvements in Chewing Gums using Lecithins (Flexibility, Hardness & Taste).*

[5] *Manual of Methods of Analysis: Beverages (Coffee, Tea, Cocoa, Chichory), Sugar & Sugar Products and Confectionary Products.* 2005. Directorate General of Health Services Ministry of Health and Family Welfare, Government of India.

[6] Anis Alam Siddiqui and Khaliduzzaman. 2012. *Studies On The Preparation of Green Mango Chewing Gum.*Bangladesh Research Publications Journal, ISSN: 1998-2003, Volume: 7, Issue: 1, Page: 01-04, May – June.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Effect of Honey on Functional Compounds and Sensory Attributes of Natural *Amla* (*Emblica officinalis* Gaertn) Milk shake

Hisham Mammu[1] and *G. Nagamaniammai[2]

[1]Department of Food and Process Engineering, School of Bioengineering, SRM Deemed to Be University, Kattankulathur-603203, INDIA.

[2] Department of Food Processing Technology, AMET Deemed to Be University, Kanathur, East Coast Road, Chennai -600 112, India. *Corresponding Author: nagamaniammai@gmail.com

Abstract: *In this paper, an attempt made on development of ready- to - drink milk shake enriched with vitamin C and other functional compounds. Freshly extracted amla juice in 5 different concentrations from 8.34 to 10.44 was mixed with remaining mixture of honey and milk at 10°C. Analysis like Total Soluble Solids (TSS), pH, acidity, ascorbic acid, total protein content, total sugar content, total phenol contentand total tannin content along with sensory attributes such as colour, taste, flavor and Overall Acceptability (OAA) of the samples performed. The results showed that OAA and other functional compounds were found to be good for sample 4 containing, 77.51% milk and approximately equal quantity of amla juice and honey. There was not a significant difference in the functional compounds at P≤ 0.05% for sample 4 with other samples. The functional compounds enriched milk shake as a natural product with extended shelf life weredeveloped and validated for regular consumption.*

Keywords:*Amla, Functional compound, Honey, Milk shake,Sensory attributes*

I. Introduction

The growing interest in new functional foods with special characteristics and health properties has led to the development of new beverages based on fruit juiceskim milk mixtures. The proliferation of ready-to-drink beverages has caused the market to focus its interest on these products.Commercial conventionally pasteurized or sterilized beverages based on a mixture of fruit juice and skim milk were evaluated nutritionally for their concentrations of vitamin C, vitamin A and phenolic compounds and their total antioxidant capacity, taking the influence of physicochemical parameters into account. Zulueta, conducted studies & they reported that main contribution to the total antioxidant capacity was provided by vitamin C, followed by phenolic compounds [1]. In order to meet the research and market demand, the work was carried out to develop *amla* based milkshake with honey as a natural preservative.

Amla is a gift of nature to mankind. It is an indispensable part of the Ayurvedic and Unani system of medicine. The ascorbic acid content of fresh amla fruit can range up to 950mg/100g which is said to highest among all fruits next only to Barbados cherry (*Malpighia glabra L.*) (1677.6 mg of vitamin C in 100 g of fruit)[2]. Amla has been reported to possess expectorant, purgative, spasmolytic, antibacterial, hypoglycemic[3], [4], hepatoprotective and hypolipidemic activity [5]. The aqueous extract has been reported to have anti-pyretic laxative and tonic properties and also showed antibacterial activity [6].

Milk is one of the most nutritious foods. It is rich in high quality protein providing all ten essential amino acids. It contributes to total daily energy intake as well as essential fatty acids, immunoglobulins, and other micronutrients. The exact components of raw milk vary by species, but it contains significant amounts of saturated fat, protein and calcium as well as vitamin C. Cow's milk has a pH ranging from 6.4 to 6.8, making it slightly acidic[7].Milk fortification began in the first half of this century. Vitamin D fortification of milk in the United Kingdom is reported to have begun in 1923; currently, milk fortification with vitamins A and D is practiced in India as per FSSAI regulation.

Honey is considered to be a natural sweetener with potential nutritional and functional benefits [8].Honey appears to be effective in killing drug-resistant biofilms which are implicated in chronic rhinosinusitis[9]. Eddy reported thatTopical honey has been used successfully in a comprehensive treatment of diabetic ulcers when the patient cannot use topical antibiotics[10].According to Ayurveda taking amla with honey increases the

medicinal effects of amla multi fold. This unique product has amla segments and shreds preserved in honey. It is delicious, pure, nutritious and full of health (Source: Market - Super sales enterprises).The work was carried out to study the effect changes in amla juice concentration on the amla milk shake with honey on its functional compounds.

II. Materials and Method

Preparation of Amla milkshake

Mature fruits of amla were procured from the Tambaram market, Tamilnadu.Dabur Honey and standardized milk were also procured from the Super markets located at the same place.Amla was washed in running tap water, then seeds were removed and the edible part were cut into pieces manually. The cut pieces were ground by food processor and juice was filtered using muslin cloth. Milk was boiled in a vessel at 101°C for 3 minutes. Cooled rapidly to 10°C then cream was removed by filtering.Initially honey was mixed thoroughly with chilled milk, further amla juice was also added to prepare amla milkshake. Optimization of the amla milkshake samples with honey was done by using various trial and error methods. The sensory analysis of the amla milkshake samples of varying concentrations was also important in the optimization process. There was a recommendation by judges of sensory analysis tests to improve the overall flavor of the product. So amla juice concentration as well as the sweetening agent honey's quantity was varied. The composition of amla milkshake samples with honey is shown in Table 1. Amla milk shake formulation was optimized based on sensory attributes and functional compounds.

Table 1. Formulation of Amla milkshake with honey

Ingredients	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Milk (%)	83.3	81.96	80.64	77.51	74.62
Amla juice (%)	8.34	9.83	11.29	10.83	10.44
Honey (%)	8.34	8.196	8.06	11.64	14.72

Total carbohydrate content, titrable acidity were determined [11]. Total phenol content[12], tannin content [13] , crude protein [14] , Sensory evaluation was conducted by 15 untrained panelists by using 9 point Hedonic scale from 1-9 as unlike extremely to like extremely [15]. Statistical analysis of the results were conductedusing paired t test. The paired t- test confidence interval and test procedures are used to analyze the differences between paired observations.

III. Result and Conclusion

Changes in Functional compounds of Amla milkshake

The results of the functional compounds of amla milkshake with honeys are shown in Table 2. The results obtained from the analysis showed that titrable acidity, ascorbic acid, tannin content, and total phenol content of the milkshake samples increases rapidly with increase in juice concentration. It is evident that the same trend was observed by work done by Dachiya [16]. There was not much change in pH and protein content of the samples. At the same time there is a drastic increase in the total sugar content, but slight increase in TSS was observed from Sample 1 to sample 5, Amla with honey based ready to serve beverage also showed same trend with increase in composition by increase in honey and amla juice [17]. The changes were due to increase in amla juice and honey concentration in the samples. The formulation was fixed as trial and error method without chemical additives, honey concentration was also increased to control curdling of milk by adding amla juice. Significant changes were observed in the all the compounds except pH and protein at $p<0.05$ level with respect to changes in the concentration of amla juice in the milk shake.

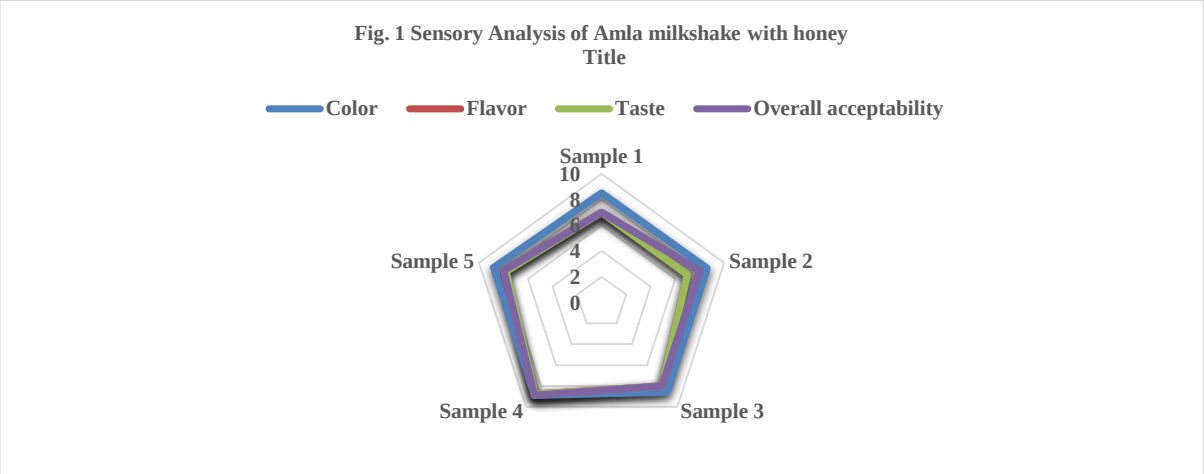
Table 2.Functional compounds of Amla milkshake with honey

Chemical analysis	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
TSS in ^{o}Brix	21.33±0.32^{a}	21.67±0.38^{b}	22.67±0.35^{c}	23.33±0.48^{d}	23.87±0.12^{e}
Titrable Acidity (%)	0.452±0.025 ^{a}	0.466±0.012 ^{b}	0.470±0.029 ^{c}	0.478±0.032 ^{d}	0.480±0.031 ^{e}
Ascorbic acid content (mg/100g)	78.94±0.23 ^{a}	78.94±0.32 ^{b}	92.1±0.45 ^{c}	92.1±0.31 ^{d}	92.1±0.48 ^{e}
pH	5.83±0.074 ^{ax}	5.88±0.069 ^{bx}	5.91±0.044 ^{cx}	5.93±0.062 ^{dx}	5.98±0.052 ^{ex}

Protein content (g/100ml)	3.09±0.022 ^{ax}	3.12±0.015 ^{bx}	3.15±0.043 ^{cx}	3.15±0.032 ^{dx}	3.16±0.049 ^{ex}
Total sugars (g/100ml)	45.31±0.27 ^{a}	47.52±0.44 ^{b}	50.28±0.38 ^{c}	52.7±0.50 ^{d}	55.1±0.48 ^{e}
Total phenols (g/100g)	0.217±0.023 ^{a}	0.221±0.019 ^{b}	0.223±0.020 ^{c}	0.224±0.017 ^{d}	0.226±0.012 ^{e}
Tannins content (%)	0.273±0.009 ^{a}	0.277±0.008 ^{b}	0.281±0.004 ^{c}	0.28±0.002 ^{d}	0.28±0.003 ^{e}

Mean values within a row with single letter are significantly ($p<0.05$) different among the respective process by paired t- test.Mean values within a row with pairs of letters are not significantly ($p<0.05$) different among respective process by paired t-test.*N=3; Mean±std. dev.

Sensory characteristics of amla milk shake with honey



The sensory attributes of amla milk shake with honey was pictatedin the Figure1. The observation from the figure undicated that the the colour of the sample was not changed much, flaour and taste of the all 5 samples results were colse with each other. Nut the overall acceptability of amla milk shake was high for sample 4 compare with other samples. The higher concentration of both honey and amla juice score lesser compare to sample 4 because acceptablity was limited by astrigent taste.

The functional compounds analysis also indicates that, there was not mush differernce between sample 4 and 5 execpt pH and protein content. Hence, sample 4 containing 77.51% mil, 10.83% amla juice and 11.64% honey sample 4 was fixed as a optimized formulation with high functional compunda and sensory characteristics. The functional compound analysis studies done by Ghosal[18] and amla post-harvest review by Goyal[19] also revealed protein, ascorbic acid and total phenol compound of amla juice incorporated beverages stored under lower temperature were stable for more than 3months. The stablity study was for optimized sample was also conducted at storge temperature at 8°C for 15days in closed galss bottles. The sample retained its orginial characteristics upto 10days without changes in the functional compounds and microbial load.

IV. Conclusion

A functional compound enriched milk shake was developed in this research. It was observed from the study that increase in amla juice concentration rapidly improved ascorbic acid, total phenol and tannin content of the milk shake. The attempt to prepare natural dairy beverage with anti-oxidants and invert sugar could be one of the most recommended healthy ready to drink milk shake for those who are suffering in chronic health issues.

References

[1]. Zulueta, A., Esteve, M. J., Frasquet, I. and Frígola, A. (2007) "Vitamin C, Vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain". Food ChemistryVolume 103, Issue 4, Pages 1365-1374.

[2]. Johnson, Paul D. (2003). "Acerola (*Malpighiaglabra* L., *M. punicifolia M. emarginata* DC.) Agriculture, Production, and Nutrition". In Artemis P. Simopoulos; C. Gopalan. *Plants in Human Health and Nutrition Policy*. 91. Karger Publishers. pp. 63–74.

[3]. Thakur, C.P and Mandal, K. (1984). "Effect of *Emblica Officinalis* on Cholesterol Induced atherosclerosis in rabbits". *Indian Journal of Medical Research*, 79, 142-146.

[4]. Jayshri, S and Jolly, C.I. (1993). "Phytochemical antibacterial and pharmacological investigations on *Monordicachiranlia*and *Emblica officinalis"*. *Indian Journal of Pharmaceutical Sciences*. 1, 6-13.

[5]. Jamwal, K.S., Sharma, I.P. and Chopra, L. (1959). "Pharmacological investigations on the fruits of *Emblica Officinalis"*. *Journal of Scientific and Industrial Research*, 18 c, 180-181.
[6]. Vinayagamoorthy, T. (1982). "Antibacterial activity of some medicinal plants of Srilanka Ceylon". *International journal of biological sciences*, 11, 50-55.
[7]. Bowen, W. H. and Lawrence, R. A. (2005). "Comparison of the Cariogenicity of Cola, Honey, Cattle Milk, Human Milk, and Sucrose". *Pediatrics* Vol. 116 No. 4, pp. 921–926.
[8]. Jones, R. (2001). "Honey and healing through the ages". Cardiff: International Bee Research Association IBRA, pp. 1-4.
[9]. ScienceDaily. (2008). Honey Effective In Killing Bacteria That Cause Chronic Sinusitis.

[10].Eddy, J. (2007). "UW study tests topical honey as a treatment for diabetic ulcers", University of Wisconsin–Madison.

[11]. AOAC, "Official Methods of Analysis,"(984.25). 17th Edn. Association of Official Analytical Chemistry, Maryland-14; 2000.

[12]. Sadasivam S, ManickamA, In: Biochemical methods for Agricultural Sciences.Wiley Eastern Ltd. New Delhi, India. 1992: 187-188.

[13]. Burns RE Method of tannin analysis for forage crop evaluation. Tech. Bull. Georgia Agric. Station, Athens. 1963; 32: 62-66.

[14]. Ranganna, S. Handbook of analysis and quality control for fruit and vegetable products. 2nd edition. *Tata Mc Graw- Hill Publishing Company Ltd. New Delhi*. 1997.

[15]. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., Journal of Biological Chemistry., 193, p.265, 1951.

[16]. Dachiya, S.P. and Dhawan, S.S. (2001)."Physico-chemical characteristics of aonla (*Emblica officinalis* Gaertn.) cv. Chakaiya", *Indian Food Pack*er, 55(6), 133.

[17]. Jain, S. and Khurdiya, D. S. (2004). "Vitamin C Enrichment of Fruit Juice Based Ready-to-Serve Beverages Through Blending of Indian Gooseberry (Emblica officinalisGaertn) Juice", *Plant Foods for Human Nutrition*, Volume 59, Number 2, pg. 63-66.

[18]. Ghosal, S (1996). "Active constituents of *Emblica officinalis:* Part 1 – The chemistry and antioxidative effects of two new hydrolysable tannins, Emblicanin A and B". *Indian Journal of Chemistry*, 35B, 941-948.

[19]. Goyal, R.K., Patil, R.T., Kingsly, A.R.P., Himanshu, W. and Pradeep, K. (2008). "Status of post-harvest technology of Aonla in India-A review". *American Journal of Food Technology*, 3: 13-23.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

BIOSENSORS FOR FOOD ALLERGENS DETECTION

Senthilrani.N[1], Jenifer.A[2], Kousalya Mangai.S[2], Subhangini Thithima[2], Vivega[2], Seema Afra[2], Mega varshini[2], Sivarajasekar. N

[1]Department of Medicine, Government Medical College and ESI Hospital, Coimbatore-641015, TN, India

[2]Kumaraguru College of Technology, Coimbatore-641049, India.

jenifer.16bt@kct.ac.in

Abstract:

In recent times food allergy is considered as one of the major health threats for sensitized individuals all over the world and, over the years, significant efforts and investments have been made by food industry to offer safe foods for allergic consumers. It is the need of the hour ,as everyone depends on a rapid, sensitive and reliable methods for tracing food allergens in commodities . So that one can minimise the risk associated with the presence of hidden allergens in food.The allergic reactions due to food falls under the category of type 1 hypersensitivity.Severe allergic reactions lead to anaphylaxis which is a life threatening allergic response which affects the whole body . Repeated exposure to the food allergens results in the risk of more severe anaphylaxis.

Keywords: *Biosensor; food allergens; QCM; aptamers; fret sensing; gluten; anaphylaxis*

I.Introduction

The daily life of the individuals with food allergy is more disrupted than in individuals with rheumatoid and coronary diseases. Family activities are usually limited for people with food allergic children. Foods that are usually sensitive to people on the average includes milk, egg, peanuts, fish, brinjal, gluten and many more. [2]Once the type of food responsible for the food allergy is diagnosed, the most efficient method is to avoid using the allergens because the reason for the development of the food allergies is not known.[3] So, in order to identify the presence of the allergen in the food, biosensors are widely used in recent times. Biosensing is the combined technology of various disciplines of science like biology, chemistry, physics and computer science.[1]The sensors are usually grouped into two : *catalytic type* and *affinity type*. There are so many limitations and in order to overcome this, the use of proteins or enzymes as a part of sensors is employed. Due to the small size of the ligands, they are replaced with the enzymes which are highly stable.

The research papers that are related to the sensors that detects the food allergens are dealt and outlined here. The detection of almost all the food allergens are based on their binding ability and their emission spectrum. These type of biosensors are effective when it detects only the target food allergen and not the other ingredients which could be essential for the food allergic patients. [4]The prevalence of the food allergy in India was about *2-3%* and most commonly affected children but now it is found to have a surprising rise.

II.Detection of gluten alergy using biosensors

The Sensors usually work on the principle of gathering by measuring photons emitted. *Celiac disease* is a type of immune reaction characterized by the *inflammatory action in the small intestine* that usually affects the genetically susceptible people(due to the inappropriate T-cell activation stimulated by peptides present in the wheat gliadin or the prolamines from the barley).[5]

The gluten proteins responsible for the allergy is of two groups. I) *soluble gliadins* in free state II) *insoluble glutenins* in their clustered state. Among the two groups, gliadins are exclusively responsible for this disease.By the use of *reversed phase HPLC* , 30 different components of gliadins proteins were discovered.[6]

There are different methodologies discovered by performing various assays and some of the different sensors are discussed here. They are i) FRET-sensing ii) Reflectivity based sensors iii)sensors based on EFLISA

(i)FRET-Sensing:

This methodology is based on the biomolecules. i.e., the use of *E.coli glutamine binding protein (GlnBP)* which is a monomeric protein. The principle is the ability of the GlnBP to bind with glutamine. Since it is believed that gliadins contain large amount of glutamine residues, it is the question that GluBP protein could bind the gliadins also. It is said that the emission spectrum obtained from the GluBP alone is initially taken and then the emission spectrum from the *fluorescence labeled GluBP PT-gliadin*. This method is an effective method since

GluBP only binds to gluten and does not bind prolamines from corn or a peptin of zein and also rice which are all important for celiac patients diet. Thus it is concluded that gluten which contains glutamine resides are the substrate for GluBP.[7]

(ii) Reflectivity based sensors :

This methodology is based on the linkage of protein to the *porous silicon wafer(Psi*) whose performance was meant to be transducer. The principle is that when exposed to the target analyte, it results in the change in *photoluminous or Reflectivity.* Due to the disadvantages in handling complex mixtures, ligand binding proteins were used in the biosensor where initially a covalent bond is formed between a analyte and the GluBP protein. After completing *passivation* process and testing, the ligand binding event takes place which can be noticed due to the large conformational change in the GluBP. This event leads to the fringe shift in the wavelength which can be visualized from the graph and the amount of the gluten in the food can be determined from the graph which should be plotted as optical variation as a function of PT-gliadins concentration.[8]

(iii) Nano structural based sensors- i.e., based on EFLISA:

This methodology is designed for determining the minimum amount of gluten content to be present in the food for celiac patients. It is based on the fluorescence emission on the *silver island films* that is coupled to *Total Intern Reflection Mode.(TIRF).* The process is enhancement which is based on the three process like formation of local field , excitation and far field radiation.This method is the utilisation of silver islands Coupled to *EFLISA (Enhanced Florescent Linked Immunosorbent Assay)*. The sensitivity for detecting gluten is enhanced to 10 times in silver islands than in glass plates. Hence by this method, *EDL (Estimation detected Limit)* is found to be *10 micro gram per milli litre* which is the clinical cut off for gliadins presence in food for celiac patients. This method is found to be a very fast and cheap wet procedure for identifying gluten-free foods.[9]

III. Detection of fish allergy using biosensor

This highly sensitive,accurate,on site,time efficient and cheap instrument helps detect allergens present in the sample. Its working is based on the fact that it comprises of a *microfluidic paper* assisted analytic device with hydrophobic channels backed up by a wax filter on filter paper and functionalized grid gold nanoparticles which help identify the substances that lead to hypersensitivity reactions in our body.

Different proteins cause allergic reactions in our body. A wide variety of people are allergic to proteins such as Arachin present in peanuts,beta-lactoglobulin in milk, tropomyosin present in shrimps.This additionally helps identify cross contamination by cockroaches.

The speciality of this instrument is based on the use of *aptamers*(biotinylated).Aptamers are short DNA or RNA oligonueotides that have specific complementary sequences for the epitope of antibodies.These aptamers are conjugated*(biotin-streptavidin interaction)*with gold nanoparticles.When the sample is added,the conjugate interacts with the allergen(protein)(if any).Later,graphene oxide is added to the sample.This gets attached to the conjugate that hasn't interacted with the allergen protein.This results in a colour change that might range from colourless to pink.Next step includes the analysis using a paper-based analytical device that utilises the principle of capillary action and a filter that helps grade the colour change.

IV.Detection of peanut allergy using biosensor

Peanuts are considered to be the most allergic food. It contains 12 different allergens identified(till date)as *arachin(ara) H1-ara H13*. Ara H1, H3 and H6 are identified as the most significant among the 13. Studies also show that Ara H8 is found to be pathogenic and considered under Class II allergens which can cause symptoms in individual with *pollen allergy.* Ara H9 is a lipid transfer protein that triggers response in a individual with *peach allergy* where the patients test positive for *Pru p 3-specific IgE antibodies*.The high stability of Ara H6 makes itself a suitable marker for the identification of peanuts in food. For a distinct detection of Ara H6,a biosensor has not been described till date. Hence the methodology of using *screen printed carbon electrodes(SPCE)* has been employed. For the detection of Ara H6 SPCE surface was coated with gold nanoparticles which results in increased binding reactions between the allergens and antibodies. Sandwich type immunoassay is the principle used here.

The process includes coating the SPCE surface with monoclonal anti Ara h6 IgG which was developed against Ara H6 using two monoclonal mouse IgG antibodies.[10]The binding of the allergens to the anti Ara H6 molecule detects the presence of peanut in the food matrices which could be visualized using the electrochemical current. Using this method, when the food samples with and without the peanut was analyzed, the food without peanut gave negative result whereas even small amount of peanut present in the sample was/can be detected. The validation studies of this chapter of using SPCE indicated that it was precise and produced accurate results.

V. Detection of soybean allergy using biosensor

Soybean additives in milk are popular because it does not involve high cultivation price and meets to need of higher protein content in the milk products. Despite being one of the 'Big Eight' causes of food allergies, soybean allergens are highly detected in milk products. With *POC technology*, common allergen Glysinin is identified by combining chromatographic lateral flow and gold labeled antibody molecule reaction. Either t-sample analytical cartridge fill or the test marker contain three wells with a sample load absorbent and conjugate reagent. These wells are associated with the sample test membrane by the charge of *run line* and a *test line.* The test trip could be used for qualitative, semi-quantitative, and quantitative detection because glysinin is the most sensitive and specific among other soy proteins or allergens. An analysis of powdered milk samples can be completed in under 10 min with the detection range is *81 to 90.0 mg/kg,* detection limit of *0.7 mg/kg* based on results verified with an optical density immune biosensor.[11]

More local and national food industries around the world are implementing regulations labelled with genetically modified (GM) food. The emerging biosensor i.e., magnetic electrochemical immunosensor incorporates with c-DNA molecule. The end point PCR is manipulated for the detection of both GM and non-GM lectin compound in soya beans. The two distinct DNA sequences expand and are captured by magnetic beads to trigger an enzyme labelled by probe measured by *chronoamperometry*. When compared, RT-PCR detection proved to be reliable, cost effective and simple. The detection limit was *61.7 per gram* for the GM sequence and *1.2 ng for lectin.*

VI. Detection of milk allery using biosensor.

Milk adulteration can be detected using *quartz crystal microbalance(QCM)* based biosensor. A quartz crystal microbalance immunosensor was developed using commercial crystal resonator and polyclonal antibody specific to cow milk protein. A particular protein at 208 KDa is discovered just in cow's milk and does not exist in goat milk. The presence of this protein can be utilized as a marker of cow's milk content in a target solution. To recognize the PSS 208 kDa protein, antibody explicit to the PSS 208 was created. The purified antibody was immobilized on top of the sensor surface on a polystyrene layer. The limit of detection is found to be 1 ppm. The concentration of antibody used must be ranging from 200 ppm to 2000 ppm. [12]

The usage of silver electrode in the crystal resonator is irrelevant for the immobilization of biomolecule. The silver electrode might react with the buffer solution used and might result in variation in the resonance frequency of the QCM due to electrode oxidation. The antibody to be immobilised in the QCM sensor is injected along with tris HCl buffer solution to the sensor surface . Then followed by BSA blocking and washing with PBS. The target solution is passed over the sensor surface .[13] Then the resonance frequency is obtained. The steady resonance frequency is obtained using a specific cow milk protein. Most of the people consume milk produced by goat,cow,donkey,buffalo,sheep,camel.Goat milk is adulterated typically utilizing cow's milk . Cows milk is broadly accessible and its cost is less expensive contrasted with goat milk. Milk is a supplement rich, white fluid nourishment delivered by the mammary organs of warm blooded animals. It is the essential wellspring of nourishment for baby and other warm blooded animals .Dairy farms produced about 730 million tones of milk in 2011,from 260 million dairy cows. The countries which are the largest exporter of milk are United States, India ,China ,Brazil. The countries which are regarded as the largest importers of milk are Russia and China . Lactose intolerance is different from allergy due to milk .[14]Lactose intolerance is a common digestive problem where the body can't process lactose, a kind of sugar primarily found in drain and dairy items. The body digests lactose utilizing a substance called lactase. This separates lactose into two sugars called glucose and galactose, which can be effectively retained into the circulation system. Individuals with lactose intolerance doesn't have the ability to synthesize lactase, so lactose remains in the stomach where it's fermented by microscopic organisms.[15] Recent statistics reveal that in the last three decades, world milk production has increased by more than 50 percent, from 500 million tones in 1983 to 769 million tones in 2013. Laboratory intensive procedures such as ELISA,PCR, spectroscopy, chromatography and HPLC are usually used in order to find any adulteration in milk but suitable only for large scale industries.

VII. Conclusion

With the scope for biosensors expanding at a rapid rate, the need to develop new technologies to quench the thirst for effective solutions is growing too. It's global market is said to reach over $22.5 billion by 2020. With 8 major allergens and sesame allergy on the rise, biosensors can come handy in providing a proactive solution for this concern. Although children tend to outgrow these allergies by the age of 5, the real problem is when there is failure of the above matter. Since there is no cure for these allergies, one needs to look for an effective measure to help prevent contact with the allergen. Because of the fact that an allergic reaction is triggered when in contact with the allergen protein, the use of monomeric proteins/antibodies/aptamers help detect the target. It is also detected using their property of fluorescence and their linkage with porous silicon wafer. Alternative biosensors are also present in our current market and have proven to be successful not only in detecting allergens but also by restoring the 'normal factor' in the lives of people with food allergies.

References

[1] Gotoh, M., Mure, H., & Shirakawa, H. (2003). U.S. Patent No. 6,503,381. Washington, DC: U.S. Patent and Trademark Office.

[2] Sicherer, S. H., & Sampson, H. A. (2010). Food allergy. Journal of allergy and clinical immunology, 125(2), S116-S125.

[3] Christie, L., Hine, R. J., Parker, J. G., & Burks, W. (2002). Food allergies in children affect nutrient intake and growth. Journal of the American Dietetic Association, 102(11), 1648-1651.

[4] Breiteneder, H., & Radauer, C. (2004). A classification of plant food allergens. Journal of allergy and clinical immunology, 113(5), 821-830.

[5] da Silva Neves, M. M. P., González-Garcia, M. B., Nouws, H. P. A., Delerue-Matos, C., Santos-Silva, A., & Costa-García, A. (2010). Celiac disease diagnosis and gluten-free food analytical control. Analytical and bioanalytical chemistry, 397(5), 1743-1753

[6] Matveeva, E., Gryczynski, Z., Malicka, J., Gryczynski, I., & Lakowicz, J. R. (2004). Metal-enhanced fluorescence immunoassays using total internal reflection and silver island-coated surfaces. Analytical biochemistry, 334(2), 303-311.

[7] Dattelbaum, J. D., & Lakowicz, J. R. (2001). Optical determination of glutamine using a genetically engineered protein. Analytical Biochemistry, 291(1), 89-95.

[8] D'Auria, S., Scire, A., Varriale, A., Scognamiglio, V., Staiano, M., Ausili, A., ... & Tanfani, F. (2005). Binding of glutamine to glutamine-binding protein from Escherichia coli induces changes in protein structure and increases protein stability. Proteins: Structure, Function, and Bioinformatics, 58(1), 80-87.

[9] Hischenhuber, C., Crevel, R., Jarry, B., Mäki, M., Moneret-Vautrin, D. A., Romano, A., ... & Ward, R. (2006). Safe amounts of gluten for patients with wheat allergy or coeliac disease. Alimentary pharmacology & therapeutics, 23(5), 559-575.

[10] Huang, Y., Bell, M. C., & Suni, I. I. (2008). Impedance biosensor for peanut protein Ara h 1. Analytical chemistry, 80(23), 9157-9161.

[11] Zeiger, R. S., Sampson, H. A., Bock, S. A., Burks Jr, A. W., Harden, K., Noone, S., ... & Wilson, G. (1999). Soy allergy in infants and children with IgE-associated cow's milk allergy. The Journal of pediatrics, 134(5), 614-622.

[12] Montagut, Y., Narbon, J. G., Jiménez, Y., March, C., Montoya, Á., & Arnau, A. (2011). QCM technology in biosensors. In Biosensors-Emerging Materials and Applications. InTech.

[13] Song, S., Wang, L., Li, J., Fan, C., & Zhao, J. (2008). Aptamer-based biosensors. TrAC Trends in Analytical Chemistry, 27(2), 108-117.

[14] Skripak, J. M., Matsui, E. C., Mudd, K., & Wood, R. A. (2007). The natural history of IgE-mediated cow's milk allergy. Journal of Allergy and Clinical Immunology, 120(5), 1172-1177.

[15] Jakobsson, I., & Lindberg, T. (1979). A prospective study of cow's milk protein intolerance in Swedish infants. Acta Paediatrica, 68(6), 853-859.

Acknowledgments

We would like to take this opportunity to thank our college,Kumaraguru College of Technology for providing us this opportunity to present thie review article and the department of biotechnology for ths wonderful opportunity and we would like to thank Dr.Sivarajasekar for motivating us throughout the process.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

DEVELOPMENT OF THERAPEUTIC RTS

Nithya Priya S***,** Anushya Menon K C, Kousalya P **a**nd Ruthi Muhinda K
Department of Biotechnology
Kumaraguru College of Technology, Coimbatore-641049, nithyapriya.n.bt@kct.ac.in

Abstract: *RTS is a beverage made from blending of natural extracts from fruits and vegetables. The current situation is that consumption of sugar-sweetened beverages has been linked to risks for obesity, diabetes, and cardio vascular diseases, therefore can replace consumption of soft drinks. The development of Hibiscus juice will be a valuable produce for health benefits. Hibiscus and Aloe vera gives a palatable taste enhancing therapeutic behavior of the product. Blending of both the ingredients gives good taste with medicinal property. It can be consumed in the form of drink which can replace the carbonated drinks. Hibiscus is edible and is delicious. Consumption of this gives relief from high blood pressure and cholesterol. The combination of the above mentioned extracts as RTS will serve as a therapeutic thirst quencher for any season.*

Keywords: *Hibiscus, Aloe vera, Ginger, RTS beverage, thirst quencher*

I. Introduction

Hibiscus *(rosa-sinensis)* is a tropical shrub which belongs to the family *Malvaceae* with red or dark red calyces [1]. A large number of these species have the ability to grow under adverse conditions and are also known for their therapeutic and nutritive value. It is widely accepted that diet rich fruits and vegetables will reduce the risk of several oxidative stress diseases including coronary heart diseases, stroke and dementia. These health benefits are ascribed to phytochemicals such as flavonoids and polyphenols[2]. The phytochemicals in the diet is supposed to contain antioxidants, which may reduce the uncontrolled free radical production. Color is an important factor which influences the acceptability of food products. This is due to the fact that consumers always associate food color with other food qualities such as freshness, ripeness and food safety. The use of flavonoids showed benefits over that of synthetic color [3]. *Aloe barbadensis* miller belongs to the family *Liliaceae*. The leaf exudate contains antraquinoes, particularly (aloin) which appear to be responsible for its bitter taste and cathartic effect [4]. It has immune boosting effects, dietary fiber and normalization of metabolic pathway. *Zingiber officinale Roscoe*, is a monocotyledon, which belong to the family *Zingiberaceae*. It is a source of valuable phytonutrients characterized by an aromatic odor and pungent taste. It had been used to treat gastritis, inflammatory diseases shown as potential antipyretic activities [5]. It contains several antioxidant compounds such as gingerol, gingediol, which possess antimicrobial activity against food spoilage organism and seems to improve the shelf life [6,7].Blended drinks are good alternatives for development of new products to provide taste, nutritional as well as medicinal properties.

II.Materials and methods

Procurement of ingredients
Hibiscus flower, aloe vera, ginger, water, sweetner.

Methology

Hibiscus flowers are collected and the petals are separated from other parts of the flower. The petals are cleaned and soaked in boiling water for 5mins. The filtered ginger and aloe vera extract was mixed with hibiscus juice and finally the sweetner was added to it**.**

Figure 1. Flow chart for the preparation of Hibiscus RTS beverages

.

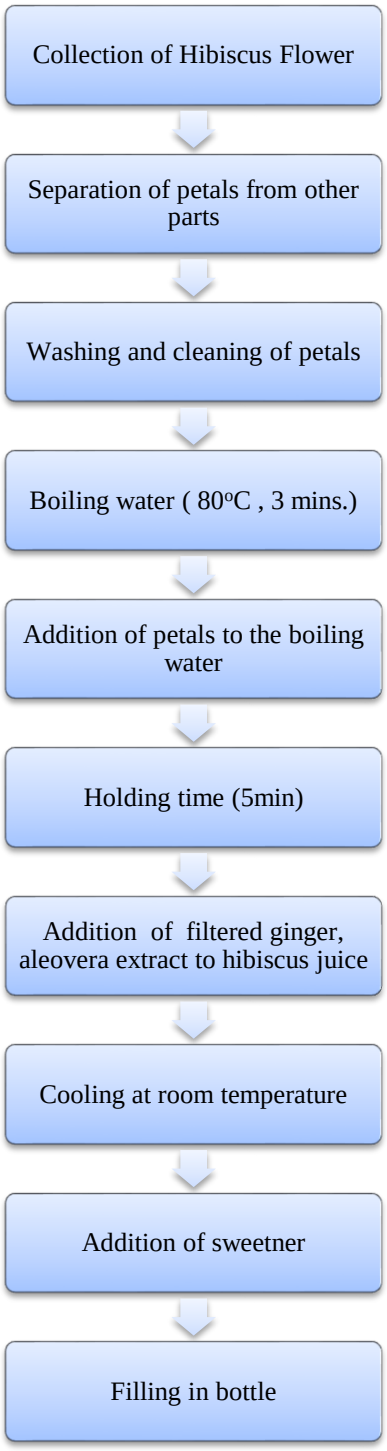


Figure 2: Hibiscus (*rosa-sinensis*)



Figure 3: RTS beverage



III.RESULT AND DISCUSSION

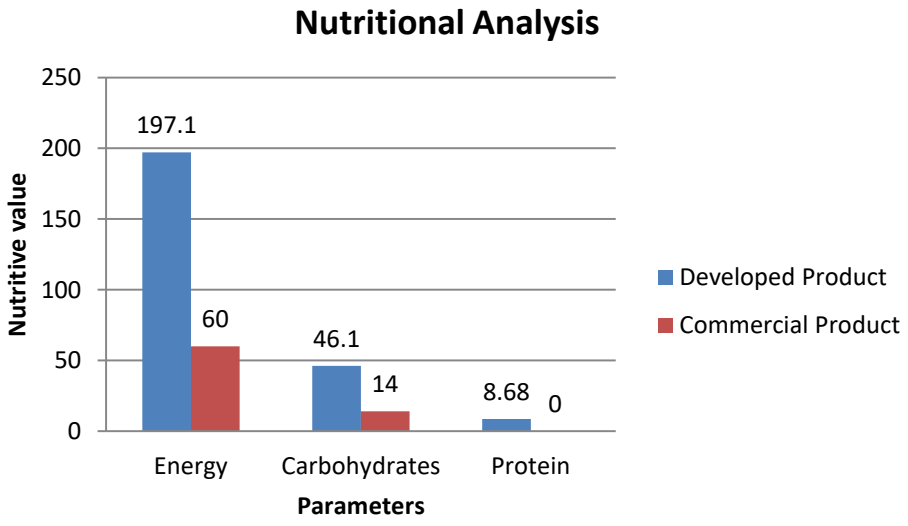
Nutrient content of the hibiscus RTS beverages:

The nutrient content of the freshly processed Hibiscus RTS beverages were analyzed and presented in Table 1. The blended RTS beverage using Hibiscus, Aloe vera, Ginger contains 197.1 Kcal of energy. The protein and carbohydrate content was found to be 46.1gm and 8.68gm respectively which is higher than the commercially available RTS beverage

Table 1: Nutritional composition of Hibiscus RTS beverages

S.NO	NUTRIENTS	RESULTS
1.	Energy	197.1Kcal
2.	Protein	46.1gm
3.	Carbohydrates	8.68gm

Figure 4: Graph comparing the nutritional analysis of Hibiscus RTS beverages and commercially available beverages.



The above graph illustrates the proportional level of carbohydrates and protein between the products. The amount of carbohydrates and proteins was found to be elevated in the developed hibiscus product than the sample A. The energy present in the product was sufficient to boost up the calories and makes the product superior.

The developed thirst quencher has many health benefits with better level of energy, this made the drink more nutritious. The quantity of energy, carbohydrates, protein, dietary fiber, iron and calcium are relatively found to be in adequate amount when compared with the product commercially available in the market. The presence of dietary fiber may ease the digestive process in the human beings. The parameters analysed in the RTS developed were pH and TSS and the values are 5.45 and 1.2º Brix, respectively using pH and refractometer. Hence the developed hibiscus product served as an alternative for carbonated beverages.

Organoleptic evaluation of hibiscus ready–to-serve-beverages:

Organoleptic characteristics observed in the freshly prepared spiced pineapple RTS beverages are presented in Table 2. The color, flavor, taste, texture, appearance and overall acceptability of the prepared ready-to-serve beverages were graded as like extremely to dislike very much with score values as 9-1. The nearly maximum score value of 8.0 were found in the beverages. The beverages obtained the highest grade of 7.8 for flavor. The freshly prepared hibiscus ready-to-serve beverages had good taste(7.9). The texture and appearance of beverage is high(8.2) when compared to other parameters. And hence the quality noted in each attribute of the beverages had influenced the overall acceptability score values(8.0)

Table 2: sensory evaluation of Hibiscus RTS beverages

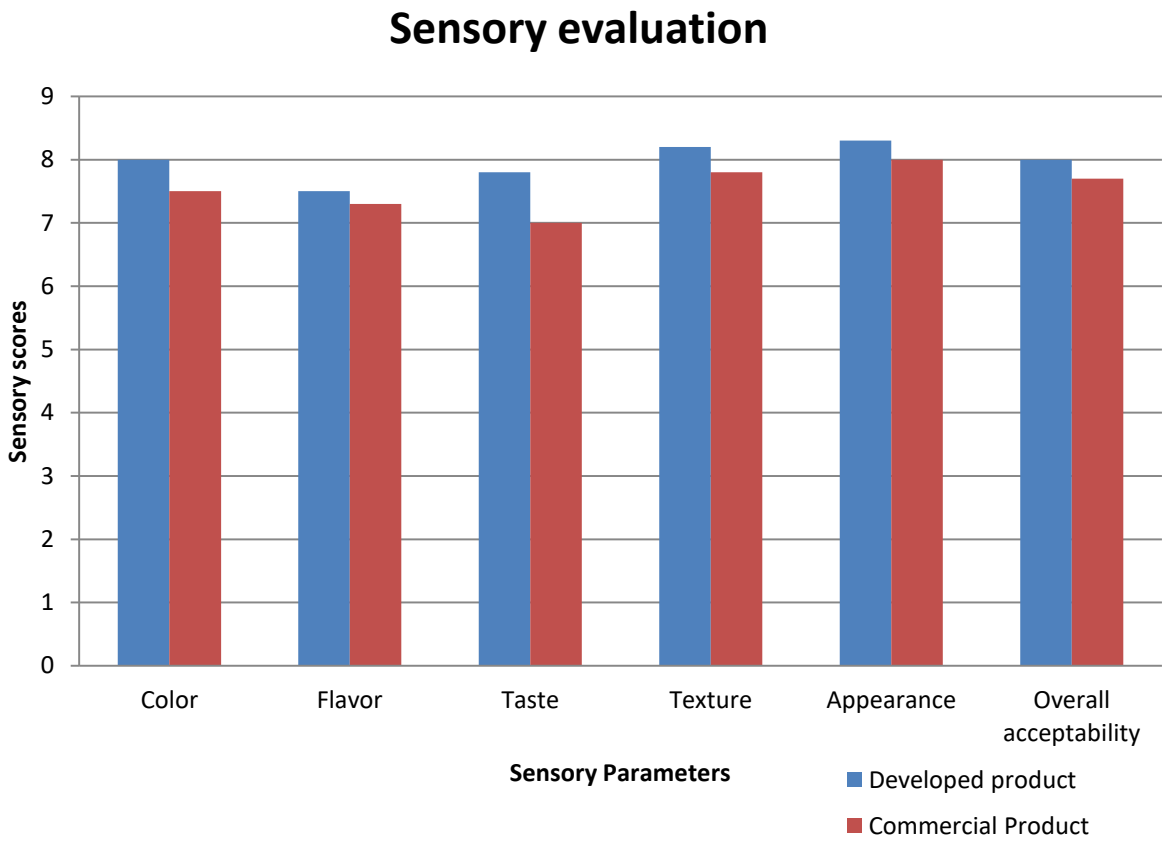
S.NO	ATTRIBUTES	SCORE
1.	Color	8.0
2.	Flavor	7.8
3.	Taste	7.9
4.	Texture	8.2
5.	Appearance	8.2
6.	Overall acceptability	8.0

Table 3: 9-point hedonic scale

Organoleptic scores	Rating
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Comparison of sensory parameters of the produced hibiscus RTS beverage and the commercially available beverage is given in figure 5. The graph represents that the color, flavor, taste, texture, appearance and overall acceptability of the produced beverage is comparatively high and hence this thirst quenching healthy nutritive beverage will be accepted by all customers in market.

Figure 5: comparison of sensory parameters of hibiscus RTS beverages and the commercially available beverage.



IV. Conclusion

It could be concluded that the presence of flavonoids gave the corresponding color to the product irrespective of the addition of flavoring agents. The bioactive compound such as polyphenol will be able to give overall health benefits. Aloe vera and ginger attributed a very good flavour for the developed RTS. Hence it can be consumed by all the age groups as a wonderful thirst quencher.

Reference

[1] Ross, I. A. (2003). Hibiscus rosa-sinensis. In *Medicinal Plants of the World* (pp. 253-266). Humana Press.

[2] Chaovanalikit, A., & Wrolstad, R. E. (2004). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal of food science*, *69*(1).

[3] Francis, F. J., & Markakis, P. C. (1989). Food colorants: anthocyanins. *Critical Reviews in Food Science & Nutrition*, *28*(4), 273-314.

[4] Dagne, E., Bisrat, D., Viljoen, A., & Van Wyk, B. E. (2000). Chemistry of Aloe species. *Current Organic Chemistry*, *4*(10), 1055-1078.

[5] Singh, G., Kapoor, I. P. S., Singh, P., de Heluani, C. S., De Lampasona, M. P., & Catalan, C. A. (2008). Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of Zingiber officinale. *Food and Chemical Toxicology*, *46*(10), 3295-3302.

[6] Singh, J. (2000). *Studies on processing of carambola (Averrhoea carambola Linn.) fruit* (Doctoral dissertation, M. Sc. thesis).

[7] CHORO, H. K. (2015). *Formulation of therapeutic ready to serve (RTS) beverages prepared from blend of Aloe vera gel and ginger juice* (Doctoral dissertation, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya).

Acknowledgments

We thank KCT management for providing us support to conduct the research work in a successful manner.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

DEVELOPMENT OF OMEGA-3 ENRICHED TRADITIONAL INDIAN SWEET

Nithya Priya S[1], Geetha P[2], Priyanka S[3], Ramakrishnan V, Veeragayathri P V, Sanjana R, Subha Priya C, Sakthi Priyadharsini A and Joel John Varghese

[1]Department of Biotechnology, Kumaraguru College of Technology, Coimbatore-641049

[2] Department of Food Processing Technology, College of Food and Dairy Technology, Koduvalli, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Alamathi Post, Chennai-600052

INDIA

[1]nithyapriya.s.bt@kct.ac.in

Abstract: *Studies in India have shown that the frequency of coronary artery disease is increasing at an alarming rate. Increasing the consumption of omega (ω)-3 fatty acids may be a particularly powerful dietary strategy to combat CAD. Omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids that must be derived from the diet, cannot be made by humans, and other mammals because of the lack of endogenous enzymes for ω-3 desaturation.2g of ALA is found in 30 g of flaxseed fibre. The benefits of ALA are seen at intakes as low as 1g/day and 2g/day is recommended for a cardio protective effect. Laddu is a traditional recipe of Indian foods with different compositions and combinations,the present study was planned to develop convenient snack food rich in omega-3 fatty acids and subjected to sensory evaluation using 9 point hedonic scale rating method.*

Keywords: *CAD, flax seed, omega-3 fatty acids, laddu, Indian snack*

I. Introduction

Omega-3 fatty acids are essential for growth and development throughout human life cycle be included in the diets of all humans. The Global Burden of Disease study estimate of age-standardized CVD death rate of 272 per 100 000 population in India is higher than the global average of 235 per 100 000 population. A very high omega-6/ omega-3 ratio is found to promote pathogenesis of many diseases like autoimmune diseases, cancer, cardiovascular diseases in western diets, whereas increased level of omega-3 PUFA have suppressive effects.(Artemis P Simopoulos, Iii, Ph, & Worth, 2008). Fish oils contain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are in combination with anti-rheumatic drugs have effect on rheumatoid arthritis, skin lesions, hyperlipidemia and toxicity of cyclosporin in case of psoriasis(Arternis P Simopoulos, 1991). Coronary artery disease (CAD) is a leading cause of death today and a major economic challenge for the health care system. The incidence, prevalence, hospitalisation, and mortality of CAD in Asian Indians are 3-4 times higher than their Western counterparts. Asian Indians are 4 times more likely to die from CAD than other Asians and blacks of south Africa. Studies in India have shown that the frequency of coronary artery disease is increasing at an alarming rate. There are now significant research data suggesting that CAD can be altered or prevented in large part by three major lifestyle changes. These three factors are nutritional modification, incorporating exercise into our daily lives and eliminating smoking. The implementation of only one of these three factors, nutritional modification may generate significant effects on CAD.

The increased use of omega (ω)-3 fatty acids is a powerful example of one such nutritional strategy that may produce significant cardiovascular benefits. Marine food products have provided the traditional dietary sources of ω-3 fatty acids. Flaxseed is an alternative to marine products. It is one of the richest sources of the plant-based ω-3 fatty acid, alpha-linolenic acid (ALA). In a comparison study between supplemented fish oil and flaxseed oil by Nalini Kaul, 2013, flaxseed oil was proved to have supplemented with sufficient ALA concentration. Based on the results of clinical trials, epidemiological investigations and experimental studies, ingestion of ALA has been suggested to have a positive impact on CVD. Because of its high ALA content, the use of flaxseed has been advocated to combat CVD (Rodriguez et al 2010). Another study proved Secoisolariciresinol diglycoside, which was isolated from flaxseeds, showed promising results as an anti-tumor substance; this in turn hints at the fact that high-fiber diets can deliver a healthy eating habits in man (Lilian U.

Thompson, 2009). Other than the intuited compounds, flaxseeds are also vital sources of phenolic compounds and high quality proteins. (B Dave Oomah, 2001)

Katare et al 2012 described that with rapidly changing global health scenario and fast realization of the ill effects of uncontrolled food processing and overmedication; plant products have gained the well deserved attention. Growing awareness about the role of diet and quest for wellness has fueled interest in foods that can work like medicine. 'Functional foods' or 'neutraceuticals' are foods or dietary components that may provide a health benefit beyond basic nutrition. Furthermore, Cora J Dillard, 2000 stated that introducing the phytochemical based diet would indeed address the safeness and efficacy of dietary foods and supplements that claim to be health promoting in the current market.

Increasing the consumption of omega (ω)-3 fatty acids may be a particularly powerful dietary strategy to combat CAD. Fish oil contains both docosahexaenoic acid and eicosapentaenoic acid. Swanson et al 2012 reported that there is strong scientific evidence from human trials that w-3 fatty acids from fish or fish oil supplements (EPA and DHA) can significantly reduce risk factors for heart disease, nonfatal and fatal myocardial infarctions. Despite the data supporting the contention that the long-chain ω-3 fatty acids EPA and DHA in fish oil have significant, beneficial cardiovascular potential, dietary endorsement has not been widespread.

Omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids that must be derived from the diet, cannot be made by humans, and other mammals because of the lack of endogenous enzymes for ω-3 desaturation. Simopoulos et al 2016 reported that due to agribusiness and modern agriculture western diets contain excessive levels of omega-6 PUFAs but very low levels of omega-3 PUFAs, leading to an unhealthy omega-6/omega-3 ratio of 20:1, instead of 1:1 that was during evolution in humans. A very high omega-6/omega-3 ratio promotes patho-genesis of many chronic diseases like cardiovascular disease, cancer, inflammatory and autoimmune disease, rheumatoid arthritis, asthma, whereas increased intake of omega-3 fatty acids (reduced omega-6/omega-3 ratio) suppresses the effect. Seafood, especially fatty fishes like salmon and tuna, are the best dietary sources of omega-3 fatty acids, although some seeds and nuts, such as flaxseed, walnuts and soybeans, also contain significant levels. But concerns about fish taste, smell, toxin content, allergies and eructation following a fish meal are some of the factors that have limited their dietary use.

II. Materials and Methods

Formulation of flax seed laddu

A) Procurement of ingredients

Flax seeds, mung beans, jaggery, elachi were used in the formulation of the Omega -3 fatty acid rich laddu.

All the ingredients were procured from the local market.

B) Composition of flax seed laddu

S.NO	INGREDIENTS	QUANTITY IN gm
1	Flax seed	125
2	Mung beans	125
3	Jaggery	500
4	Ghee	50
5	Elachi for flavour	Required amount

C) Re-treatment and preparation

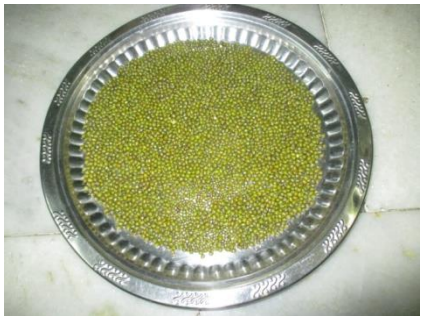
Ingredients used for laddu were accurately weighed. Flax seeds were cleaned, roasted and powdered. Mung beans were cleaned and roasted separately and powdered. The fine Powder's were mixed with the powdered jaggery and elachi to form a Flax seed laddu along with the essence of ghee.

The proximate analysis of the product was analysed using AOAC methods. The nutrients analysed include calcium, phosphorus, Iron, copper, manganese and Zinc per 100 g of the product.

The sensory evaluation of the product was performed using 9-point hedonic scale with the parameters like appearance, flavour , taste, texture and colour.

D) Methodology of flax seed Laddu

- The flax seeds, mung beans and Elachi were taken separately.





- All the ingredients were roasted until golden brown under low flame.



- Ingredients were kept aside and allowed it to cool down. After 5 minutes the roasted ingredients were mixed with Jaggary.



- After well mixed with Jaggary, the mixers were ground into fine powder.



- The ghee was melted with mild heat and mix it with the fine powder to form omega-3 rich laddu



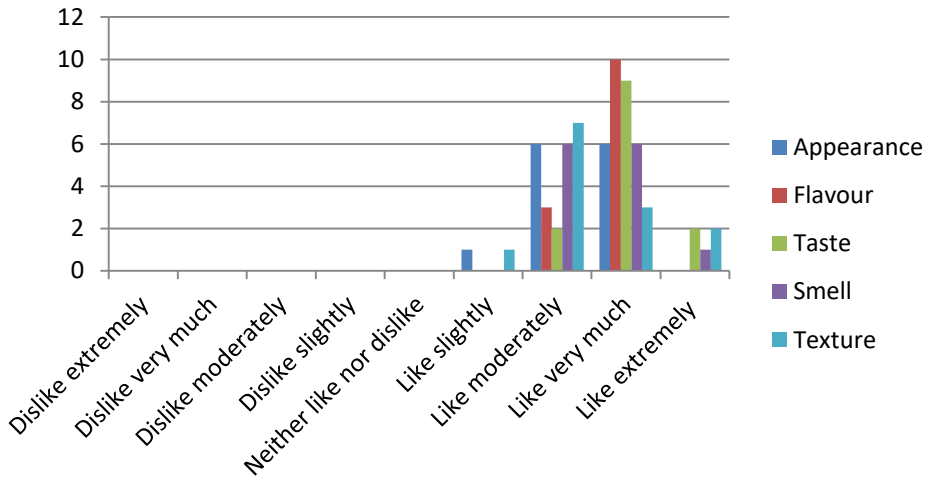




III. Results

The proximate analysis for the product was performed by AOAC methods.

Nutrients	Composition(per 100 g of the product)
Calcium	231.6 mg
Phosphorous	375 mg
Iron	4.89 mg
Copper	1.27 mg
Manganese	1,88 mg
Zinc	3.28 mg



The main reason behind making this recipe was its nutritive value. The recipe is a rich source of protein and energy. It was basically made for pregnant and lactating women, but anyone can consume it for better health. Sharma and Goyal, 2017 reported similar results.

IV. Discussion

Flax seeds are an important food stuff loaded with nutrients as the nutritive value of seeds are very high. Most beneficial seeds are found to have vitamins and mineral contents that compliment a healthy body. Seeds can be made healthier and nutritious by changing the main ingredient of a food to the same, or incorporating any nutritious component into the snacks. Some call it one of the most powerful plant foods on the planet. Because of their content of omega-3 fatty acids, flaxseeds promote a lower ratio of omega-6 to omega-3. A lower ratio of omega-6 to omega-3 fatty acids may significantly reduce the risk of various chronic diseases. Other known trace vitamins in Flaxseeds are Vitamin B6 and minerals such as magnesium, phosphorus and copper. There are several evidences that the presence n-3 series PUFAs may help to reduce the risk of heart diseases, cancer, stroke and diabetes. The vital part is through food supplementation, the developed laddus can be supplemented for people to combat cardio vascular disease.

Reference

[1] Dillard, C. J., German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of Science of Food and Agriculture*, 80(12), 1744-1756.

[2] Katare, C., Saxena, S., Agrawal, S., Prasad, G. B. K. S., & Bisen, P. S. (2012). Flax seed: a potential medicinal food. *Journal of Nutrition & Food Science*, *2*(120), 2.

[3] Kaul, N., Kreml, R., Austria, J. A., Richard, M. N., Edel, A. L., Dibrov, E. (2013). A comparison of Fish Oil, Flaxseed Oil and hhempseed Oil Supplementation on Selected Parameters of Cardiovascular Health in Healthy Volunteers. *Jounral of American College of Nutrition*, 27(1), 51-58.

[4] Oomah, B. D. (2001). Flaxseed as a functional food source. *Journal of Science of Food and Agriculture*, 81(9), 889-894

[5] Rodriguez-Leyva, D., Bassett, C. M., McCullough, R., & Pierce, G. N. (2010). The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. *Canadian Journal of Cardiology*, *26*(9), 489-496.

[6] Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *The American journal of clinical nutrition*, *54*(3), 438-463.

[7] Simopoulos, A. P. (2003). Omega-3 fatty acids and cancer. *Indoor and Built Environment*, *12*(6), 405-412.

[8] Simopoulos, A. P. (2016). An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*, *8*(3), 128.

[9] Sharma, L., & Goyal, K. To study the organoleptic properties of Ladoos made from variations of Flax seeds and multigrain flour.

[10] Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in nutrition*, *3*(1), 1-7.

[11] Thompson, L. U., Seidl, M. M., Rickard, S. E., Orcheson, L. J., & Fong, H. H. S. (2009). Antitumorigenic effect of a mammalian lignan precursor from flaxseed. *Journal of Nutrition and Cancer*, 26(20), 159-165.

Acknowledgments

We thank Kumaraguru College of Technology, Coimbatore Management for providing moral and technical support.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

IMAGING TECHNIQUES IN FOOD PROCESSING

Pradeepika.T , Sivapriya.S , Gayathri.V, Keerthana.K.J, Sivarajasekar. N

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu 641049, India

tpradeepika15@gmail.com

Abstract*: Food safety and quality maintenance are inevitable part in food processing industries. Quality assessment is based on various parameters such as color, texture, aroma, sweetness, firmness etc., Earlier days, the quality assessment was done manually, which is tedious and time consuming. Despite the use of imaging techniques in various sectors, its contribution in food processing sector is a remarkable one. Contamination of food is a major problem which causes various human health issues. In most cases, the contamination is not visible to the naked eye wherein these techniques play a vital role in detecting the contamination at initial stage. There are wide range of imaging techniques out of which hyperspectral imaging has a huge role. In this paper we have discussed the application of these techniques in analyzing quality and safety of various food and food products.*

Keyword: *Quality; safety; imaging techniques; contamination; hyperspectral imaging*

I. Introduction

Vegetables and fresh fruits are easily contaminated with pathogens during harvest, package, storage and food preparation. This causes a range of food – borne illness[1].Food technology is a broad field which not only involves process and production. It also includes regular monitoring of the process for proper safety and to maintain good quality of the product[2]. Food quality is directly related to human health. Quality is basically defined as the sum of all the attributes that leads to the synthesis of products which I acceptable by the consumers when they are combined. Smell, texture, aroma and appearance are the various attributes that must be considered for food quality assessment[3]. The human panel test is usually carried out in our country in order to ensure food safety such as dairy product evaluation, wine quality analysis etc. In order to maintain safety and quality rapid and more precise analytical techniques are required. The automation in this field actually increases the productivity of good quality foods. This benefits both the production sector and the consuming sector.In many food industries still the food quality inspection is carried out by the food inspectors manually[4]. This process is tedious and consumes more amount of time. In order to overcome these demerits, various imaging techniques accompanied with computers combined with mechanical devices have been evolved[5].

A. Earlier unfortunate incidents due to lack of food safety:

- Mexico and Central America were the largest suppliers of Cantaloupes to United States and Canada during 2001 – 2003. During that period there was huge outbreak of *Salmonella.* Food and Drug Administration (FDA) in 2002 reported that 2 people were dead in California and around 18 people were hospitalized due to this outbreak.
- Similarly in 1997, many children in a school were subjected to Hepatitis who consumed strawberries. It was reported that around 1.7 million pounds of frozen strawberries were supplied to the school for the children lunch.[6]

The conventional methods of maintaining food safety and quality include drying, fermentation, low temperature storage, salting. Drying is the most important method in food preservation. It is the removal of water content in the food material which serves as a base for the growth of microorganisms. This process enhances the stability of the food. The difficulties associated with this process is the change in physical and senatorial qualities of the food are changed. Fermentation is the most common method used to maintain the food quality. This process, by the release of certain metabolites, CO trolls the growth of microorganisms and changes the conditions of the food. Another conventional method is that of low temperature storage. The microorganisms present in the food grow and die at optimum temperatures. So to control the growth of microorganisms, a low temperature can be maintained. In such conditions the cell division is stopped and hence less growth of microorganisms. The difficulties in this process is when there is a sudden temperature change, it leads to the increased growth of few

pathogens. Following is the process of salting. In olden days salting was an inevitable process used to prolong the shelf life of foods. The reason for using salt as a preservative is that it drains moisture from the food. Salting was mainly done in areas where the climate was not suitable to dry the food in sun. Apart from draining moisture away from food it also helps in the removal of microorganisms. The disadvantage of this method is the increased use of sugar and salt, leading to health complications.

Hyper spectral imaging is the most widely used tool for the identification of contamination, safety analysis and food quality. This HIS follow through in three modes they are fluorescence, reflectance and transmission. Even-though there are three modes the HIS usually prefers the reflectance mode. The cost of Hyperspectral imaging is high so many HIS related food researches are prepared for the low cost. Therefore, many developments are going to take place in HSI equipment manufacture at lower cost and improvement in process speed[2]. They are well suited for predicting the food samples like meat. They helps to identify the pH, H2O holding capacity and tenderness of meat products. It gives better accuracy, sorting and classification than spectroscopy in food products. The HSI has many applications like quantification, segregation of the product. It can also provide greater benefits in processing industry(meat,food) for both quality and quantity analysis by using multivariate techniques. For monitoring the quality of the meat the HSI technique gives greater platform. The very important difficult application feature to be noted is superior quality authentication, discriminating the meat qualities, false processed meat detection, overall quality monitoring and their compositions[7]. In the past two decades the HSI has tremendous development in the detection/testing of food products. It acts as a major tool in food industry, and gives more accurate results in sorting and classification. However, HIS is used effectively and their improvements were taken place in research, industries and laboratories[4].

Even-though HSI has many benefits, ultrasound technology plays a major role in food. Likewise, HSI the ultrasound also plays a major role in analysis of food, quality and processing of food. For the detection of contamination in diary or canned foods, structural and physicochemical properties of diary products. Due to their low cost and portability the ultrasounds are used in many laboratories and factories. The production is maximized hence they are known as green technology. They have many applications in safety, processing and preservation of food. [8].These imaging techniques has many advantages over the conventional/ traditional methods. They are time saving and it's a single time investment which could reduce the manufacturing cost.

II. Emerging imaging techniques

Food safety and quality plays an important role in food processing industry. Conventional method of quality and safety analysis of food is a time consuming process. The imaging techniques like hyperspectral imaging, fluorescence imaging etc..are the emerging techniques which has a great application in food processing industry.

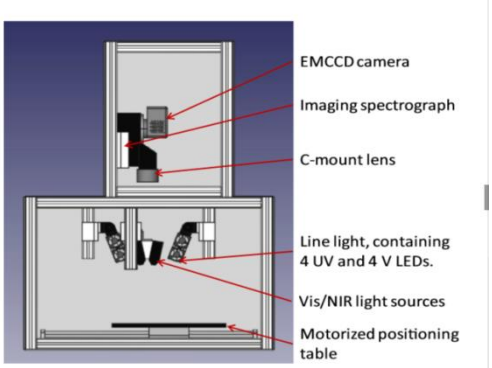
A. Hyperspectral imaging

The hyperspectral imaging technique (cheng2013) is a spectroscopic imaging technique (analytical) which combines traditional imaging and spectroscopy to get both spectral and spatial information of an object. The image obtained using this technique is usually known as hypercubes, a 3-D cubes which are of 100 contiguous wavebands for every spatial position of the object. This imaging technique has been widely used in several food processing industry to maintain food quality and safety[1].The images obtained are large in size and its data are highly correlated. The advantages are speed, ease of use, reasonable start-up cost, non-destructiveness. The HIS is being coupled with some chemometrics to increase the precision of analysis[9].[10]discussed about three types of HIS : Ultraviolet HIS,V HIS, Vis/NIR HIS. [11]reported that Vis/NIR hyperspectral imaging technique could be used to differentiate between fresh and frozen-thawed fish.[12]investigated the soluble solids content and firmness of the apple using this technique within 5 selected spectrum bands (680nm – 1060nm).

B. Backscattering imaging

Backscattering imaging is a technique which is the reflection of waves or radiation back to direction from which they came. The objects which are transparent or semi-transparent can reflect waves or radiation which pass through them. Such objects can be analyzed through the backscattering. Food substances are assumed to be semi-transparent which can allow the light passage and can reflect it back i.e. backscattering imaging , the texture and color of the food can be studied[1]. The application of backscattering in food industries are analyzing SSC and firmness from which the ripening stage of fruit can be identified. Some temperate crops like plums, apples and several fruits and vegetables were analyzed by using this technique.[13]

Figure 1 Push broom hyperspectral imaging system [13]



C. Magnetic resonance imaging

NMR is a technology where it measures the magnetic properties of spin which relates to the physical or chemical properties of the object being analyzed. Magnetic resonance imaging analyze the food by utilizing the water content in the food, the hydrogen molecule which align the protons in the direction of magnetic field when placed in it. The nuclear magnetic resonance imaging signals been released as electromagnetic radiation. During the imaging process, radio frequency has been recorded from which the properties of the food was studied. MRI is being used in quality control of foods such as meat product, cereals, fruits & vegetables. The food quality mainly concerned about the state of water.[1]

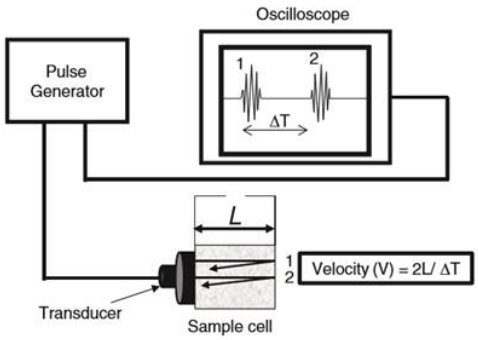
D. X-ray imaging

X-ray is an electromagnetic wave, nowadays being widely used in food processing industry to eliminate the contaminated foods. Soft X-ray imaging system was designed based on the density of the particular food product. When the X-rays are passed through the food, depending upon the density some energy is adsorbed and comes out as a signal. The signal on reaching sensor is converted in to image of the food. On analyzing the image, the contaminant in the food appeared as dark grey[1].[14]reported the presence of foreign objects in some packed dry foods using this technique. This technique can detect contaminants or foreign objects whose density is greater than water.

E. Ultrasound imaging

Ultrasound imaging techniques uses mechanical waves whose frequencies are in the range 20kHz – 20,000kHz.This technique is a easy, cheap and post-image-processing on compared with other techniques. The UI principle is same as that of X-ray imaging system. The application of this technique is mainly on food packaging and foreign objects. [15]reported that using non-contact air-instability compensation ultrasound imaging technique to find internal disorder and foreign objects in poultry and cheese.

Figure 2 A schematic diagram of ultrasonic velocity pulse- echo technique [8]



III. Applications

The application of these techniques on several food and food products are as below:

A. Apple

Apple's spectral range ranges from 430 nm- 930 nm when checked in a hyperspectral imaging system and has a spectral resolution of 10 nm. The spatial resolution is larger than 1 mm. The hyperspectral imaging system can measure reflectance and fluorescence with the help of ultraviolet emission. The hyperspectral imaging system has a significance that can be distinguished based on the fluorescence and reflectance test of normal fresh apple and apple affected by fungus and also helpful in conduction food quality research[16].

For many fresh fruits including apples , firmness and soluble solid content (SSC)are inevitable quality attributes. The spectral images reflected back from the fruit by the backscattering of light from a focused beam ranging from 680 - 1060 nm. The range of scattering numerical were used to predict the fruit firmness and SSC. A neural network method was formulated to relate the mena and standard deviation to determine the firmness and SSC. This is done with the help of multispectral imaging techniques which is helpful in measuring the sweetness and firmness of apple[12].

It is still a tedious job to distinguish between the rotten and sound tissues of apple in an automatic grading system. To over come this, hyperspectral imaging techniques were used along with spectral analysis techniques. Chemometric and pattern recognition. Methods were employed in the spectral analysis technique. To select the right candidate, successive projection analysis (SPA) was conducted. This was done in the region of interest in the good and rotten tissues. The selected candidate's efficiency was measured by binary particle least square discriminant analysis (PLS-DA). In the image processing domain,in order to verify if the wavelengths were efficient, principle component analysis (PCA), minimal noise fraction (MNF) was employed . On a total, all the methods were accomplished by hyperspectral imaging techniques[17][18].

B. Grape seeds

The appropriate harvest period of grape is usually found by sugar content in the pulp of the berry. Actually, the harvest period should be estimated based on the stage of maturation of grape seeds. There are more number of sensory and chemical analyses available to assess the appropriate harvest period. But the chemical and sensory analyses actually consumes more time and is destructive. In order to overcome these disadvantages, hyper spectral imaging serves to be an alternative technology. To carry out this experiment 2 red varieties of grape seeds namely Tempranillo and Syrah, also one white variety grape seed known as Zalema in two kinds of soil were selected. The features were assessed based on the reflection from the Nearby-Infrared (NIR) spectra by using principal component analysis and discriminant analysis. The possibility to distinguish between the variety of seeds and even the type of soil was done using this hyper spectral imaging method.[19]

C. Fish

To determine whether the fish has been frozen-thawed, an imaging technique was used. For this purpose, Visible and near Infrared (VIS/NIR) hyperspectral imaging technique was used as a non-destructive and rapid method. To carry out this experiment, about 108 halibut (Psetta maxima) fillets were used. Out of these 108 fishes, 48 were fresh fishes and 60 were frozen-thawed (F-T). The imaging was done using push-broom hyperspectral imaging system. The spectral range was between 380 and 1030 nm. The mean spectral data was generated with the aid of ROI image(Region of Interest).The ROI was selected from the centre of the image. By carrying out principal component analysis, Dimension reduction was done on the ROI image. Around 97% of the band spectra variance was revealed in the first three principal components (PC's).To differentiate between the fresh and Frozen-Thawed varieties of fish vector machine classification was developed namely: a)Textural variable b)Spectral variable c)combination of textural and Spectral variables. Average correct satisfactory rate was obtained for (c) which was superior to (a) and (b).[11]

D. Green tea

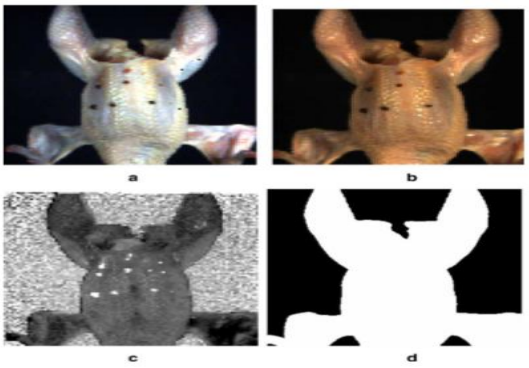
The green tea categories were sorted using Multi-Spectral Imaging technique. Three different wavelengths ranging between 580nm and 800nm were used to capture around more than 300 images using the multi-spectral digital camera. For the image texture features, the entropy values of images were obtained. The correction answer rate was up to 100% in case of least squares support vector machine (LS-SVM) with radial basis function kernel. This was better than those of LS-SVM with linear kernel, partial least squares and radial

basis function neural networks, respectively. This advantages of multi-spectral imaging process is that is non-destructive in nature. It is rapid, simple and easy to operate.[20]

E. Poultry

The hyperspectral imaging system is capable of detecting contamination on the ingesta of poultry carcasses and the waste matter on the surface. The hyperspectral imaging system uses visible spectroscopy or near infrared spectroscopy for the key wavelengths, it also consists of a camera to scan and the image processing software. The different diet was taken place for the detection of contamination. To detect the surface waste and ingesta of the poultry carcasses the pre-processing methods such as calibration of spatial, spectral and percent reflectance were used. In this band width ratio I565/I517 it provides the high accuracy of 96.4% to 99%.In this the three band equation provides increased accuracy the two band equation. Thus, the detection of high accuracy contaminants was given by spectral smoothing method[21].

Figure 3 Procedure of HIS image processing of a poultry carcass: (a) color composite; (b) calibrated color image; (c) ratio image (calibrated and smoothed) ; (d) background mask [21]



F. Spinach leaves

For *Spinaciaoleracea* (spinach leaves) the ultraviolet and violet spectral fluorescent imaging gives better accuracy than the visible or near infrared hyperspectral imaging. In the hyperspectral imaging three configurations were compared they were, UV(HIS),V(HIS) and visible or infrared(HIS).PLSDA models were used for the detection of contamination. At the 1:10 dilution the fecal contamination has 100% accuracy in the HSI configuration of both fluorescence also 1:20 dilution the violet has 99% accuracy in HIS configuration and at 1:30 dilution it has 87% accuracy in HIS configuration. The detection of fecal contamination in spinach leaves can reduce illness like food borne and related[10].

G. Pork meat

The spoilage of the meat usually occurs due to the bacteria. The multispectral imaging device was used to find out the degree of spoilage. Samples were stored at $0\text{-}20^0$ C for different time under aerobic and modified atmospheric packages. The Viable cells were counted by traditional method for different samples stored at different temperature and time. The device used 18 different wavelengths to determine the spoilage degree. The sensory analysis were carried out before and after cooking the meat by analyzing color, smell and taste. The dark color of the multispectral image shows that the meat is spoiled and the light color indicates fresh or semi-fresh meat. The bacterial growth was found to increase progressively on increasing the temperature which was also confirmed by sensory analysis and bacterial count testing (8 log cfu/g). [22]reported that the multispectral imaging can be used to detect the spoilage degree which founds to be time consuming .

H. Cantaloupes and strawberries

The fecal contamination of cantaloupe and strawberries resulted in *salmonella* in human beings.[6] examined the contamination by applying the diluted cow dung of different volume on the fresh cantaloupes and strawberries. The dilution ranges from 1:10 to 1:500 where as the volume is 10 – 40 µL was applied on to them in counter clockwise direction. The lower dilution feces were visible to the naked eye where as at higher dilution the fecal contamination were not visible to the naked eye. Hyperspectral imaging can be used to find out the contamination at the beginning stage. It may also reduce the risk of *salmonella*.

I. Cereals

The impurities in the cereals such as wood, straw, cellulosic materials, plastic items should be removed before processing. It takes more time when it is done in manual. [23]examined the NIR hyperspectral imaging along with some chemometric tools. The process was time saving and almost most of the impurities were removed from the cereals.

IV. Conclusion

Food safety and quality needs to be balanced well in any emerging food processing industry. Using these imaging techniques, quality maintenance will be very ease and time saving process. The automation could also be included in these techniques which would reduce the labour cost.

Abbreviations and Acronyms

HIS – Hyperspectral Imaging System
V HIS – V Fluorescence Hyperspectral Imaging System
Vis – Visible
NIR – Near – Infra red
NMR – Nuclear Magnetic Resonance
UI – Ultrasound Imaging

References

[1] Chen, Q., Zhang, C., Zhao, J., & Ouyang, Q. (2013). Recent advances in emerging imaging techniques for non-destructive detection of food quality and safety. *TrAC Trends in Analytical Chemistry*, *52*, 261-274.
[2] Gowen, A. A., O'Donnell, C., Cullen, P. J., Downey, G., & Frias, J. M. (2007). Hyperspectral imaging–an emerging process analytical tool for food quality and safety control. *Trends in food science & technology*, *18*(12), 590-598.
[3] Brosnan, T., & Sun, D. W. (2004). Improving quality inspection of food products by computer vision—a review. *Journal of food engineering*, *61*(1), 3-16.
[4] ElMasry, G., Kamruzzaman, M., Sun, D. W., & Allen, P. (2012). Principles and applications of hyperspectral imaging in quality evaluation of agro-food products: a review. *Critical reviews in food science and nutrition*, *52*(11), 999-1023.
[5] Du, C. J., & Sun, D. W. (2004). Recent developments in the applications of image processing techniques for food quality evaluation. *Trends in food science & technology*, *15*(5), 230-249.
[6] Vargas, A. M., Kim, M. S., Tao, Y., Lefcourt, A., & Chen, Y. R. (2004). Safety inspection of cantaloupes and strawberries using multispectral fluorescence imaging techniques.In *2004 ASAE Annual Meeting* (p. 1).American Society of Agricultural and Biological Engineers.
[7] ElMasry, G., Barbin, D. F., Sun, D. W., & Allen, P. (2012). Meat quality evaluation by hyperspectral imaging technique: an overview. *Critical Reviews in Food Science and Nutrition*, *52*(8), 689-711.
[8] Awad, T. S., Moharram, H. A., Shaltout, O. E., Asker, D., & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: A review. *Food research international*, *48*(2), 410-427.
[9] Amigo, J. M., Martí, I., &Gowen, A. (2013). Hyperspectral imaging and chemometrics: a perfect combination for the analysis of food structure, composition and quality. In *Data handling in science and technology* (Vol. 28, pp. 343-370). Elsevier
[10] Everard, C. D., Kim, M. S., & Lee, H. (2014). A comparison of hyperspectral reflectance and fluorescence imaging techniques for detection of contaminants on spinach leaves. *Journal of Food Engineering*, *143*, 139-145.
[11] Zhu, F., Zhang, D., He, Y., Liu, F., & Sun, D. W. (2013). Application of visible and near infrared hyperspectral imaging to differentiate between fresh and frozen–thawed fish fillets. *Food and Bioprocess Technology*, *6*(10), 2931-2937.
[12] Lu, R. (2004). Multispectral imaging for predicting firmness and soluble solids content of apple fruit. *Postharvest Biology and Technology*, *31*(2), 147-157.
[13] Adebayo, S. E., Hashim, N., Abdan, K., & Hanafi, M. (2016). Application and potential of backscattering imaging techniques in agricultural and food processing–A review. *Journal of Food Engineering*, *169*, 155-164.
[14] Kwon, J. S., Lee, J. M., & Kim, W. Y. (2008, April). Real-time detection of foreign objects using X-ray imaging for dry food manufacturing line.In *Consumer Electronics, 2008.ISCE 2008. IEEE International Symposium on* (pp. 1-4). IEEE.
[15] Cho, B. K., &Irudayaraj, J. M. K. (2003). Foreign object and internal disorder detection in food materials using noncontact ultrasound imaging.*Journal of Food Science*, *68*(3), 967-974.
[16] Lu, R. (2003). Imaging spectroscopy for assessing internal quality of apple fruit.In *2003 ASAE Annual Meeting* (p. 1). American Society of Agricultural and Biological Engineers
[17] Zhang, B., Fan, S., Li, J., Huang, W., Zhao, C., Qian, M., & Zheng, L. (2015). Detection of early rottenness on apples by using hyperspectral imaging combined with spectral analysis and image processing. *Food analytical methods*, *8*(8), 2075-2086.
[18] Zhang, B., Fan, S., Li, J., Huang, W., Zhao, C., Qian, M., & Zheng, L. (2015). Detection of early rottenness on apples by using hyperspectral imaging combined with spectral analysis and image processing. *Food analytical methods*, *8*(8), 2075-2086.
[19] Rodríguez-Pulido, F. J., Barbin, D. F., Sun, D. W., Gordillo, B., González-Miret, M. L., & Heredia, F. J. (2013). Grape seed characterization by NIR hyperspectral imaging. *Postharvest Biology and Technology*, *76*, 74-82.
[20] Wu, D., Yang, H., Chen, X., He, Y., & Li, X. (2008). Application of image texture for the sorting of tea categories using multi-spectral imaging technique and support vector machine. *Journal of food engineering*, *88*(4), 474-483.
[21] Park, B., Lawrence, K. C., Windham, W. R., Smith, D. P., &Feldner, P. W. (2002, November). Hyperspectral imaging for food processing automation.In *Imaging Spectrometry VIII* (Vol. 4816, pp. 308-317).International Society for Optics and Photonics.

[22] Dissing, B. S., Papadopoulou, O. S., Tassou, C., Ersbøll, B. K., Carstensen, J. M., Panagou, E. Z., &Nychas, G. J. (2013). Using multispectral imaging for spoilage detection of pork meat. *Food and Bioprocess Technology*, *6*(9), 2268-2279.
[23] Pierna, J. F., Vermeulen, P., Amand, O., Tossens, A., Dardenne, P., &Baeten, V. (2012). NIR hyperspectral imaging spectroscopy and chemometrics for the detection of undesirable substances in food and feed. *Chemometrics and Intelligent Laboratory Systems*, *117*, 233-239.

Acknowledgments

Authors are sincerely thankful to Dr. N. Sivarajasekar, Assistant Professor-II, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore- 641 049, for their contribution and guidelines regarding our work.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

Microcontrollers in Food Process Engineering

Surendhiran P, Bala subramaniam D, Nithesh kumar V, Somasundaram K, Karthick balaji K.R, Sivarajasekar. N

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore-641 049, India

surendhiran.16bt@kct.ac.in

Abstract: In this paper we are going to see about a uses of machine(microcontrollers) in various food processing industries. Starting from the initial these machines play a major role in vertical cropping. The cultivated in rural areas are mostly exploit to the cities and town due to the demand. In order to more production of crop the cultivation of those has to be increased. The crops are cultivated in a vertically stacked layer in order to save money and space for cultivation of other crops. Next mill of that product cultivated from the field is required. In this place they play major role by creating reduce the damage of the grains during extraction process. They also helps in improve the quality and quantity at low cost, low power by using automation techniques of VLSI based Microcontroller programming. If it is a fluid product like milk pasteurization of that is an important one to remove the contaminants. Usually for liquid food items like milk pasteurization has been done at high voltage ~15kv and frequency between 250Hz-30khz After that mixing of ingredients, temperature humidity and CO gas regulation, drying of food products, color sorting, detecting the quality of air, water and fruit we given as input, and also the quality of food product we get at the end of the process. Then packing and storage of those products in an appropriate manner all those things are important for food industry.

Keywords: Food processing-vertical cropping-milling-pasteurization-regulation- temperature- humidity-CO- Color sorting - Nutrients quality - packing - storage.

I. Introduction

Vertical farming:

Crops are planted in a vertical stacked layer. Compare to the conventional method of horizontal-based farming the vertical farming increased crop production, conservation of resources, impact on human health, urban growth, energy sustainability, etc. In this additional farmland is not needed and form clean surrounding around us, and solve the food crisis in growing cities and suburbs. Using controlled-environment agriculture (CEA) technology, to control environmental factors.

Rice Milling:

Next milling of rice. Many of the rice processing units are of the traditional huller type and are inefficient. VLSI Based Rice Mill are having high capacity, low cost, and are effective compare to the older one.

Pasteurization:

Pulsed Electric Field (PEF) in Pasteurization of foods under non-thermal technic. This technology in which the food need to be pasteurized is placed between two electrodes. Compare to the traditional method of pasteurization PEF technology decreases sensory malfunction and quality & quantity of the food product. In this technic harmful microorganisms are inactivated.eg: bacteria. In this pulse waves are used.

Mixing:

Once pasteurization is complete mixing of ingredients is the next step. Mixing is the process where two or more food items are mixed together is carried out manually. For making good mixer or to obtain good taste of the food after mixing one should have enough knowledge of what are the ingredients to be mixed. Sometimes it is very critical to handle appropriate quantity of particular food items. Spices, salt, oil.

Humidity & Pressure:

Humidity & Pressure play a major role in the production of food products .Monitoring of these has a advantage in plant growth protection, food industries and their quality and also working condition of many sensor belongs to these monitoring unit. One of the easy to use hardware (Arduino) is used here for monitoring all these parameters in a simple form.

Temperature:

Same like the humidity & pressure the temperature of the food processing unit has to be maintained for successive production of the product. It is not the new method of regulation it is as same as in the ancient method of preservation of food in cold and hot temperature. All the living of earth had certain mechanisms to maintain the temperature of its own body. Before maintaining the temperature first of all calculate the value of

their present in our body. Electrical sensors was invented by Sir Humphrey Davy in 1821 along with him T.J Seebeck work in it. It had placed a benchmark in development of the world. Warren Johnson in 1883 discovered a new type of temperature sensor using bimetal. Using this many industrial application had develop because of its handier behavior. But Glass thermometer is more than this one.

Detecting the qualities of fruit:

In industries quality of fruit is very important. For this certain detecting sensors are used. In industry the fruits are allow to move on the conveyor belt. The detectors measure the dimension, color and size of fruit based on the encrypted data. Any of the fruit find with abnormal condition these detectors remove them from the conveyor belt. These fruits are collected in a separated basket. This is done automatically by microcontroller inside the industrial equipment. Here detection is done by two different ways. Frist they are detected by the size and weight of the fruit. Another one is detecting fruit based on color & grading. Propose of using this type of microcontroller based detecting system is to combine the above two ways of detection of fruits.

Color sorting:

In industries color sorting is done by microcontrollers. It detect the color of the fruit by sensor build along with them. Next transfer the obtain data to the Arduino board. Then based on the information it adjust the motor moves the ball slider to in a such a direction to remove the immature or low quality fruit. The motor is placed just below the ball slider. Major colors programmed for sorting plants are red, blue, green. The slider eventually comes to the normal position after every balls are placed.

Detection of chemicals on food:

For the detection of chemicals and biological components in food industry biosensors are very useful. These biosensors calculated the data and process the output. They have advantages like sugar detection in our body, Detection of gas in water management, and also in pharmaceutical industries. For these kind of behavior they used in food industry to extract chemicals from their raw materials. Sensor used for this kind of purpose are usually cost effective and always available in local market.

Packing:

Quality and safety of the food product is maintained only by proper packing technics. Instructions are programmed in electronic device namely PLC (Programmable Logic controller). It scans memory, input and output in determined manner. Packaging machines pack the food products produced by the industries. Packing is done mainly for beverages and also for other food items in order to maintain the quality while transport.

Storage:

In ancient dates the harvested foods are storage in cold places in order to keep them safe. But today due to the massive revolution in the food industries production has greatly affect the ancient method involvement. In food industry after packing when there is excess of product produced they stored in cold storage which or not be consumed immediately but could store well. For cold storage the temperature and humidity has to be maintain properly. Now a day wireless sensor made us to monitor a particular or a specific function instead of take of care all the process.

Content:

Vertical cropping:

In this the farmer can able to see visual data. The farmer can able to use the machine in both manner (automatic & manual). In which he can farm automatically by machine and irrigate them manually. The materials used to realize this design include:

a. Sensors:

Soil moisture sensor with digital outputs is to be placed in the soil, and allow to take readings. The output from these sensors is used to determine the switching on and off of the solenoid valves. The light sensor monitor the light intensity of the environment and adjust it based on the need of the plant. Data from the Temperature sensor allow to maintain the temperature of the farm. (Fig.2)

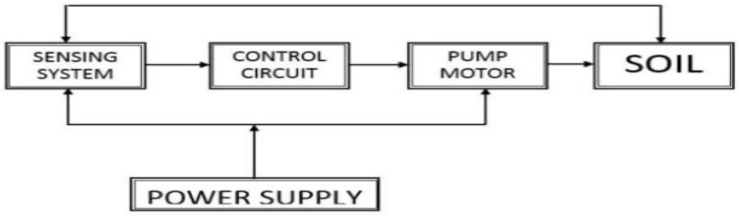


Fig.1: Block diagram of vertical cropping mechanism.[1]

b. Water pump:

Data from the soil moisture sensor allow them to pump the water. Here 12v water pump is used. Consequently it activate the water sprinkler.

c. Power supply:

AC voltage stepped down from 239.6V to 15V and rectified to 12V DC for solenoid valves, water pump and fans and 5V DC for micro-controller and liquid crystal display.

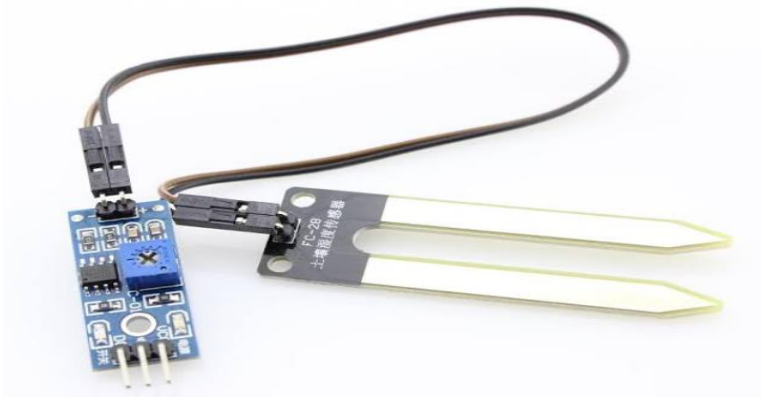


Fig .2: Sensor used in the vertical cropping [1]

Milling of rice:

Paddy in its raw form cannot be consumed by human beings. It needs to be processed. Rice milling is the process of remove hull and barns to get rice. It involve the following steps:

PROCESS DEFINITION:

Pre Cleaning: In this all the unfilled grains and impurities are removed from paddy.

De-stoning: Stones of small sizes are removed from paddy.

Parboiling (Optional): Starch content of the rice grains are gelatinize to improve the nutrients quality. Also milling recovery percent is increased during polishing and deshelling.

Husking: Husk are removed from paddy.

Husk Aspiration: In this husk of unhusk rice/brown rice are removed.

Paddy Separation: Unhusked rice are separated from brown rice.

Whitening: Germ or bran layer of brown rice are removed.

Polishing: Here removing of remaining bran particles and polishing of processed rice is happened.

Length Grading: Large and small broken are separated from head rice.

Blending: Customer requires processed with predetermined broken rice

Weighing and bagging: Transport the processed rice to the customer.

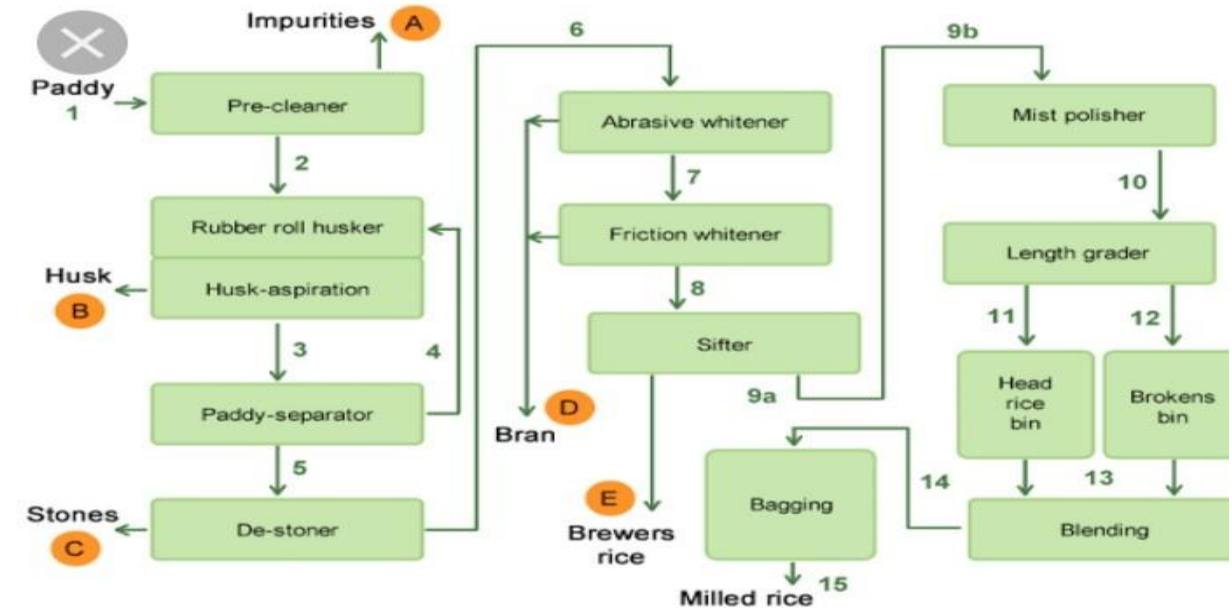


Fig.3: Block diagram of Mini milling of rice in industries.[2]

TABLE SHOWS THE EQUIPMENT AND ITS POWER CONSUPTION DURING MILLING PROCESS[2]:

S.NO	Equipment	Electric Motor(HP)
1.	Pre-cleaner	5
2.	Rubber roll husker	15
3.	Paddy separator	5
4.	Destoner, Abrasive whitener ,friction whitener	7.5
5.	Mist polisher	45
6.	Length grader	5
7.	Blending	7.5
8.	Internal	10
9	Sub total	100

Power:
The total power requirement for the rice mill is 75kw and above 90HP is required for perfect milling.



Fig.4: Equipment used for milling of rice.[2]

Pasteurization:
Fly back transformer:
It has air gap inside the couple of U shaped ferrite cores. Number of winding inside the transformer is two. At one side of the core the two windings at wounded. At the right side or the lower part of the transformer the secondary circuit is fitted.
Treatment Chamber:
Electrical field strength generated by the flow of liquid food in high voltage generated is determined by this chamber. It is made up of stainless steel plate and in the shape of cylinder covered on the top and also it is safe for all food products.

Electroporation:
The accumulation of charges around the cell eventually increase the charge around the cell and make the cell membrane more permeable. This result in breakdown of cell either in reversible or irreversible form.

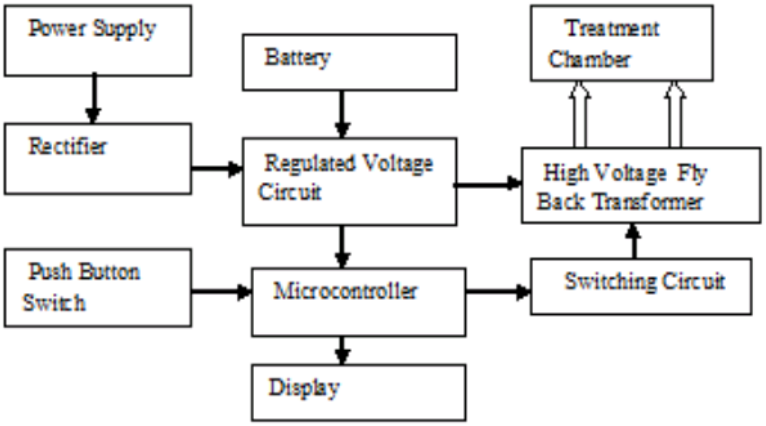


Fig.5: Block diagram showing the process inside the processing unit.[3]

Mixing:

Flow of working-

Microcontroller ATmega328 is used here because it is inbuilt with many sensors and ADC (Analogy Digital Converter). The ATmega328 is a low-power CMOS (a type of transistor) 8-bit microcontroller. RISC architecture is the base for ATmega328. RISC is enhanced by AVR

Hardware description:

Microcontroller ATmega328:

The ATmega328 for single clock cycle it approaching 1 MIPS (Million instruction Per Second) per MHz (Megahertz) it helps the system to improve power consumption versus processing speed.

Humidity and Pressure regulation:

Sensor-DHT11 is used here for humidity and temperature regulation. The sensor detect the slight changes in the value around the environment where they are fitted. It measure temperature range between 0-50 celsius and humidity ranging between 20-90% RH. Their value accuracy percentage for temperature is ±2 % and humidity is ±5 %. And voltage used between 3V-5.5V. This sensor measure the humidity value in relative humidity.

Using electrical resistance obtain between electrodes the sensor measure the moisture in the air. In this moisture and substrate attach each other and conductivity of the two electrodes increases due to the ionization of the moisture and substrate complex. The relative humidity is equal to the electrode resistance changes by absorbed moisture.

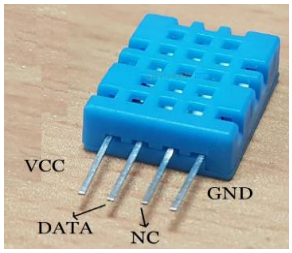


Fig.6: Sensor used for humidity and pressure detection [4]

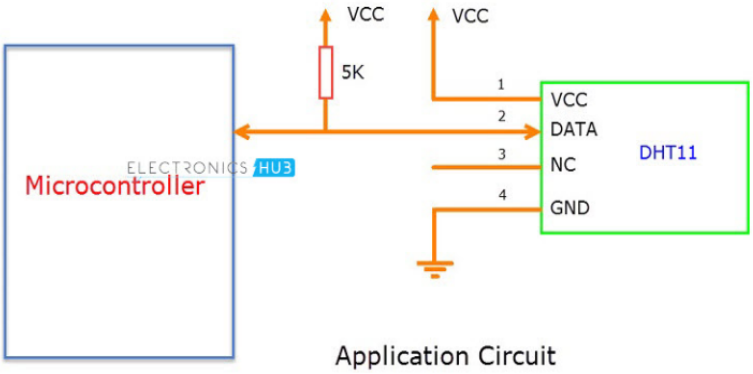


Fig.7: Circuit diagram of the process.[4]

Temperature:

System Description:

TC74 measure the temperature and transfer it to the microcontroller. It is temperature sensing unit in this system. It consists of ON and OFF switch for DC motors. In industries this works between the temperature range of -40 to 125 celuies and a very low operating current of less than 250µA.

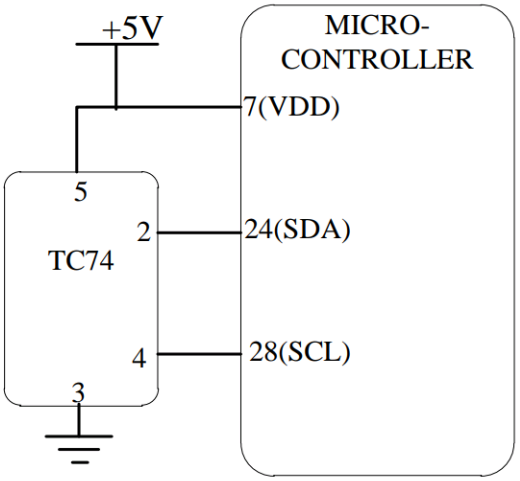


Fig.8: Circuit diagram of temperature sensing equipment.[5]

Detection of fruit quality:

SENSORS:

For detection of fruit quality, sensors play a major role. Here three sensors are used the first one is for detection of height and second one is for color detection and last one is for weight for fruit.

AT89C52 MICROCONTROLLER:

Different code given by the 2 sensors is sent to the microcontroller. By using the power of DC motor the microcontroller detect the color and size of the fruit and collect them in a separate basket if it is unqualified.

MOTOR DRIVER:

DC motor is used for the movement of conveyer belt and relay drivers used to drive the motors.

DC MOTOR:

DC motor is the o/p is this system. The distribution and the conveyer belt are powered by the DC motor.

POWER SUPPLY:

The hole system require above +5Vdc & +6Vdc for efficient function.

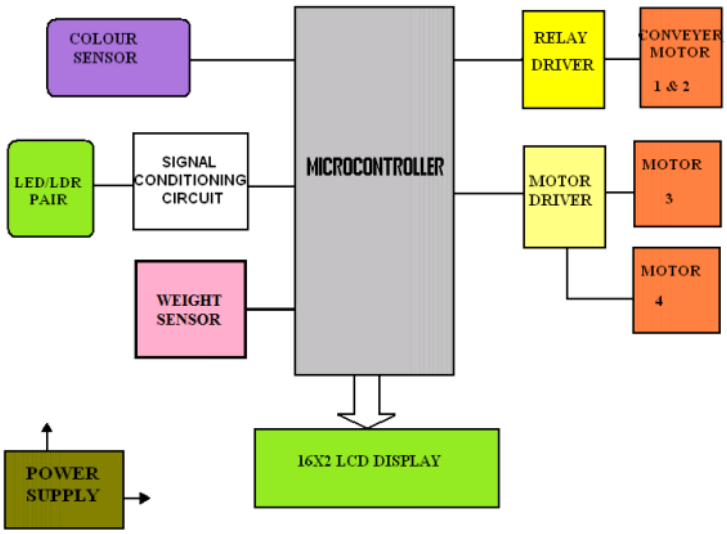


Fig.9: Block diagram of fruit quality detection unit.[6]

Color sorting:

Color are waves just like electromagnetic wave. But looks like real colour due to the interpret of our brain cells. For analyse the robot compare the value of standardised colour and their reflection on them in the normal light. The standardized colors are red, green, blue. Arduino take data and analyse the colour of the object. Arduino software is used in this process. For recognition and sorting mechanisms programing codes are created and encoded in the microcontroller. Arduino Uno is connected by the connecting wires. They act as servo, color sensor as well as microcontrollers. To detect the defect in all the direction in all the direction the motor moves the ball around right and left. For detecting purpose all the works are encoded in the hardware. Arduino UNO is used as software. The below show the hardware and software connection of the entire system.

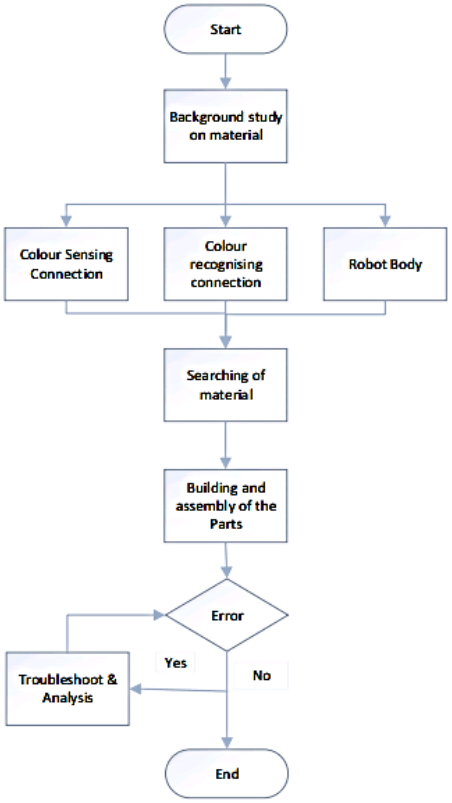


Fig. 1. Hardware flowchart.

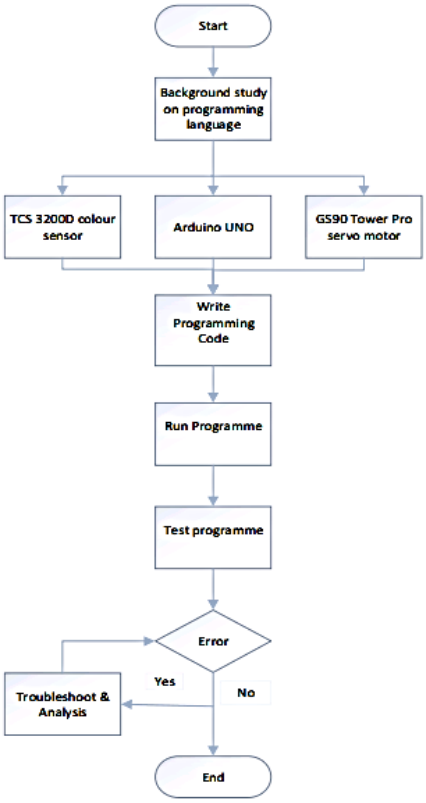


Fig. 2. Software flowchart.

Fig.10: Flowchart of the color sorting unit used in industries.[7]

Detection of chemicals in food:

Input:

Here the input will be a biosensor device which is proposed to be the size of a paper pin, the biosensor should inject it into the target.

Bio-receptor:

For target analyze biosensors are widely used. Based on the classification of bio-receptors biosensors are of various types. Some of the bio-receptors bio-sensors are antibody/antigen, enzymes, nucleic acids, protein biosensors etc.:

Bio-transducer:

A bio-transducer is a component of a biosensor system which converts the biochemical signal to electronic signal. Various types of biosensors can be classified depending upon the classification of bio-transducer. Some of the bio-transducers which can be used in these specific applications are transducer, potentiometric transducer and optical transducer.

Transmitter:

Biosensors are plugged the Bluetooth, Wi-fi or any other transmitting device so that it can transmitted the data to the outer device.

Output:

We would view the result through the smart phones. With the help if these smart phones we could easily obtain the result.



Fig.11: Block diagram for chemical detection of fruits.[8]

Packing:

VIBRATOR

Laboratory and industrial shaker usually use linear and rotary motion for vibrating. Often electrical motors are used with an unbalanced mass on its driveshaft. Food industries vibrating hoppers are usually use.

LOAD CELL

The object weight is calculated by this unit. The signal magnitude is equal to force being measured. Electric signals is created by the transducer in here load cells act as a transducer. The different types of them are hydraulic, pneumatic, strain gauge load cells. Apart from them digital load cell is fast, precise and reliable. In industry load cells are the main part require for packing and filling.

WEIGHT CONTROLLER

Weight controller is also a microcontroller. It display the load cells values as output. Two digital input can to be given to them. In industry for measuring accurate weight weight controllers are used.

PLC:

For performing various functions in industries at the same time Programmable Logic Controllers are used. They are used for automatic electromechanical function. Input and output devices like switches and motors connected with the PLC for proper controlling of them. By continually scanning a program the PLC can work properly.three steps are mainly involve in scanning cycle:

1. Input status checking
2. Program execution
3. Showing the result

HMI

For a given task the HMI act as an interaction face between the user and the programme. This make control system more interactive.It had a another name Man Machine Interface (MMI). It is different and specific for manufacturing and process controller. It gives us the visual representation of the control system and also real time value acquisition. Computerized simulation mainly depend upon this.

RS-232

RS 232 is used in short distance computer communication. RS 232 belongs to asynchronous communication method. It is simple and available all around the world.

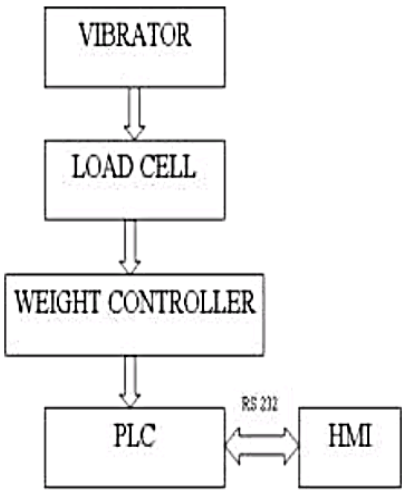


Fig.12: Block diagram of how the packing system is working[9]

Storage:

SYSTEM OVERVIEW

Zigbee and Bluetooth are two wireless sensor used on this system. Using relay switching microcontroller control both system. The microcontroller and the relay connected together once the relay comes to ON position the system works on Bluetooth basis while the relay is off the whole system works on Zigbee. Separate sensors are used for detecting humidity and temperature. The monitor collect the values from the sensors. We can control the humidity/temperature via Bluetooth/Wi-fi networking.

Sensor unit:

It contain different and specific sensors for the detection of temperature and humidity of surrounding. The sensors detect the values in analog form and convert to digital form by digital convertor in microcontroller. Then the values is received by the monitoring unit.

Monitoring unit:

Here for receiving data from sensor both Zigbee and Bluetooth are connected to the monitor. At the monitoring unit values or the data collected can be stored. In computer the data is stored by ZigBee but in Laptop it is by Bluetooth. RS232 serial communication form the communication bridge between the Zigbee and Computer. For proper transmission of data the computer and Android is should be connected with the Bluetooth module.

Conclusion

All these study about the microcontroller in food industry is for better understanding of the technic and gadgets available to overcame our ancient methods and the drawback in it. It also helps to know about the programming language for the use of Microcontrollers in food industry based on specificity. The methods and uses of microcontrollers mention above is being pratice in our food and dairy industries but our lake of knowledge towards them we don't know anything about their uses and performance.

Reference

[1] Shomefun, T. E., Awosope, C., & Diagi, E. (2018). Microcontroller-Based Vertical Farming Automation System. International Journal of Electrical and Computer Engineering (IJECE), 8(4).

[2] Bishop, R. H. (2002). The Mechatronics Handbook, -2 Volume Set. CRC press.

[3] Qin, B. L., Barbosa-Canovas, G. V., Swanson, B. G., Pedrow, P. D., & Olsen, R. G. (1998). Inactivating microorganisms using a pulsed electric field continuous treatment system. IEEE Transactions on Industry Applications, 34(1), 43-50.

[4] Dangi, N. (2018). Monitoring environmental parameters: humidity and temperature using Arduino based microcontroller and sensors: Microcontroller based building monitoring system.

[5] Javanmard, M., Abbas, K. A., & Arvin, F. (2009). A microcontroller-based monitoring system for batch tea dryer. Journal of Agricultural Science, 1(2), 101.

[6] Al Ohali, Y. (2011). Computer vision based date fruit grading system: Design and implementation. Journal of King Saud University-Computer and Information Sciences, 23(1), 29-36.

[7] Shen, L. J., & Hassan, I. (2015). Design and Development of Colour Sorting Robot. JOURNAL OF ENGINEERING SCIENCE AND TECHNOLOGY, 10, 71-81.

[8] Kothari, S., & Channe, H. (2015). Detection of Nutrients and chemicals in food products using sensors in smart phones. International Journal Of Engineering And Computer Science, 4(04).

[9] Kanimozh, D., & Nantheni, B. (2015). PLC controlled automatic food packaging machine. International Journal of En gineering Trends and Technology (IJETT), 30(1), 33-6.

[10] Kaushik, S., & Singh, C. (2013). Monitoring and controlling in food storage system using wireless sensor networks based on zigbee & bluetooth modules. International Journal of Multidisciplinary in Cryptology and Information Security, 2(3).

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA
(An Association Unifying the Sciences, Engineering, and Applied Research)

CONVERSION OF ALKALINE BATTERIES INTO FERTILIZERS

[1]Hari Chandra Mouli.R, [2]Kanimozhi.Ra, [3]Ms.S.Sharmiladevi M.E., M.B.A., [4]Mr.O.Prasanth kuttan, CEO
[1,2]U.G. Students, Department of Environmental engineering,
Park College of Technology, Karumathampatti, Coimbatore, Tamilnadu, India.
[3]Assistant professor, Department of Environmental engineering,
Park College of Technology, Karumathampatti, Coimbatore, Tamilnadu, India.
[4]CEO of Green Era Recyclers, Coimbatore, Tamilnadu, India.
Kanirama0@gmail.com

Abstract*: The alkaline batteries were used all over the world by consumers. The used batteries have been thrown away as a waste into the land and this causes a major problem to environment. Related to many literature studies we go through over of enhancing plant's growth by reducing zinc (Zn) deficiency in particular crops (corn). The government has also established the rules and regulations for a proper management of batteries (Batteries management and handling rules, 2001). The normal recycling processes are over going only on pyrometallurgical or hydrometallurgical recycling processes. But these recycling processes might cause major issues to atmospheric and to the environment by using some of chemicals or by burning processes. So the studies has been concentrated on converting of alkaline batteries into fertilizer by general shredding process and recovering of micronutrients such as Zn and Manganese dioxide powder (MnO2) for field to grow plants in efficient as well as effective way. As per the studies we went through is recovering Zn and MnO2 powder from alkaline batteries , are not consisting of any hazardous content or causing any other contaminants to the soil as well as to plant growth, it is the eco-friendly approach.*

Keywords*: Alkaline batteries, Recycling, Micronutrients (Zn and MnO2), Enhancing crop's growth, Eco-friendly.*

1.Introduction

The Alkaline batteries were generated at a maximum level of amount used by the consumers worldwide and thrown away as a waste. The alkaline batteries were collected around 80% in Swedish at Renova Company for recycling. The alkaline batteries as a waste without a proper disposal and recycling process were thrown away, it will result a major problems to the environment and for recycling process too. The recyclers are recycling the electronic equipment, but they have not concentrated more on its components present inside the batteries. The micronutrients are very important to plant growth and health, and one of the more important micronutrients is zinc.

The components present inside the batteries will be functioning as a catalyst in the soil of land for enhancing the growth of plants. In this, corn plants were selected for our application in field growth. This study shows that micronutrients are very important to plant health and contribute greatly to yield. The granules of Zn and MnO2 are incorporated into the soil. As the Alkaline batteries are not containing of any hazardous substances or other contaminants. It is the safest move to utilize these Zn and Manganese dioxide powder on to the land for enhancing the crops growth and to improve a nutrient rich soil, with a high yield of plant growth. This is an Eco-friendly approach too. This paper also describes that the "Wealth out of Waste" conceptual approach.

2. Alkaline batteries components

The Alkaline batteries began in 1980s and were used all over the world. The Alkaline batteries are made up of steel as a container. Zinc (Zn) act as anode and Manganese dioxide (MnO2) act as cathode, and it will get charged through positive and negative terminal reactions which the batteries are used to operate the TV remotes, torch lights, watches, etc, to be in working condition. When these batteries charging process gets over it will be thrown away as a waste. Its components such as follows:

Container: - Steel holds the entire battery together.

Cathode: - Is made up of Manganese dioxide mixed with carbon (graphite). This becomes the positive terminal on top of the battery.

Separator; - This material separates the anode from cathode and keeps the reaction from occurring, unless the powered device is on and the circuit thereby closed.

Anode: - Is made up of powdered Zinc.
The Anode, Electrolyte and Collector are installed in the battery container last, and then battery is selected.
Electrolyte: - Is potassium hydroxide (KOH) in a solution of water. It helps ions and electrons flow.
Collector: - Is a Brass pin in centre of anode. It collects current and leads to negative terminal on bottom of battery.
The alkaline battery is available in two design configurations:

1. Large-size cylindrical batteries and
2. Miniature button batteries.

This alkaline battery consists of:

- Zinc (Zn) and
- Manganese dioxide powder (MnO_2).

In this we are concentrating on the alkaline batteries powder of Zn and MnO_2 powder to be utilized as a catalyst for giving a plant with enhancing yield of growth.

3. Zinc deficiency in soil affects the plants growth

Zinc deficient soil can be found throughout the world with low soil organic matter and a soil pH higher than 7.0. Any deficiency or toxicity has to be addressed before crop damage is irreversible or else it leads to negative impact in crop growth and quality. Zn deficiency is also identified in calcareous parent materials which consist of eroded areas in soils. The erosion exposes the high calcium carbonate subsoil which has low Zn availability. The resulting of yellowing leaves is also a common sign of nitrogen deficiency as well as zinc deficiency symptoms of plants.

4. Properties

A. Zinc properties (Regarding corn growth)

Zinc, one of the essential micronutrients and an important constituent of several enzymes and proteins, is only needed by plants in small quantities. However, it is crucial to plant development, as it plays a significant part in a wide range of processes.
The normal range for zinc in plant tissue is 15-60 ppm and in the growing medium between 0.10-0.20 ppm. Zn sources must be at least 40-50% water soluble to be reasonably effective nutrient sources. Over the years, micronutrients, especially Zn, have been applied in many forms and by many methods including: broadcast applications to the soil then incorporation into the soil before planting, banding below and to the side of the seed before or at the time of planting, and of directly treating seeds or plant seedling roots with Zn. In this paper, the last said point is

B. Soil properties (Regarding corn growth)

The soil properties are the major properties to be concentrated on growing some crops or plants in that certain region. There is a saying of "When soil is healthy, the crops will make you wealthy". So, the soil properties will play a major role to grow a crop with efficient as well as effective yield.
Corn (Zea mays) growing soils must have a soil temperature ranges between 60 and 65 degree Fahrenheit. The ideal soil for growing corn is well-drained, rich, moist and with a sandy loam soil. In addition to this for making up a nutrient rich soil the compost, leaves can also be added to improve its soil quality in heavy clay soil in particular. The best soil pH will range between 5.8 and 6.8 for a best growth of corn. Even the moisture content is also simultaneously very important as soil temperature in soil for plant growth.

5. Climate considerations for corn growth

The corn grows only in summer months and they are sensitive to frost. If the corn is planted too early then the entire corn plant can be lost. And to be safe, the corn should be planted at least two weeks after the last spring frost date. The harvesting time for corn will vary according to their growth duration and with types of corn were planted.

6. Experimental process

The process includes a major part of collection of some waste alkaline batteries from the recycler company for our experimental process.

- The collected batteries are broken with help of recycler by electrical blade and then the powder inside the alkaline batteries will be recovered by two members for a small experimental purpose in this project.
- Actually the main recovering process of this batteries powder is by collecting, shredding, vibratory screening, magnetic separator, again a vibratory screening and at last the powder of Zn and MnO_2 will be recovered by a machinery processes.
- And then the powder of Zn and MnO_2 are tested for project purpose.
- Later, the soil must be tested and then the corn seeds with catalyst (i.e. mentioned as a fertilizer, it was actually a catalyst which enhances the growth of plants yield.) is added with different ratios according to

identify the measures of appropriate level of catalyst ratio to be added for a good yield and knowing at which ratio the improvement in soil nutrients and plants efficiency growth is occurring and the variation of corn plant at different proportions of catalyst have been added will be recorded.

- The plants weight, height, kernel growth and its weight also calculated for analysing the plants growth too.
- These steps must be done after the soil testing is completed fully, and then a testing report, the conformation of whether that soil on the land can be accurately used for growing crop (corn) or not must be analysed with help of report.

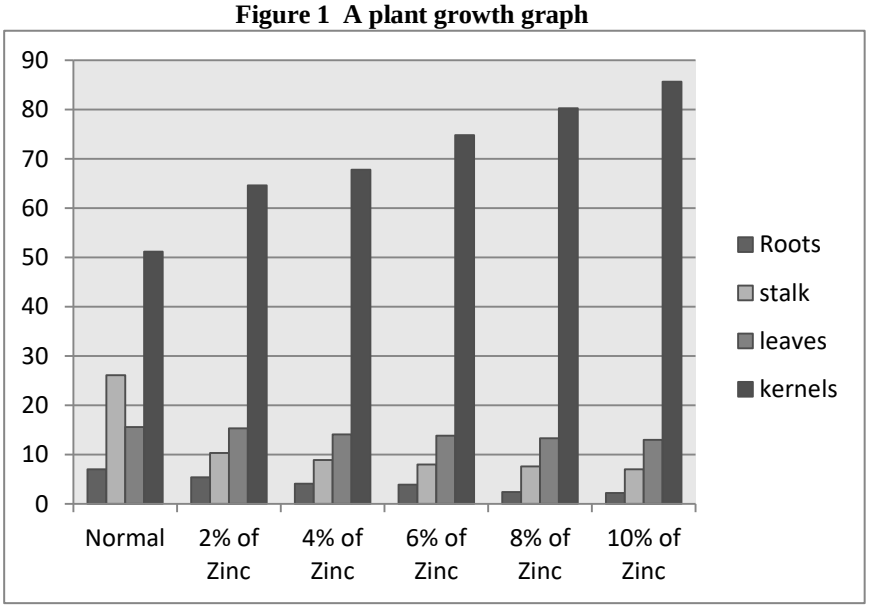
Table and Variation of plant growth

Table.1 Variation of plant growth (corn)

Applied Percentage of Zn and MnO_2 (/metre)	Yield of plant (Corn)				Soil area (m)
	Roots height (cm)	Stalk height (cm)	Leaves height (cm)	Kernel height(cm)	
0%	7	26.1	15.6	51.2	1
2%	5.4	10.3	15.3	64.6	1
4%	4.1	8.9	14.1	67.8	1
6%	3.9	8	13.8	74.8	1
8%	2.4	7.6	13.3	80.3	1
10%	2.2	7	13	85.7	1

The above table describes the variation of plant yield comparison of normal growth of corn with fertilizer (Zn and MnO_2) applied growth of corn plant with different percentage applications. The yield of corn plant with application of Zn and MnO_2 in the soil is efficient when compared with the normal corn growth. The soil area occupied for corn growth where 1 meter for each plant in experimental basis to attain a efficient and nutrient rich soil as well as corn yield as a result.

Figure 1 A plant growth graph



7. Variations in corn growth

The corn growth can be determined by measuring its height in (cm) such as roots, stalk, leaves and kernel height. The up taking of Zn content in plant will be the major part of analyzing the soil nutrient improvement and the plant growth as well. The variation takes place while the percentage is changed and the Zn content up take in plants get varied and its growth and yield of crop (Corn) will vary accordingly. As the result the variation can be analysed physically and to select the most efficient level of zinc utilization percentage to soil in land.

Conclusion

The corn plant can vary in their yield when the land was taken for about certain meters, with different proportional ratios of Zn and MnO_2 powder to soil. As a result the highest and efficient yield of corn plant was recorded at 6% or 8% level of powder get added in soil for growth. Which will be a best range to apply the alkaline batteries recovered powder (Zn & MnO_2) to that certain type of soil with a certain level of ratio is needed to be recorded and implemented to a large scale proportion of catalyst utilization for land farming of corn cultivation basis. This powder is not causing any contaminants or hazardous nature to the soil or to plant.

References

[1] Mansoor et al, Studying the soil properties and root growth of corn under different combination of soil bulk densities, vol. 6, No. 3, 2016.
[2] Panneerselvam et al, Response of maize to soil applied zinc fertilizer under varying available zinc status of soil, vol. 7(7), 934-944, July 2014.
[3] Jim Camberato and Stephen Maloney, Zinc deficiency in corn, July 2012.
[4] R. Bradley Lindenmayer, Zinc fertilization: A Review of scientific literature, April 2007.
[5] Denise et.al, An overview on the current processes for the recycling of batteries, Received 22 February 2004; accepted 31 March 2004.
[6] M. Maftoun and N. Kaeimian, Relative efficiency of two zinc sources for maize (Zea mays) in two calcareous soils from an arid area of Iran, Received 22 January 1988, accepted 28 May 1989.
[7] Book on household batteries and the environment.

Acknowledgments

We are here by thank our guides for guiding us towards successful path and by sharing their greatful and valuable ideas with us. It is useful to gain more knowledge in this part of area about plant growth and many studies we went through.

American International Journal of Research in Science, Technology, Engineering & Mathematics

Available online at http://www.iasir.net

ISSN (Print): 2328-3491, ISSN (Online): 2328-3580, ISSN (CD-ROM): 2328-3629

AIJRSTEM is a refereed, indexed, peer-reviewed, multidisciplinary and open access journal published by International Association of Scientific Innovation and Research (IASIR), USA

(An Association Unifying the Sciences, Engineering, and Applied Research)

EFFICIENCY RATE ANALYSIS OF TUBERS USING ZERO WASTAGE-REVIEW

Caroline Arunagiri[1], Aishwarya Jayakumar[2], Fahima firdouse Thajudeen[3], Pooja Kanakarajan[4], Preethaa Arokiyaaswamy[5], Rajshri Naveen[6]

Department of Food Processing and Preservation Technology, SCHOOL OF ENGINEERING

Avinashilingam School Of Engineering ,Varapalayam, Coimbatore-641043,Tamilnadu, INDIA.

Caroline.srmite@gmail.com

Abstract*: Fruits and vegetables are the most utilized commodity among all the foods. Globally, 1.3 billion tonnes of food gets wasted every year. In this study the complete utilization of produce is analysed by designing a running prototype. The prototype includes three main components which gives the desired end product with less or minimal time consumption. Efficiency rate (Π) of tubers was compared both manually and mechanically. The skin of tubers are peeled and dried using Zero wastage model at optimum temperature for medicinal purposes. The produce is peeled, sliced and dried automatically at optimum rpm and speed.Economical rate of individual machine were considered to design the automatic Zero wastage model.This prototype mainly aims at minimizing the by product waste during peeling, drying and slicing/cutting process of any produce*

Keywords*: zero wastage model, tuber, shear force, prototype, utilization of by product*

I.Introduction

The tuber crops constitute an important group of staple foods for million in the tropics especially for people living in the African and the south American countries. The annual production of tubers is approximately 836 million tonnes globally[1]. Asia is the leading producer of tubers followed by Africa, Europe and America[1]. Tubers are categorised as starchy roots, tubers, rhizomes, corms and stem. A variety of tubers are consumed globally, but the major ones are ginger, potatoes, sweet potatoes, beetroots and cassava. The tubers are also used as animal feed, for manufacturing starch and beer (fermented beverage [2].

The tubers are the second major source of starch next to cereals. In spite of being a major calorie supplier in population's diet, they also contain bioactive constituents such as phenolic compounds, bioactive proteins, phytic acids, saponins [3]. The tubers show proximate composition between 16 – 20% and the fat and protein is scarce, between 0-2 %[4]. Among a huge variety of tubers, ginger (*Zingiberofficinale*) is used as a medicinal plant universally. Ginger is used both fresh and dried. Dried ginger is produced from mature rhizome and is used for the extraction of both oil and oleoresins[5]. Ginger is also known for its antioxidant and anti inflammatory properties[5]. The cassava and the sweet potatoes are a rich source of vitamin C and also the β carotene. The tubers are also rich in fibres. The cassava peel is also used for starch extraction[4].

The water content of tubers ranges70-80%, the carbohydrate ranges between 16 – 20% and the fat and protein is scarce, between 0-2 %[4]. Among a huge variety of tubers, ginger (*Zingiberofficinale*) is used as a medicinal plant universally. Ginger is used both fresh and dried. Dried ginger is produced from mature rhizome and is used for the extraction of both oil and oleoresins[5]. Ginger is also known for its antioxidant and anti inflammatory properties[5]. The cassava and the sweet potatoes are a rich source of vitamin C and also the β carotene. The tubers are also rich infibres. The cassava peel is also used for starch extraction[4].

	Energy (Kcals)	Moisture (g)	Protein (g)	Fat (g)	Minerals (g)	Carbohydrates (g)	Fibre (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)
Arrow root flour	334	16	0	0	0	83	-	10	20	1
Banana rhizome	51	85	0	0	1	12	1	25	10	1
Beet root	43	88	2	0	1	9	1	18	55	1
Carrot	48	86	1	0	1	11	1	80	530	1
Colocasia	97	73	3	0	2	21	1	40	140	0
Khamalu	79	80	1	0	1	18	0	16	31	0
Mango ginger	53	85	1	1	1	10	1	25	90	3
Onion big	50	87	1	0	0	11	1	47	50	1
Onion small	59	84	2	0	1	13	1	40	60	1
Parsnip	101	72	1	0	1	23	2	50	40	0
Radish pink	32	91	1	0	1	7	1	50	20	0
Radish rat-tailed	25	92	1	0	1	4	1	78	24	-
Radish table	16	95	0	0	1	3	1	20	20	1
Radish white	17	94	1	0	1	3	1	35	22	0
Sweet potato	120	68	1	0	1	28	1	46	50	0
Tapioca	157	59	1	0	1	38	1	50	40	1
Potato	97	75	2	0	1	23	0	10	40	0

Table1: Nutrient content of tuber crops

The tubers are irregular in shape .Due to the irregular shape of the tubers, it becomes extremely difficult to cut and peel the tubers. Also it consumes a lot of time, labour and also results in mechanical damage. The tubers contain a very high amount of moisture. Therefore it is very important to remove the moisture for extended shelf life and better storage conditions. Minimal processing of ginger, when done mechanically becomes both time and cost efficient. 50-60% of the production cost can be minimised through automation. Automation also helps prevent mechanical damages and flesh loss. The tuber peels which are also high in phenolic compound can be utilised through this plant as a result of which the tubers can be completely utilised for starch extraction, aromapathy treatment and others.

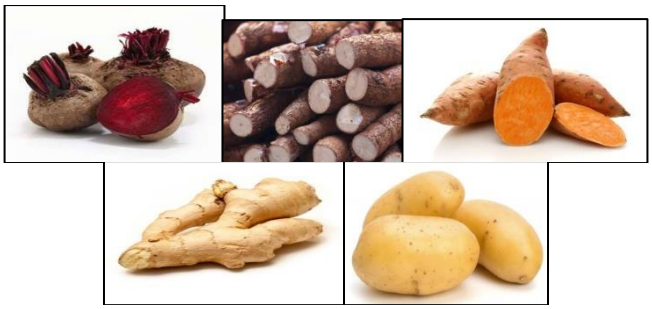


Figure1.variety of tubers

II. Materials and methods:

Materials:

The unit mainly consists of 3 major operations such as cutting, peeling and dehydration. All the three operations are in a single system or equipment with a continuous process. Those operations are operated in a series throughout. The materials used in the unit are as follows:

1.hopper-made up of stainless steel
2.peeling roller
3.automated cutting tool
4.dehydrator setup.
5.a battery-12 v for the units power backup and continuous power supply.

Cutting:

Cutting, slicing, dicing, and shredding are many of the non-thermal food operation techniques for size reduction[6]. This process mainly reduces the preparation time by the consumers. The traditional cutting systems involve knives, hopper, discs and counter shear Cutting is carried out in order to remove the inedible and discolored portions from foods using knife, chopper or slicer[7]. Another relevant factor in the cutting process is the cutting tools used. The main factors include sharpness of the knife blades will directly influence the storage life, whereas dull knives cause bruises and damage to the tissue layers. The most common geometric shapes of fresh-cut vegetables and fruits are: disc, baton, cube, shred and slice. The shapes depend on the types of product and their requirement.

The kitchen knife method remains the most common method of cutting tubers and cannot support large quantities as required by different industries The problems associated with this method are low speed which leads to poor output and low income generation, hand injury, non-uniformity of slice thickness, high production time Currently slicing of tuber crops such as ginger is done mostly manually using knives. This poses danger to individual for injuries inflicted while slicing the ginger fingers with sharp knives while holding them. Knife slicing results in minimal deformation and rupture of the fruits and vegetables cell producing slices of non-uniform size, shape, thickness. Size reduction reduces the time and energy required for drying. Slicing and dicing are the two common pulverizing types which has been practiced.

Many mechanisms have been devised for the mechanized slicing for different capacities. These include the rotary blades, serrated blades, motorized reciprocating principle with fixed blades, stationary cutting blade and two revolving impellers, pneumatic cylinder piston with reciprocating motion, stationery cutters and rotating centralizing/locating slit plate concept[8].

The moisture content and 8he cross sectional areas of the fruits and vegetables have significant influence on the cutting energy. The cutting efficiency is directly proportional to the cross sectional area and inversely proportional to the moisture content. Other parameters influencing cutting are the cutting velocity, shear angle of cut and bevel angle of the knife[9].

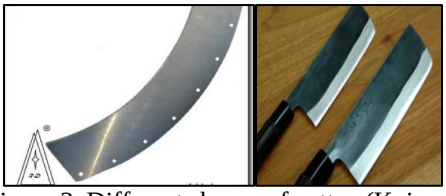


Figure 2 .Different shapes of cutters(Knives)

Peeling:

Removal of the peel is the most important unit operation in the processing of any vegetable. Peeling is defined as the process of removing the inedible portion from the tuber. Some tubers such as ginger and turmeric are irregular in shape and the peeling becomes crucial. Usually tubers are peeled manually with knife which is a very tedious process and leads to flesh loss.

The efficiency of manual peeling was reported to be 21.8 kg/hr[10]. To meet up with the difficulties and the wastage caused by manual peeling mechanical peeling system was designed. The mechanical peeling system of a zero wastage unit consists of a peeling drum. The principle behind the peeling system is *abrasion* peeling[11]. The system consists of a rotating drum and protrusions inside the drum. The drum is rotated at a speed of 40 rpm and the capacity of the machine is around 100kg/hr[12].

The protrusions are normally milling cutters. When the tubers are automated into the peeling system, the drum rotates and the peels are detached from the flesh by abrasion. The mechanical peeling is more efficient than the manual ones. The peeling system is operated with the help of an AC motor. The peeling efficiency through a mechanical peeling is 78% whereas the peeling loss is only 6%[12] .The major advantage of using the mechanised peeling system is the minimal damage to fruits and vegetables, uniform peeling, improved time efficiency, minimised labour costs. Peeling efficiency is given by the ratio of the weight of peel removed by machine to the total weight of peel[13]

P.E= weight of peel removed by machine × 100

Total weight of peel



Figure 3.Commercially used peelers

Dehydration:

Medium sized, healthy and disease-free gingers and other tubers such as beetroot, sweet potato werepurchased from the local market at Coimbatore. These ginger,beetroot and sweet potato were washed, peeled and cut mechanically in the zero wastage unit. Fresh as well as dehydrated tubers samples were analyzed for moisture, ash, crude fibre and volatile oil content[16][17]. The prepared ginger slices were dehydrated in a hot air oven at 55-60degreeCelsius and 60-65 % relative humidity (RH)[18] based on preliminary trials and the prepared beetroot and sweet potato slices were dehydrated in a hot air oven at 50 -120 degree Celsiusfor about 3 to 4 hrs. The moisture loss was determined by weighing the samples at regular intervals. The dehydration ratio was determined by water loss percentage at different time intervals. The tubers are dehydrated such that the moisture is reduced to 5-6%.

The advantages of using the hot air oven are: they use dry heat for the operation, they work well with a digitally controlled interface which manage the heat. It also has a double-walled construction which helps to keep the heat and they conserve energy,there is a supreme thermal efficiency and they provide a uniform dehydration or drying throughout the operation[14][15].



Figure 4.Commercially used dehydrator

III. Results and discussions:

Fruits and vegetables are the most commonly utilized commodities among all crops. They are consumed raw, minimally processed, or even as completely processed, due to their nutritional benefits and health-improving compounds. With the growth in population and drastic change in diet habits, the production and processing of fruits and vegetables, have increased very significantly to fulfill these increasing demands. Significant food losses and waste in the processing industries are becoming a serious economical, nutritional, and environmental problem. The United Nations Food and Agriculture Organization (FAO) has concluded that, losses and waste in fruits and vegetables are the highest among all types of foods, and will eventually reach up to 60%. The processing operations of fruits and vegetables produce significant wastes of by-products, now constitute about 25% to 30% of a whole commodity group. The waste is composed mainly of seed, skin, rind, juice and pomace, containing good sources of bioactive compounds, such as carotenoids, polyphenols, dietary fibers, vitamins, enzymes, and oils, among others. These phytochemicals can be utilized in different field of food industries, for the development of functional or enriched foods, the health industry for medicines and pharmaceuticals, and the textile industry, among others. The use of waste from these good Industry for the production of various bioactive components is an important step toward sustainable development and zero waste.

This device the Zero Waste Unit has been developed to overcome the difficulties faced by small or large scale restaurants and even household works, which are normally man powered. Mostly the tuber crops we use daily are cutting by using human hand. This needs more time and high working load. In order to avoid all such disadvantages, this machine has been designed in such a way that it can be used for cutting the tuber crops very smoothly without any impact force. Whilst gentle handling to ensure less damage to crops is always one of the important considerations when designing a new unit for the food industry, increasing productivity and minimizing labor requirements is always one of the key essentials. In most cases, a reduction in labor requirements coupled with an increase in automation, means that the unit can run more continuously for maximum throughput.

This is an era of automation where it is broadly stated as replacement of manual effort by mechanical power in all modes of automation. The operation remains an essential part of the system although with changing demands on physical input as the degree of mechanization is increased. Advantages commonly attributed to automation include higher production rates and increased productivity. With the development of this Zero Waste Unit a large amount of labor intense work can be minimized and alternatively reduce wastage due to inefficiency. This unit mainly focuses on the application on tuber crops such as beetroot, ginger and sweet potato.

Reference:

[1][1 FAOSTAT,2013,http://faostat3.fao.org
[2] Food and Agriculture Organization (FAO), Roots, Tubers, Plantains and Bananas in Human Nutrition,vol.24 of Food and Nutrition Series, Food and Agriculture Organization, Rome, Italy,1990.
[3] M. Naczk and F. Shahidi, "Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis," Journal of PharmaceuticalandBiomedicalAnalysis, vol.41, pp.1523–1542, 2006.
[4]Anomachandrasekara, ThamiliniJoshepkumar "roots and tubers as functional foods" international journal of food science, 2016
[5]Ajibola O.O. (2000) "Adding value to the farmer's harvest" ObafemiAwolowo University Lecture Series No 145.
[6]Washing, Peeling and Cutting of Fresh-Cut Fruits and Vegetables : M.R. Tapia MM. M. Gutierrez-Pacheco, F. J. Vazquez-Armenta, G. A. González Aguilar, J. F. Ayala Zaval, Mohammad Shafiur Rahman and Mohammed WasimSiddiqu
[7]Technologies in Fresh-Cut Fruit and Vegetables G. R Velderrain-Rodríguez , A. E. Quirós-Sauceda , G. A. González Aguilar , Mohammed WasimSiddiqui , and J. F. Ayala Zavala
[8] Advances in minimal processing of fruits and vegetables: a review WasimSiddiqui, IviChakraborty, J F Ayala-Zavala and R S Dhua
[9]Fresh-cut fruits and vegetables: Critical factors influencing microbiology and novel approaches to prevent microbial risks, OvaisShafiqQadri,BasharatYousuf &Abhaya Kumar Srivastava, FatihYildiz
[10]Odigbo, E.U. (1983) – Model III batch process cassava peeling machine presented at International Conference of Agricultureal Engineering Paper No 88.406
[11] Emadi, B., Abbaspour-Fard, M.H. and Yarlagadda, P. K. D. V. (2008). Mechanical peeling of pumpkins. Part 1: Using an abrasive-cutter brush. J. FoodEngg., 89: 448-452.

[12]K.K.singh, B.D Shukla"abrasive peeling of potatoes ", journal of food engineering 26(4):431-442,1995.
[13]Igbeka, J.C. (1985) "Mechanization of tuber (cassava) peeling
[14]1.JJ Kelly, in: Handbook of Industrial Drying, AS Mujumdar, Ed., Marcel Dekker, New York, 1987.
[15]JG Moore, in: Handbook of Industrial Drying, AS Mujumdar, Ed., Marcel Dekker, New York, 1987.
[16]pruthi, JS spices and condiments (new book trust, new Delhi 1987).
[17]mantri AR, and Agarwal YC , effect of process variation on ginger quality .
[18]Yiljep, Y., G. Fumen and E. Ajisegiri. 2005. The effects of peeling, splitting and drying on ginger quality. Agricultural Engineering International: the CIGR EJournal, Manuscript FP 05 009. Vol. VII. December.