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**‘Innovations in Bio Chemical and Food Technology – 2020’
(IBCFT-20)**

14th March 2020



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Research Culture Society & Publication

2nd National Conference on “Innovations in Bio Chemical and Food Technology – 2020”

(IBCFT-20)

14th March 2020

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2nd National Conference on
“Innovations in Bio Chemical and
Food Technology – 2020”
(IBCFT-20)
14th March 2020

(Conference Proceedings – Special Issue)

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Foreward - From the Principal's desk

Kumaraguru College of Technology, an acclaimed and illustrious institution is an autonomous and self-financing college affiliated to Anna University. The motto of the institution is "CHARACTER IS LIFE" and each individual of the college abides to it. The institution provides a challenging and technologically advanced learning environment to the students. The college is accredited by NAAC and many programmes by NBA. The architecture and ambience serves to enhance a transcendental learning context.

The Department of Biotechnology, established in the year 2002 has been ever brimming with new innovations and serves to nurture thoughts and to provide solutions to the issues faced in our country. The department always strives towards progress through its numerous projects, research and development initiatives which have received funding by various government agencies like DST, DBT, AICTE, FIST etc. The main limelight is on three upcoming fields like medical biotechnology, Environmental biotechnology and food biotechnology.

I am happy that the National conference on "Innovations in Bio Chemical and Food Technology - 2020 (IBCFT - 20)" is being organized for the students all over the country who have a thirst for knowledge and learning. This conference will definitely help them excel in their field of choice. This is a wonderful opportunity for all the students to exhibit and inculcate various skills and also a learning ground for budding biotechnologists and I suggest everyone to kindly make use of the conference. My best wishes to the team and all the participants!



(Principal - Dr. J. Srinivasan)

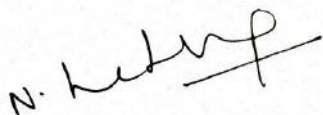
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Foreward - From the HoD's desk

The Department of Biotechnology, established in the year 2002, has been open to research, innovation and development since then. The Centre offers both Full time and part time research programmes serving as stepping stones towards Ph.D and Research. The department holds research and development budget of Rs 2.1 Crores obtained through various government-recognized funding agencies like DBT, DST, AICTE and UGC. The department is recognized by FIST-DST. The department has been awarded with 3 Indian patents and has filed for 6 patents. It holds numerous skill development and consultancy services in specific areas like herbal technology, enzymes for industrial applications, wastewater treatment and so on. Major areas of specialization include Medical, Food and Environment Biotechnology.

To promote more such endeavors, the department of biotechnology is organizing a National Conference on Innovations in Bio Chemical and Food Technology – 2020 (IBCFT-20), which aims to explore recent advancements and research trends in the fields of Bio, chemical and food technology to provide solutions to challenging issues faced in our society. The conference covers multiple themes like, Chemical science and engineering which includes separation process, process modeling and so on. Food science and technology covers food microbiology, food chemistry etc. Bioscience and technology encompasses biopharmaceuticals, bioremediation, bioresource etc.

This wonderful opportunity should be made use by all aspiring students to propagate their knowledge through interaction and learning. Kudos and best wishes to the whole team and all the participants!



(HOD - Dr. N. Saraswathy)

Date: 14.03.2020

Foreward - From the Organizing Secretary

On behalf of the organizing committee, I demit a great privilege in furnishing the proceedings of the National Conference on Innovations in bio chemical and Food Technology organized by Kumaraguru College of Technology, Coimbatore. Recent explorations in Laboratory for Bioremediation Research led to many project developments in the arena of Bio-energy, Food Processing and waste water treatment techniques and these works have been published in many high impact and reputed journals. In this juncture our academic researchers have found three significant areas of which on which this national conference is proposed to. The main objective of the "IBCFT-2020" Conference is to provide a forum for presentation and explore recent trends and advancements in the field of Bio, Chemical and Food Technology which brings suitable and economic solutions via innovative and cost-effective approaches. The topics of technical program have been divided into 3 thematic sessions and several sub-thematic related topics. More than 250 abstracts were received from various participants across the country. Out of them about 100 papers have been selected for paper presentation and about 25 of them have been chosen for poster presentation. All the nominated oral presentations are published in ICI indexed in UGC approved "International journal for innovative research in multidisciplinary field" as a special issue. All the papers appearing in the proceedings were peer-reviewed by renowned reviewers in those fields. A special attention was paid to ensure the high quality of the accepted papers.

We are grateful to the Institute of Engineers India, Student chapter, KCT who assisted to promote and also support our conference. We also address our sincere thanks to the speakers and members of this Conference.

Finally, extending a hearty welcome to Kumaraguru College of Technology, Coimbatore and wishing a blissful experience at the Conference.



(Organising Secretary - Dr. N. Sivarajasekar)

ABOUT THE INSTITUTION

Kumaraguru College of Technology (KCT) is an autonomous, self-financing Engineering college, affiliated to Anna University. KCT was started in the year 1984 with the able guidance and patronage of *Arutselvar* Dr. N. Mahalingam, with the mission of providing aspiring students of technical education, a challenging learning environment.

The college is accredited by NAAC and several programmes offered by KCT are accredited by NBA. The institution is scored good rankings in Teaching Learning Resources, outreach and the National Institutional Ranking Framework (NIRF) accepted by the MHRD.

ABOUT THE DEPARTMENT

The Department of Biotechnology was established in the year 2002. The department has been recognized as the center for research in biotechnology focusing on research and development; the center offers both full-time and part-time research programmes leading to Ph.D. The department holds R&D budget of Rs.2.1 Crores obtained through various government funding agencies like DST, DBT, AICTE and etc.

The department focuses on three major thrust areas – Medical Biotechnology, Environmental Technology and Food Biotechnology.

To reflect the improvement in the research, the department has currently awarded 5 Indian patents, filed 3 patents and offers consultancy services in focused areas like herbal technology, enzymes for industrial applications, fiber analysis, wastewater treatment and antimicrobial testing.

Currently Ph.D scholars are working in the fields of electrosorption, liquid membrane, membrane bioreactors, foldscope, and hydrogels.

**2nd National Conference on
Innovations in Bio Chemical
and Food Technology - 2020
(IBCFT-20)**

Saturday 14 March 2020, Kumaraguru College of Technology, Coimbatore

ABOUT THE CONFERENCE

This conference aims to explore the recent advancements and research trends in the fields of Bio, chemical and Food Technology. These are the interdisciplinary fields which brings suitable and economical solutions via innovative and cost effective approaches.

This unique national conference will provide a platform for researchers present their latest findings and learn about all the important developments in their field.

The conference themes are but not limited to

Chemical Science and Engineering

- Separation process
- Process modelling
- Waste water treatment
- Product design and development
- Nanotechnology
- Drug design and delivery
- Petroleum refinery and petrochemicals

Food Science and Technology

- Food processing
- Food preservation
- Food microbiology
- Food chemistry
- Food biotechnology
- Agricultural engineering

Bioscience and Technology

- Bioproduct / process
- Bioresource
- Bioenergy
- Biopharmaceuticals
- Bioremediation
- Environmental biotechnology
- Computational biology
- Industrial microbiology
- Cell Biology and Genetics

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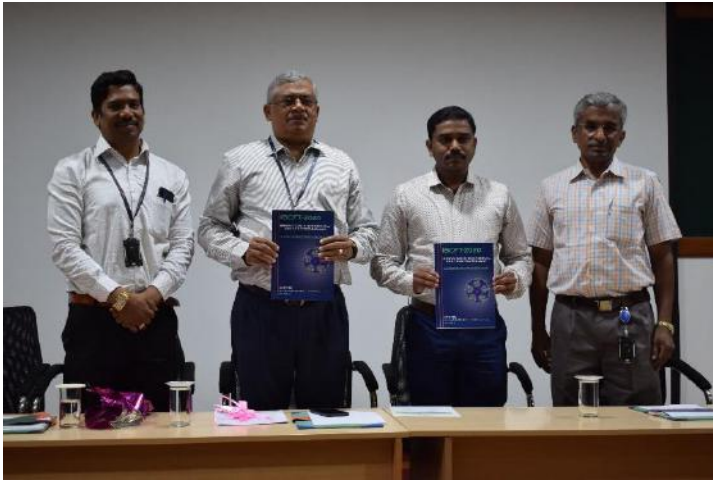


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2nd National Conference on
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14th March 2020

Department of Biotechnology, Kumaraguru College of Technology, Tamil Nadu, India

**Comparative study on the Fermentative Production of Lovastatin from
Monascus purpureus with different agricultural residues**

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Abstract: Lovastatin ($C_{24}H_{36}O_5$), a potent drug for lowering blood cholesterol acts by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) which catalyzes the rate limiting step of cholesterol biosynthesis. Lovastatin was the first statin to obtain approval from the United States Food and Drug Administration in August 1987. The various industrially important and lovastatin producing microorganisms were screened and *Monascus purpureus* MTCC 369 obtained from the Institute of Microbial Technology, Chandigarh, India was found to produce higher concentration of lovastatin. The production of lovastatin using *Monascus purpureus* MTCC 369 was carried out in solid state fermentation using various solid substrates. Barley, long grain rice and sago starch were found to be the suitable substrates producing maximum lovastatin of 193.7 mg, 190.2 mg and 180.9 mg per gram of dry solids. These substrates were further used in various combinations as designed by the central composite design (CCD) for lovastatin production using *M. purpureus*.

Keywords: Lovastatin, fermentation, cholesterol, agricultural substrates

INTRODUCTION:

Lovastatin, a specific and potent competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) is a powerful serum cholesterol lowering drug in humans and other species (Alberts 1988). It is formerly called as mevinolin; monacolin K, and mevacor® and it is a fungal secondary metabolite which inhibit HMG-CoA reductase (E.C 1.1.1.34), the first committed enzyme of cholesterol biosynthesis (Manzoni et al., 1998). 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. The history of statin began in 1987 when the lovastatin received Food and Drug Administration (FDA) approval in the USA (Manzoni and Rollini, 2002). Mevastatin was the first statin to be reported as a fungal secondary metabolite followed by lovastatin (Endo, 1979). *Monascus ruber* produces an active methylated form of compactin known as monacolin K (lovastatin; mevinolin) in liquid fermentation (Endo, 1979).

Lovastatin is produced by a variety of filamentous fungi and some of the industrially important microbial sources for the production of lovastatin are *Monascus* sp, *Aspergillus* sp. and *Penicillium* sp. In particular, *M. purpureus*, *M. ruber*, *M. pilosus*, *M. vitreus* and *M. pubigerus* were found to be the most significant producers of lovastatin (Negishi et al., 1986). Red mold species also produces pigments like: rubropunctatin (red color), monascorubrin (red color), monascin (yellow color), ankaflavin (yellow color), rubropunctamine (purple color) and monascorubramine (purple color) which is widely used to replace synthetic food dyes by natural colorants (Manzoni et al., 1998; Chang et al., 2002). There are different types of statins currently available, which can be classified broadly into natural statins (obtained directly by fermentation), semisynthetic and synthetic. Lovastatin and pravastatin are natural statins, while simvastatin is a semisynthetic and atorvastatin and fluvastatin are synthetic statins (Manzoni and Rollini, 2002).

Response surface methodology (RSM), is a collection of statistical and mathematical techniques useful for developing; improving and optimizing processes. It also has important applications in designs, development and formulation of new products, as well as improvement of existing product designs (Myers and Montgomery, 1995). Response surface methodology has been successfully utilized to optimize the compositions of microbiological media (Oh et al., 1995), improving fermentation process (Lee and Chen, 1997) and product development (Gomes and Malcata, 1998). The objective of the present study is to screen the various agricultural residues and to optimize the significant parameters using RSM to maximize lovastatin production by *M. purpureus* efficiently.

MATERIALS:

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 *M. purpureus* MTCC 369 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.

Culture conditions and Inoculum preparation

Actively growing slants were taken and spore suspension of *M. purpureus* MTCC 369 was prepared using sterile water. 10% spore suspension was inoculated to conical flasks containing the basal medium: 100 g dextrose, 10 g peptone, 2 g KNO₃, 2 g NH₄H₂PO₄, 0.5 g MgSO₄·7H₂O and 0.1 g CaCl₂ in 1000 mL of distilled water, adjusted to pH 6. These cultures were incubated at 30°C for 48 h in a shaking incubator at 120 rpm.

Solid state fermentation

All the sources were purchased from local market and used as the solid substrates in the experiments. Five grams 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 MnSO₄·H₂O, 0.73 g/L was added. The contents of the flasks were mixed and autoclaved at 121°C at 15 psi for 20 min. Fermentation was carried out at 30°C with initial moisture content of 40% and inoculum size of 10% (v/v) and pH at 6.0 for 14 days with *Monascus purpureus*, 10 days with *Pleurotus ostreatus* and 7 days with *Aspergillus terreus*.

METHOD:

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 30°C. 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 C₁₈ 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 45°C. Binary gradient system was used and the samples were injected manually using Rheodyne injector of 20 µL. The mobile phase used was acetonitrile and 0.1% orthophosphoric acid in the ratio of 60:40 respectively. The eluent was pumped at a flow rate of 1.5 ml min⁻¹. Pharmaceutical grade lovastatin (lactone form) tablets containing 40 mg lovastatin per tablet were obtained from Merck Laboratories. Various concentrations of lovastatin dissolved in acetonitrile was prepared and analyzed in HPLC. The equation of the standard curve for the various concentrations of lovastatin (Y) versus peak area (X) is $Y = 44250 X$ with $R^2 = 0.993$. As lovastatin is produced as a mixture of lactone and free β-hydroxyacid form, the standards were prepared in both the forms. The lactone form of lovastatin is converted to the beta hydroxyacid form by dissolving the tablets in a mixture of 0.1 N NaOH and ethanol (1:1 by volume) and heating at 50°C for 20 min and then neutralizing with HCl. The retention time of lovastatin in its beta hydroxyacid form elutes at 6.5 min and the chromatography of a fermented sample is shown in Fig. 1.

DISCUSSION:

Screening of various agricultural residues for lovastatin production by M. Purpureus:

In order to screen the best solid substrates for lovastatin production by *M. purpureus*, the following substrates were experimented; including besan flour (*Cicer aritinum*), jaggery (*Saccharum officinarum*), palm jaggery (*Caryota urens*), black gram (*Vigna mungo*), green gram (*Vigna radiata*), barley (*Hordeum vulgare*), sago (*Manihot esculanta*), ground nut cake (*Arachis hypogaea*), sesame waste (*Sesamum indicum*), millet (*Pennisetum Typhoides*), ragi (*Eleusine coracana*), wheat bran, rice bran (*Oryza Sativa Francais*), jack fruit seed and long grain rice (*Oryza sativa indica*). Among the fifteen different solid substrates tested, maximum lovastatin of 219.2 mg, 193.7 mg, 180.9 mg, 110.7 mg and 70 mg g⁻¹ of dry solids was obtained from long grain rice, barley, sago, besan flour and millet respectively. From Fig. 1, it is evident that long grain rice, barley and sago are the better substrates for the lovastatin production via SSF process.

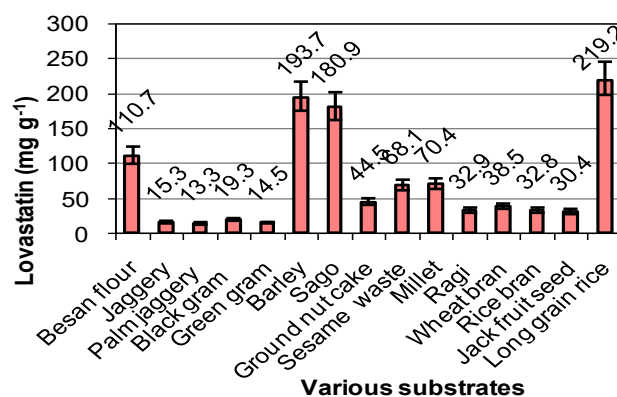


Fig. 1 Lovastatin production from various solid substrates using *M. purpureus* in SSF

The objective of the work is to enhance the production of lovastatin using *M. purpureus* in mixed substrate SSF using various solid substrates and to optimize the combination of the solid substrates by RSM. Long grain rice, barley, and sago starch were found to be the best substrates producing maximum lovastatin of 219.2 mg, 193.7 mg and 180.9 mg per gram of dry solids. These substrates were further used in various combinations as designed by CCD for enhancing the lovastatin production using *M. purpureus*.

Table 1 Experimental range and levels of the independent variables

Variables with designate	Coded values				
	-1.682	-1	0	1	1.682
Barley (g/10 mL)	6.59	10	15	20	23.41
Long grain rice (g/10 mL)	6.59	10	15	20	23.41
Sago starch (g/10 mL)	6.59	10	15	20	23.41

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/g)	
	x_1 Barley	x_2 Long grain rice	x_3 Sago starch	Experimental	Predicted by RSM
1	-1	-1	-1	132.5	104.82
2	1	-1	-1	85.7	73.52
3	-1	1	-1	156.6	150.52
4	1	1	-1	86.8	89.06
5	-1	-1	1	162.4	158.56
6	1	-1	1	159.63	164.1
7	-1	1	1	95.46	106.02
8	1	1	1	55.33	81.40
9	-1.682	0	0	68.41	83.71
10	1.682	0	0	49.75	36.68
11	0	-1.682	0	151.97	174.52
12	0	1.682	0	163.67	143.4
13	0	0	-1.682	81.34	106.59
14	0	0	1.682	168.23	145.29
15	0	0	0	91.12	98.84
16	0	0	0	88.75	98.84
17	0	0	0	92.01	98.84
18	0	0	0	90.56	98.84
19	0	0	0	122.55	98.84
20	0	0	0	108.41	98.84

ANALYSIS:

Mixed substrate SSF could be used to formulate economical substrates for commercial production. The experimental and the predicted values were presented along with the CCD experimental design in Table 1. Multiple regression analysis of the CCD experimental design gives the following quadratic polynomial equation for the biosynthesis of lovastatin:

$$Y = 98.84 - 13.98 x_1 - 9.25 x_2 + 11.52 x_3 - 13.66 x_1^2 + 21.25 x_2^2 + 9.57 x_3^2 - 7.54 x_1 x_2 + 9.21 x_1 x_3 - 24.56 x_2 x_3 \quad (1)$$

where, Y is the amount of lovastatin produced (mg g^{-1} dry substrate), x_1 is the coded value of variable X_1 (long grain rice), x_2 is the coded value of variable X_2 (barley), and x_3 is the coded value of variable X_3 (sago) in equation (1).

FINDINGS:

The results of the regression analysis from the data of central composite design experiments were shown in Table.3. Here the linear effect of x_1 , quadratic effect of x_1 and x_2 , squared effect of x_2 and interactive effects of $x_2 x_3$ were found to be significant as the P -value is less than 0.05 as shown in Table 3. The optimal values of the test variables in the coded units were found to be: $x_1 = -0.1257$, $x_2 = -1.6856$ and $x_3 = 1.5651$ with the predicted maximum lovastatin production of 279.2 mg/g of dry solids. The real values of the independent variables were long grain rice = 14.37 g, barley = 6.61 g and sago = 7.17 g.

Table 3 Regression results from the data of central composite designed experiments

Model Terms	Coefficient	Standard error of coefficient	t -value	P -value
Constant	98.84	9.299	10.629	0.000
x_1	-13.98	6.169	-2.266	0.047
x_2	-9.25	6.169	-1.500	0.165
x_3	11.52	6.169	1.868	0.091
$x_1 x_1$	-13.66	6.006	-2.274	0.046
$x_2 x_2$	21.25	6.006	3.539	0.005
$x_3 x_3$	9.57	6.006	1.594	0.142
$x_1 x_2$	-7.54	8.061	-0.936	0.371
$x_1 x_3$	9.21	8.061	1.143	0.280
$x_2 x_3$	-24.56	8.061	-3.046	0.012

Table 4 Analysis of variance values for the fitted quadratic polynomial model of lovastatin production by *M. purpureus*

Source	Degree of freedom	Sum of squares	Mean square	F -value	P -value
Regression	9	22894.9	2543.9	4.89	0.010
Linear	3	5650.1	1883.4	3.62	0.053
Square	3	11286.8	3762.3	7.24	0.007
Interaction	3	5958.0	1986.0	3.82	0.046
Residual error	10	5197.9	519.8	-	-
Lack of Fit	5	4267.6	853.5	4.59	0.060
Error	5	930.3	186.1	-	-
Total	19	28092.9	-	-	-

RESULT:

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(A), Fig. 2 (B) and Fig. 2 (C). In Fig. 2(A), the contour plot is not elliptical, indicating that there are fewer interactions among the independent variables x_1 and x_2 . The

response surface plot and the contour plot shown in Fig. 2(B) depict the effects of barley and sago on lovastatin production at fixed concentration of long grain rice (15 g). The interaction effects of the substrates barley and sago was also not significant as the contour plot was not elliptical and the P value is greater than 0.05. Fig. 2 (C) shows the effect of interaction between the substrates long grain rice and sago at a fixed concentration level of barley (15 g).

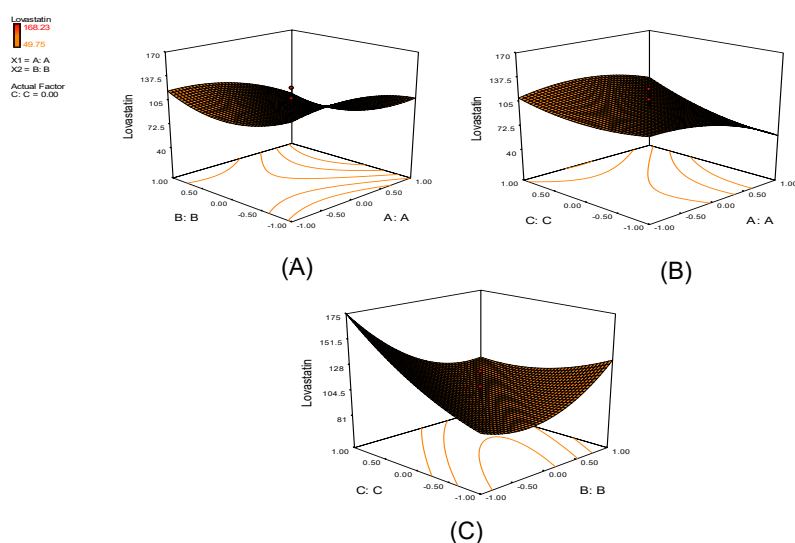


Fig. 2 Response surface plot and the contour plot showing the effects of interaction between the variables (A) Long grain rice and Barley (B) Long grain rice and Sago (C) Barley and Sago

It could be well identified from the Fig. 5 (C) that the contour plot is elliptical and the interaction effect is statistically significant. From the plot of mean lovastatin production and the experimental levels of barley shown in Fig. 6 (A), it was apparent that there was a marked reduction in the lovastatin production when the concentration is increased from coded level -1 (10 g per 10 mL) to coded level +1.682 (23.41 g per 10 mL). 10 g per 10 mL of barley was found to be the optimum amount of barley where the production of lovastatin is found to be high. From the mean lovastatin production and experimental range for long grain rice (Fig. 6 (B)), the maximum lovastatin production was observed at two points, lower level of 6.59 g per 10 mL and at a higher level of 23.41 g per 10 mL. But there was an increase in the lovastatin production at highest factor setting of long grain rice which showed that further increase in long grain rice concentration would increase the lovastatin production, however the optimum concentration of the medium components can be determined by using the polynomial equation. From the plot of the mean lovastatin production and the experimental range of sago concentration (Fig. 6 (C)), the maximum mean lovastatin production was observed at the higher factor setting.

RECOMMENDATIONS:

As lovastatin is having a great potential in lowering the blood cholesterol by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) which catalyzes the rate limiting step of cholesterol biosynthesis, various agricultural residues can also be used as the substitutes in addition to the regular nutritional supplements for the production.

CONCLUSION / SUMMARY:

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 predicted 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 286.7 mg g⁻¹ was obtained which is found to be higher than the predicted lovastatin production of 279.2 mg g⁻¹ at 14 days of fermentation validating the proposed model.

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Statistical optimization of xylanase production from *Cellulomonas fimi*

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Abstract: The present work deals with the production of xylanase by *Cellulomonas fimi* which was enhanced by media optimization. A Plackett-Burman based statistical screening procedure and Central composite design (CCD) was used to identify and determine the optimal concentrations of the nutrients which influences the xylanase production. From the experiments, twelve nutrient components were screened and the results revealed that Peptone $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, K_2HPO_4 , and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were the most significant nutrient components. The optimum conditions are Peptone – 0.56 g/L, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.0016 g/L, K_2HPO_4 – 3.55 g/L, and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.38 g/L. At these optimized conditions, the maximum xylanase activity was found to 5.82 IU/ml.

Key Words: Xylanase, Plackett-Burman design, Tapioca stem, *Cellulomonas fimi*, central composite design, response surface methodology

INTRODUCTION:

The plant cell wall is mainly consists of cellulose; hemicellulose (mainly xylan) and lignin (Bisson et al., 2002). Xylan is the major hemicellulosic constituent. It is the next to cellulose It consists of of 1, 4- linked β -D-xylopyranose units (Annamalai et al., 2009).. Xylanases are one of the hydrolytic enzymes which break the β -1, 4 glycosidic bonds backbone of the complex plant cell wall polysaccharide xylan. Xylanases enzymes produced by a various microorganisms including actinomycetes, yeast, bacteria, and fungi bring hydrolysis of hemicelluloses (Lakshmi et al., 2009). Various researcher used micro organism to produce xylanase are *Streptomyces albus* and *Streptomyces chromofuscus* (Riffat et al., 2005), *Streptomyces lividans* (Ragauskas et al., 1994), *Tricoderma reesi* (Tenkanen et al., 1992), *Bacillus pumilus* (Battan et al., 2007) and *Bacillus subtilis* (Saleem and Akhtar, 2002). Industrial production of enzymes on large scale is associated mainly with substrate. The use of agriculture residues as low-cost substrates for the production of industrial enzymes is a significant way to reduce production cost.

In the present study the production of hydrolytic enzyme xylanase by *Cellulomonas fimi* was improved by optimization medium components. Initially a large number medium components were screened and insignificant ones were eliminated in order to obtain a smaller and more convenient set of factors.

The remaining factors could be optimized by a response surface modeling. Finally, after model building and optimization, the predicted optimum was verified.

MICROORGANISM AND CULTURE MEDIA

Cellulomonas fimi used in this study was purchased from the National Chemical Laboratory, Pune, India. Stock cultures were maintained on nutrient agar slants which contain 1 g of beef extract, 0.5 g of NaCl, 1 g of peptone, 2 g of agar, in 100 ml of distilled water, pH 7.0 to 7.5, at room temperature. The subculturing was performed every 15 days to assure its viability. The inoculum was prepared by adding 5.0 mL of sterile distilled water to the agar slants and shaking vigorously. The spore suspension that obtained was adjusted to 1×10^7 spores per mL and used as the inoculums.

SUBSTRATE PREPARATION

100 g of the washed ground wheat straw was treated separately with 2000 mL of 2% NaOH solution and autoclaved at 121°C for 30 minutes. Then it was filtered, washed with distilled water and excess alkali present was neutralized with phosphoric acid. Again it was filtered and the residue material was dried at 65°C to constant weight in a hot air oven. To the cellulosic material obtained, the same volume of distilled water was added and heated at 121°C for 30 minutes.

The suspension was filtered and the solid material was dried at 65°C to constant weight in a hot air oven (Muthuvelayudham and Viruthagiri, 2010). The dried wheat strawpowder was used as the carbon source.

CULTIVATION OF *CELLULOMONAS FIMI*

Alkali pretreated wheat strawpowder was used as substrate for xylanase production. Fermentation was carried out in Erlenmeyer flasks (250 mL) with 10 g of alkali pretreated wheat strawpowder in 100 ml of enzyme production medium, the composition of media varied according to the experimental design described in this work. pH of the medium was adjusted to 7.0 with 1 mol NaOH or 1 mol HCl. Each flask was covered with hydrophobic cotton and autoclaved at 121 °C for 20 min. After cooling, each flask was inoculated with 10% v/v of inoculum incubated at 28 °C on rotary shaker (150 rpm). After incubation, the samples were withdrawn at regular time intervals and the samples were filtered through GD-120 glassfiber filter disks (Whatman) to remove the residual insoluble substrate. Then, the liquid content obtained after filtration was centrifuged at 10,000×g for 10 min at 4 °C to separate the cells. The cell-free supernatant was analyzed for enzyme activity. During the preliminary screening process, the experiments were carried out for 96 hours, and it was found that at 72 hours the maximum enzymes production was obtained. Hence, experiments were carried out for 72 hours. All the experiments were carried out in triplicate and the average values are reported.

XYLANASE ASSAY

The reaction mixture containing 0.5 ml of suitably diluted enzyme solution, 1 ml citrate phosphate buffer (pH 7.0) and 0.5 ml of xylan (oat spelt) was incubated at 50 °C for 5 min (Bailey *et al.*, 1992). Xylose released by xylanase was assayed by DNS (Dinitrosalicylic acid) method using xylose as standard (Miller, 1959). The enzyme activity was expressed in international units and was defined as the amount of enzyme required to release one μ mol of reducing sugar (xylose) per min.

SCREENING OF MEDIUM COMPONENTS BY PLACKETT-BURMAN (PB) EXPERIMENTAL DESIGN

The main application of Plackett-Burman experimental design is to identify the most significant ingredients of the medium on the xylanase producing capability of *Cellulomonas fimi*. When this kind of statistical experimental design is employed it is assumed that no interactions between different factors occur in the range of variables under consideration (Plackett and Burman, 1946). A total of twelve variables were considered for screening (Table 1) in 20 experimental runs (Table 2) and insignificant variables were eliminated in order to obtain a smaller, manageable set of factors. Each factor examined in two levels: -1 for low level and +1 for high level of each factor are listed in Table 1. Twelve variables (Table 1) were screened.

Table 1 Nutrient screening using a Plackett-Burman design

Nutrient Code	Nutrient (g/L)	Low (-1)	High (+1)
A	Peptone	0.25	1.25
B	Yeast extract	0.5	3.5
C	MnSO ₄ .7H ₂ O	0.0010	0.002
D	KH ₂ PO ₄	2.0	4.0
E	K ₂ H PO ₄	1.0	5.0
F	NaNO ₃	0.5	1.0
G	CoCl ₂ .6H ₂ O	0.02	2.0
H	MgSO ₄ .7H ₂ O	0.3	0.5
I	CaCl ₂ .2H ₂ O	0.3	0.5
J	(NH ₄) ₂ SO ₄	0.06	0.1
K	Corn steep liquor	0.4	0.8
L	FeSO ₄ .7H ₂ O	0.005	0.1

A standardized Pareto chart consists of bars with a length proportional to the absolute value of the estimated effects, divided by the standard error. The bars are exhibited in the order of the size of the effects, with the largest effect on top.

The Pareto chart illustrates the order of significance of the variables affecting xylanase production at p≤0.05 (Figure 1). From the Pareto chart we found that the variables namely) the variables namely peptone, MnSO₄.7H₂O, K₂HPO₄ and MgSO₄.7H₂O was found to be significant. These nutrients were selected for further optimization to attain a maximum production of xylanase.

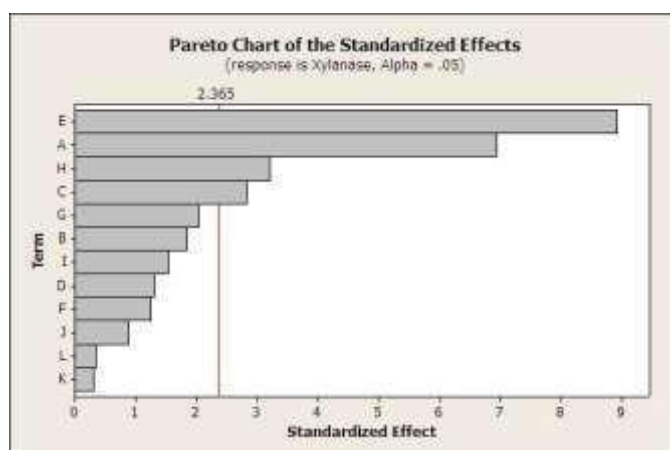


Figure 1 Pareto chart showing the effect of media components on xylanase activity by *Cellulomonas fimi* NCIM-5015 using wheat straw

Optimization of nutrients for the xylanase production by *Cellulomonas fimi* NCIM 5015 using wheat straw

The CCD of RSM was employed to optimize the selected four significant nutrient components, namely peptone, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ which enhances the xylanase production. The four independent variables were studied at five different levels (Table. 2) and a set of 30 experiments were carried out. The results were analyzed by ANOVA (Table 3).

Table 2 Ranges of the independent variables used in RSM

Variable		Levels (g/L)					
		Code	-2	-1	0	1	2
1.	Peptone	X_1	0.25	0.5	0.75	1.0	1.25
2.	$\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$	X_2	0.0010	0.00125	0.0015	0.00175	0.002
3.	K_2HPO_4	X_3	1	2	3	4	5
4.	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	X_4	0.3	0.35	0.4	0.45	0.5

The second-order regression equations provided the levels of xylanase as the function of peptone, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ which can be presented in terms of coded factors as in the following equation(1):

$$Y = +5.71 - 0.23 X_1 + 0.046 X_2 - 0.011 X_3 + 0.025 X_4 - 0.62 X_1 X_2 - 0.21 X_1 X_3 + 0.16 X_1 X_4 + 0.13 X_2 X_3 - 0.043 X_2 X_4 - 0.22 X_3 X_4 - 0.47 X_1^2 - 0.59 X_2^2 - 0.26 X_3^2 - 0.33 X_4^2 \quad \dots(1)$$

Where Y represents the activity of xylanase respectively. X_1 , X_2 , X_3 and X_4 are the coded values of peptone, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ respectively. ANOVA xylanase are shown in Table 3.

Table 3. Analysis of variance (ANOVA) for xylanase by *Cellulomonas fimi* NCIM-5015 using wheat straw

Source	Coefficient factor	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	5.71	25.140645	14	1.7957604	24.804652	< 0.0001
X_1	-0.23125	1.2834375	1	1.2834375	17.72799	0.0008
X_2	0.04625	0.0513375	1	0.0513375	0.7091196	0.4130
X_3	-0.01125	0.0030375	1	0.0030375	0.0419567	0.8405
X_4	0.0245833	0.0145042	1	0.0145042	0.2003446	0.6608
$X_1 X_2$	-0.619375	6.1380063	1	6.1380063	84.783646	< 0.0001
$X_1 X_3$	-0.214375	0.7353063	1	0.7353063	10.156709	0.0061
$X_1 X_4$	0.160625	0.4128063	1	0.4128063	5.7020501	0.0305
$X_2 X_3$	0.131875	0.2782563	1	0.2782563	3.8435248	0.0688
$X_2 X_4$	-0.043125	0.0297563	1	0.0297563	0.41102	0.5311
$X_3 X_4$	-0.218125	0.7612563	1	0.7612563	10.515154	0.0055
X_1^2	-0.4748958	6.1858574	1	6.1858574	85.444609	< 0.0001
X_2^2	-0.5948958	9.7070003	1	9.7070003	134.08179	< 0.0001
X_3^2	-0.2611458	1.8705503	1	1.8705503	25.837718	0.0001

X_4^2	-0.3348958	3.0762574	1	3.0762574	42.492026	< 0.0001
Residual		1.0859417	15	0.0723961		
Lack of Fit		0.8299417	10	0.0829942	1.6209798	0.3096
Pure Error		0.256	5	0.0512		
Cor Total		26.226587	29			

Std. Dev. - 0.27, R-Squared - 0.9586, Mean - 4.38, Adj R-Squared - 0.9199, C.V. % - 6.15, Pred R Squared - 0.8037, PRESS - 5.1, Adeq Precision - 14.384

A model can be considered reasonably reproducible if CV is not greater than 10%. Usually, the higher the value of CV, the lower is the reliability of experiment. Here, a lower value of CV (6.15%) for xylanase indicated a greater reliability of the experiments performed. The closer the values of R^2 to 1, the better the model would explain the variability between the experimental and the model predicted values. The coefficient of determination (R^2) for xylanase activity was calculated as 95.86 % variability of the response, and only about 4.14 % of the total variation cannot be explained by the model. The predicted R^2 value of xylanase activity was 80.37 % and has a reasonable agreement with the adjusted R^2 value of xylanase activity is 91.99 % . "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Adeq Precision of 14.38 for xylanase indicates an adequate signal. These models can be used to navigate the design space. The statistical significance of (equ.4.2.1 and 4.2.2) was checked by F - test, the results of ANOVA are shown in Table 4.2.6. The results demonstrated that the model is highly significant and is evident from Fischer's F -test with a low probability value (P model $> F$ less than 0.05) .

In the present work, the linear effects of X_1 , the interactive effects of X_1X_2 , X_1X_3 , X_1X_4 , X_3X_4 and square effects of X_1^2 , X_2^2 , X_3^2 and X_4^2 are significant model terms for xylanase production. To test the fit of the model equation, the regression-based determination coefficient R^2 was evaluated. The nearer the values of R^2 to 1, the model would explain better for variability of experimental values to the predicted values. The above models can be used to predict the xylanase productions within the limits of the experimental factors. The interaction effect of the variables on xylanase production was investigated by plotting the 3D response surfaces with the vertical (Z) axis representing enzyme activity (response) yield and two horizontal axes representing the coded levels of two explanatory factors, while maintaining other variables at their median levels are shown in Fig2(a) - 2(f).

EFFECT OF PEPTONE

Fig.2(a), 2(b), 2(c) shows the dependency xylanase on peptone. The xylanase activity increases with increase in peptone up to 0.56 g/L and thereafter xylanase activity decreases with further increase in peptone.

EFFECT OF $MnSO_4 \cdot 7H_2O$

The same trend was observed in Fig 2(a), 2(d), 2(e) which shows the dependency of xylanase on $MnSO_4 \cdot 7H_2O$. The xylanase activity increases with increase in $MnSO_4 \cdot 7H_2O$ up to 0.0016 g/L and thereafter xylanase activity decreases with further increase in $MnSO_4 \cdot 7H_2O$.

EFFECT OF K_2HPO_4

Fig.2(b), 2(d), 2(f) shows the dependency of xylanase on K_2HPO_4 . The xylanase activity increases with increase in K_2HPO_4 up to 3.55 g/L and thereafter xylanase activity decreases with further increase in K_2HPO_4 .

EFFECT OF $MgSO_4 \cdot 7H_2O$

The dependency xylanase on $MgSO_4 \cdot 7H_2O$ was shown in Fig 2(c), 2(e), 2(f). The xylanase activity increases with increase in $MgSO_4 \cdot 7H_2O$ up to 0.38 g/L and thereafter xylanase activity decreases with further increase in $MgSO_4 \cdot 7H_2O$.

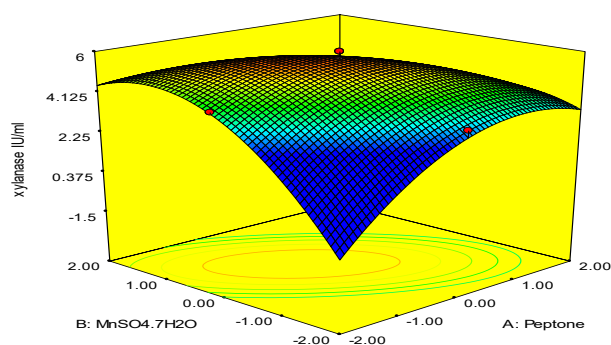
Validation of the experimental model for production of xylanase by *Cellulomonas fimi* on wheat straw

The optimum conditions for the maximum production of xylanase was determined by response surface analysis and also estimated by optimizer tool using statistical software package "Minitab 15". The optimum conditions are Peptone – 0.56 g/L, $MnSO_4 \cdot 7H_2O$ – 0.0016 g/L, K_2HPO_4 – 3.55 g/L, and $MgSO_4 \cdot 7H_2O$ – 0.38 g/L.

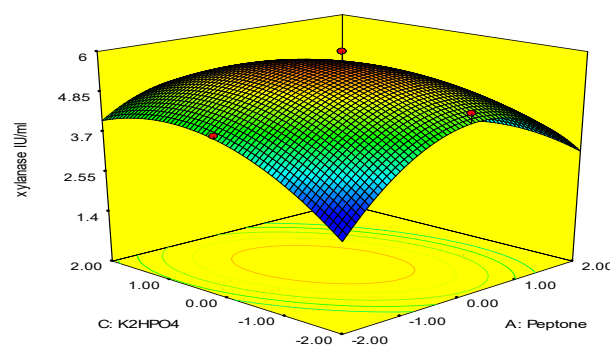
Validation of the experimental model was done by carrying out the batch experiment under optimal operating conditions. The experiments were done in triplicate and the results were compared. The xylanase activity obtained from experiments was very close to the actual response predicted by the regression model, which proved the validity of the model. The percentages of error between experimental and model predicted values of xylanase activity is 0.47. At these optimized conditions, the maximum xylanase activity was found to 5.82 IU/ml.

Validation of the experimental model for production xylanase by *Cellulomonas fimi* on wheat straw

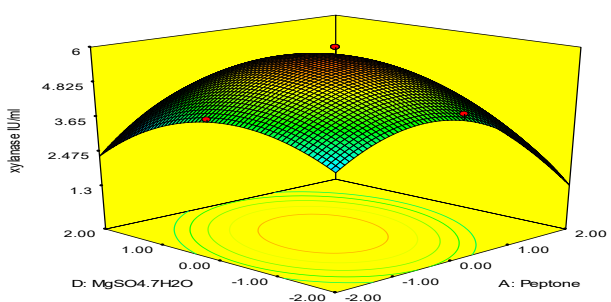
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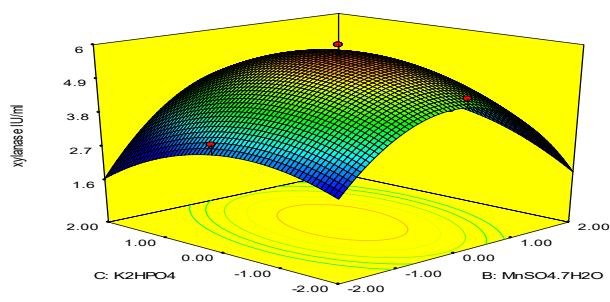
(a): Peptone and $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$



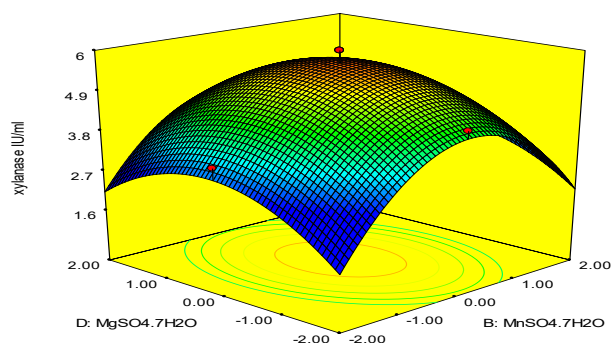
(b): Peptone and K_2HPO_4



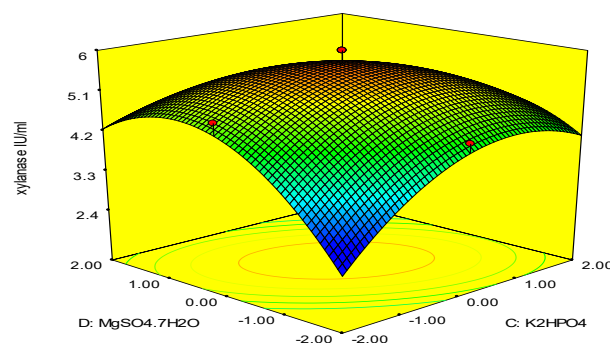
(c): Peptone and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$



(d): $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ and K_2HPO_4



(e): $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$



(f): K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

Fig2. Three-dimensional response surface plot for xylanase production by *Cellulomonas fimi* NCIM 5015 using wheat straw showing the interactive effects of medium components

Validation of the experimental model was done by carrying out the batch experiment under optimal operating conditions. The experiments were done in triplicate and the results were compared. The xylanase activity obtained from experiments was very close to the actual response predicted by the regression model, which proved the validity of the model. The percentages of error between experimental and model predicted values of xylanase activities is 0.47. At these optimized conditions, the maximum xylanase activity was found to be 5.82 IU/ml.

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Bio Degradation Kinetics of Synthetic Dyes

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Abstract: Reactive dyes are most commonly used dyes in textile industries which are found commonly in various textile industries effluents. Treatment of effluents from textile industries poses a threat to the environment and bio treatment with white rot fungus *Trametes hirsuta* seems to be a very good option. The decolorization kinetics of Reactive red 111 and Reactive red 251 by *Trametes hirsuta* was studied under optimum process conditions and the modeling was attempted using different kinetics models namely Logistic model for microbial growth and Substrate Utilization Kinetics, Half-and-first order kinetic models for dye decolorization kinetics were tested. Logistic models gave better prediction of biomass concentration with high R² values of 0.975 for Reactive red 111 and 0.968 for Reactive red 251 were obtained. The half order model and substrate utilization kinetics were found to be the best fit when compared to the first order model for both the dyes studied.

Key Words: Kinetics, Half-and-first order; RR111; RR 251, Decolorization.

INTRODUCTION:

Dyes are versatile chemicals among various dyes commercially in use azo dyes find the largest applications and uses as 60-70% of all dyes stuff in use and production fall in this group [1]. On the basis of chemical composition there are various classes of azo dyes; reactive dyes the most important of them. Most of the reactive dyes (80-95%) have azo group as chromogen [2,3]. Reactive dyes are coloured compounds that contain one or two functional groups capable of forming covalent bonds with the active sites in fibres [2,3]. From the available bibliography it can be estimated that approximately 75% of the dyes, discharged by textile processing industries, belonging to the classes of the reactive (~36%), acid (~25%) and direct dyes (~15%) [4]. 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 [5]. 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 [6]. The presence of very low concentrations of dyes in effluent is highly visible and undesirable [7]. 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 [8,9]. 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 [10]. Therefore, the removal of colour from textile and dyestuff manufacturing industry wastewater represents a major environmental concern.

MATERIALS AND METHOD:

Experiments are conducted under laboratory conditions to study the potential of fungal culture for decolourizing different reactive dyes such as reactive red 251 and reactive red 111 used in textile processing industry.

Microorganism and growth conditions

A pure strain of *Trametes hirsuta* (MTCC-136), a white-rot fungi in lyophilized form is procured from Institute of Microbial Type Culture Collection Centre, Chandigarh, India.

Table 1. Composition of the media used for the growth of *T. hirsuta*

Medium Component	Medium Composition (g/l)	Temperature (°C)	pH
Yeast Extract	5	30	5.8
Glucose	10		

Agar	15		
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The fungal culture is grown on nutrient agar slant medium containing yeast extract, glucose and agar. Table: 1 shows the composition of the media used for the growth of microorganism. The medium is sterilized at 1.1 kg_f/cm² gauge pressure at 121°C for 15 min. The organism on the slants is allowed to grow for 10 days at 30°C and stored at 4°C for further use. Microorganisms are periodically observed under microscope for any possible contamination of other morphological changes.

Preparation of Laccase production medium

Laccase, which are extracellular secretion of white rot fungus, is able to decolourize dyes by the help of a specially designed media which had the following composition given in Table 2. Cultures are incubated at 30°C and pH of the medium is adjusted to 5.0. [11].

Table 2 Composition of Laccase production medium used for the decolourization of dyes

Medium Component	Medium Composition (g/l)
Wheat bran flakes	4.50
Yeast extract	1.50
Glucose	1.00
Ammonium chloride	0.25
Thiamine dichloride	0.05
Potassium hydrogen phosphate	0.20
Magnesium sulphate	0.05
Calcium chloride	0.01
Potassium chloride	0.05

Dyes

Reactive dyes are the dyes, which are mostly used in the textile industries. Preliminary experiments are performed to test the decolourizing ability of *Trametes hirsuta* against most common reactive dyes such as reactive orange 122, reactive red 2, reactive golden yellow, reactive blue 4, reactive violet 5 R, reactive red 111 and reactive red 251. Maximum decolourization is observed only for two reactive dyes namely reactive red 111 and reactive red 251 respectively and selected for further decolourization studies.

Preparation of aqueous dye solution

Stock dye solutions are prepared by dissolving 1 g of respective dyes in one litre of double distilled water and autoclaved. Solution of the desired concentrations is obtained by successive dilution.

Estimation of Biomass Concentration

Centrifuge tubes are well washed and dried in an oven to remove all the moisture. Weights of empty dry centrifuge tubes are found out using electronic balance. 10 ml of the broth is taken in the centrifuge tube and centrifuged for 20min. The settled biomass is made free of water and it is kept in the oven to remove all moisture. The weight of the biomass is found from the difference in measured weights.

Determination of dye concentration

The dye concentration in raw and treated samples is determined by UV-Spectrophotometer. The residual dye concentration in the medium is determined by measuring the absorbance at 550 nm for Reactive red 111 and at 549 nm for Reactive red 251 using UV-Spectrophotometer (ELICO BL 198). A calibration chart is drawn between absorbance and standard solutions for various concentrations of dye. From the noted absorbance value the initial concentration, the concentration of the treated dye samples are determined.

Decolourization Kinetics for Logistic Growth Model

The most widely used unstructured models to describe cell growth are the Monod kinetic model and the Logistic equation. The Logistic equation is a substrate independent model. It account for the inhibition of growth, which occur in many batch processes. Verlhurst in 1844 and Pearl and Reed in 1920 contributed to a theory which included an inhibiting factor to population growth. Assuming that inhibition is proportional to x^2 , they used

$$\frac{dx}{dt} = kx(1 - \beta x) \quad x(0) = x_o \quad \dots (1)$$

Where x is the biomass concentration (g/l), k is the rate constant (h^{-1}), and β is the Logistic constant. The Logistic curve is sigmoidal and leads to a stationary population of size $x_s = 1/\beta$. Rate of growth of cell is proportional to the cell mass concentration present at that time. The rate will stop when the cell mass concentration reaches stationary phase. When the cell mass concentration is near the stationary phase rate will slow down. That is, growth rate also depends on how far the cell mass concentration at a given point of time is away from the cell mass concentration at stationary phase. Eq. (1) is a Riccati equation which can be easily integrated to give the Logistic curve.

$$x = \frac{x_o e^{kt}}{1 - \beta x_o (1 - e^{kt})} \quad \dots (2)$$

Where x_o is the initial biomass concentration (g/l) and t is time (h). Monod and the other models predict that the growth will stop only when the limiting substrate concentration is exhausted. In reality due to the accumulation of toxic metabolites or due to inhibition, the growth may stop even when substrate is present. These conditions are taken care of by the Logistic model. The advantage of this model for dye decolourization is that it provides the exponential phase and endogenous metabolic phase accurately [13].

Half- and First-order Kinetic Model

Decolourization kinetics occurring in the natural environment is based on the empiric approach, reflecting the rudimentary level of the microbial population and their activity in an ecosystem. When the substrate is totally available, the biodegradation will solely depend on the microbial activity, following a logarithmic growth. On the other hand, the first order kinetics link to the population density is more realistic [14]. This approach described by equation below is used to study the decolourization kinetics;

$$-\frac{dC}{dt} = KC^n \quad \dots (3)$$

where C , the concentration of the substrate (dye); t , time and K , the rate constant of substrate disappearance and n , the order of reaction.

The half-order kinetic equation is given by

$$C^{1/2} = C_o^{1/2} - \frac{K_{1/2} t}{2} \quad \dots (4)$$

Where $K_{1/2}$, the half-order reaction rate constant

Substrate Utilization Kinetics

The substrate utilization kinetics is the modified form of the Leudeking-Piret model which can be used for substrate utilization kinetics. Substrate consumption depends on the magnitude of three sink terms, the instantaneous biomass growth rate, the instantaneous product formation (Dye degradation) rate and a biomass maintenance function. Therefore, substrate consumption can be described by the following equation [13]:

$$-\frac{ds}{dt} = \frac{1}{Y_{x/s}} \frac{dx}{dt} + \frac{1}{Y_{p/s}} \frac{dp}{dt} + k_e x \quad \dots (5)$$

where $Y_{x/s}$ and $Y_{p/s}$ are the yield coefficient for the biomass and product (dye degraded), respectively and K_e is the specific maintenance coefficient. The substrate consumption rate can be modeled using Leudeking-Piret like equation that neglects the amount of carbon substrate used for product formation and maintenance constant, the model equation becomes:

$$-\frac{ds}{dt} = \frac{1}{Y_{x/s}} \frac{dx}{dt} \quad \dots (6)$$

Integrating Eq. (5.7) with two initial conditions, $x = x_o(t=0)$ and $s = s_o(t=0)$ gives Eq. (5.8).

$$s = s_o - \frac{1}{Y_{x/s}} (x - x_o) \quad \dots (7)$$

RESULTS AND DISCUSSIONS:

The ability of microorganisms to degrade reactive dyes is generally correlated with the ability to synthesize enzymes which are affected by environmental factors such as pH, temperature and dye concentration. The decolorization experiments at optimum conditions shows that rapid decolourization is observed within 24 h due to extracellular Laccase enzyme produced by the white rot fungus *T. hirsuta*. Although other enzymes are secreted in negligible quantity, Laccase enzyme alone is responsible for decolourization, because of using Laccase production medium. It takes 9 days for the decolourization of reactive red 251 and 10 days for reactive red 111. It may be due to complexity in dye structure. The experimental studies observed that *Trametes hirsuta* showing lesser activity towards decolourization of reactive red 251 than reactive red 111 and reactive black 5 tested. The organism is not able to decolorize reactive red 251 at a concentration greater than 180 mg/l. The dye is expected to become toxic to the microorganism at higher concentrations (>180 mg/l) whereas for other dyes, *T.hirsuta* is able to grow at higher concentrations and an appreciable decolourization is observed.

From the experimental results it is found that the dye concentrations significantly affected the decolourization potential of fungi. The initial dye concentration provides an important driving force to overcome all mass transfer resistances of the dye between the aqueous and solid phases. Maximum percentage decolourization of 84% and 77%; and the maximum Laccase enzyme concentration of 90U/l and 76U/l is obtained for reactive red 111 and for reactive red 251 respectively. The extent of colour removal is not consistent with all the dyes. Even though the same amount of the inoculum is used for all the dyes, the differences found in the decolourization characteristics for the individual dyes are attributed to the dissimilarity in specificities and structures of different dyes. The similar observation regarding the dye degradation by the white rot fungus *Pleurotus florida* has been observed [12]. The validity of Substrate utilization kinetics, Half- and First-order kinetic models for the decolourization of reactive red 111 and reactive red 251 are tested using solver in MS Excel and the results are compared between experimental and predicted dye concentrations for the decolourization of reactive red 111 and reactive red 251 using different kinetic models are shown in Fig 3 and in Fig 4. The simulation results are useful to predict the dynamics of dye utilization and are well suited for the decolourization of dyes studied. The half order model and substrate utilization kinetics are found to be the best fit when compared to first order model for the two dyes studied.

Table 3 Model parameters for the decolourization of dyes

Dyes	Logistic model		Half order model		First order model		Substrate Utilization kinetics	
	K (h ⁻¹)	β (l/g)	K _{1/2} (mg ^{1/2} /l ^{1/2} h)	R ²	K (h ⁻¹)	R ²	Y _{x/s} (g/mg)	R ²
Reactive red 111	0.019	0.154	0.232	0.992	0.250	0.977	0.883	0.987
Reactive red 251	0.023	0.114	0.369	0.983	0.194	0.976	0.816	0.996

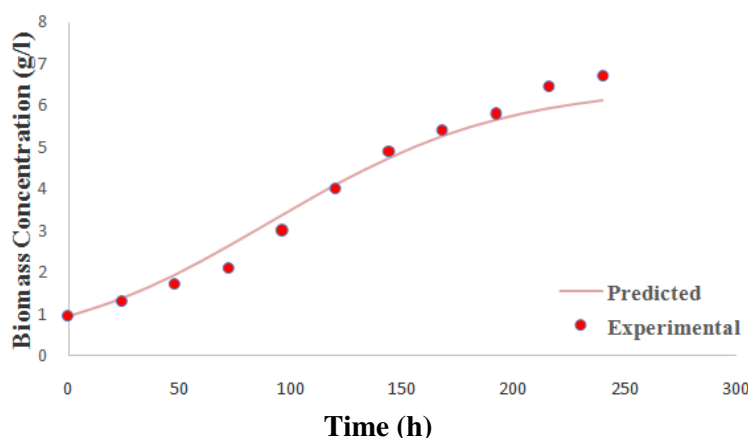


Figure 1. Comparison between experimental and predicted microbial growth using Logistic growth model for the decolourization of Reactive red 111 by *T. hirsuta*

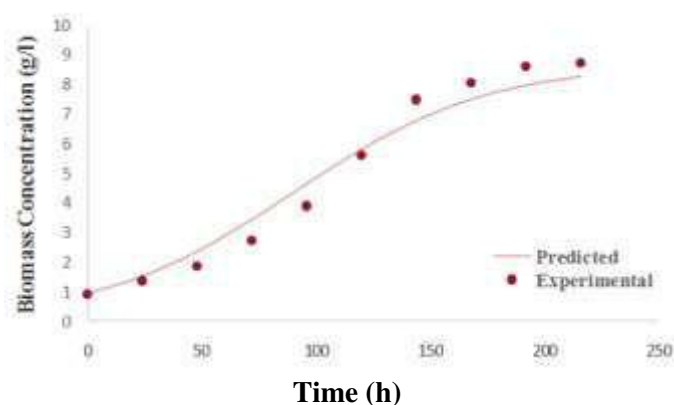


Figure 2. Comparison between experimental and predicted microbial growth using Logistic growth model for the decolourization of Reactive red 251 by *T. hirsuta*

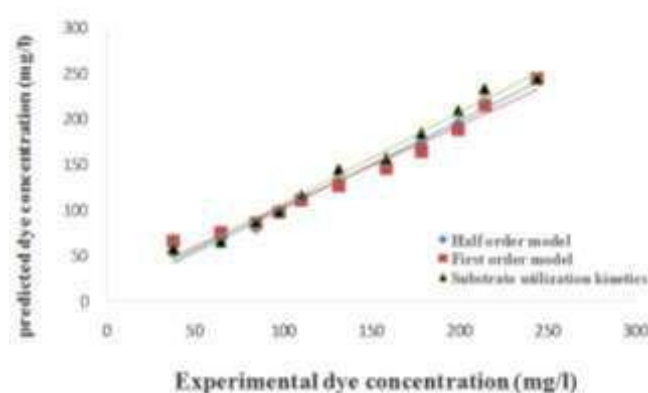


Figure 3. Predicted dye concentration Vs Experimental dye concentration for the decolourization of reactive red 111 using different kinetic models

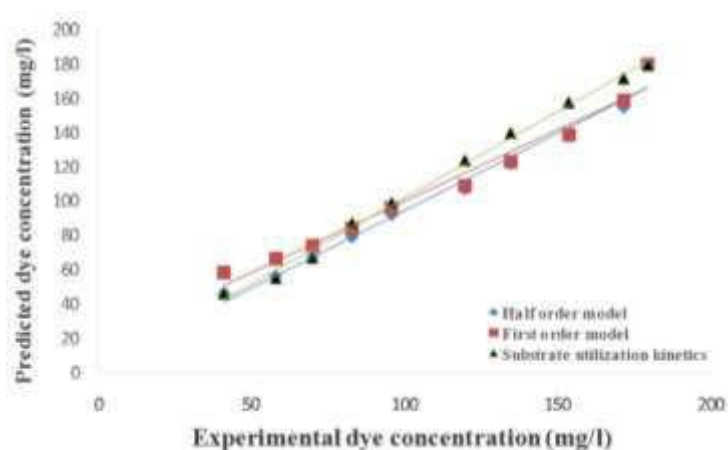


Figure 4. Predicted dye concentration Vs Experimental dye concentration for the decolourization of reactive red 251 using different kinetic models

CONCLUSION:

Treatment of effluents from textile industries poses a threat to the environment and bio treatment with white rot fungi *Trametes hirsuta* seems to be a very good option. Decolourization of reactive red 111 and reactive red 251 by suspended cells of *Trametes hirsuta* is studied in a batch reactor. The Batch decolourization kinetics of Reactive red 111 and Reactive red 251 by *Trametes hirsuta* are studied under optimum process conditions obtained from CCD using RSM and modeling is attempted using different kinetic models namely Logistic model for microbial growth and Substrate Utilization Kinetics, Half-and-first order kinetic models for dye decolourization kinetics are tested. Logistic models gives better prediction of biomass concentration with high R^2 values of 0.975 for reactive red 111 and 0.968 for

reactive red 251 are obtained. The simulation results from dye decolourization kinetics are useful to predict the dynamics of dye utilization and well suited for the decolourization of dyes studied. The half order model and substrate utilization kinetics are found to be the best fit when compared to the first order model for all the two dyes studied.

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**EFFECT OF *Aloe vera* L. AGAINST ISOLATED BIOFILM BACTERIA
FROM GINGIVAL INFLAMMATION**

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Abstract: Dental plaque is the primary etiology for chronic gingivitis, which typically develops within 10 to 21 days in the absence of plaque control. Approximately 50% of the population over the age of 30 has some form of gingivitis. Micro-organisms colonize a variety of surfaces of the human body, such as skin, the respiratory and digestive tracts and the oral cavity in the form of biofilms. Biofilms consisting of *Actinomyces* oris, *Candida albicans*, *Fusobacterium nucleatum*, *Streptococcus mutans* and *Veillo nelladispar*. The role of dental plaque at the interfaces of tooth causes the main basis of gingival inflammation, which could lead eventually to periodontitis. In the present study five different bacterial colonies were observed. Isolates were identified by cultural, morphological and biochemical characteristics. The isolated bacterial stains were identified such as *Bacillus subtilis*, *Streptococcus mitis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Key Words: gingival inflammation, biofilm, *Staphylococcus aureus*, *Aloe vera* L. Dental plaque, antibacterial activity.

INTRODUCTION:

Biofilms are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers. Many bacterial species form biofilms, and their study has revealed them to be complex and diverse. The structural and physiological complexity of biofilms has led to the idea that they are coordinated and cooperative groups, analogous to multicellular organisms [1].

LITERATURE REVIEW:

Researchers have estimated that 60-80 percent of microbial infections in the body are caused by bacteria growing as a biofilm – as opposed to planktonic (free-floating) bacteria. Some external biofilm, namely chronic wounds and dental plaque, can be manually removed. Because of their inaccessibility and heightened resistance to certain antibiotic combinations and dosages, internal biofilm are more difficult to eradicate.

MATERIALS:

Sample Collection

The gingival inflammation samples were collected from infected patients at Nannilam, Thiruvavur District, Tamilnadu. The samples were inoculated in Nutrient broth (HiMedia, India) and viable cells were enumerated.

Enumeration of Viable Cell Count

The overnight broth culture was serially diluted with autoclaved distilled water upto 10⁻⁶ dilution and 100 µl of each dilution was spread on to Nutrient agar (HiMedia) plates and incubated overnight at 37°C. After 12 -18 hours incubation the number of viable colonies were counted using total viable plate count method [2].

$$C.F.U/mL \text{ original sample} = \text{Number of colonies /plate} \times (1/mL \text{ aliquot plated}) \times \text{dilution factor}$$

METHOD:

Screening and Conformation of Biofilm Forming Bacteria Microtitre plate biofilm assay[3]

Isolated colonies with visually distinguishable morphologies were randomly selected and isolated by directly streaking on Nutrient agar plates and incubated for another 12-18 h. The isolated colonies were then restreaked after incubation onto nutrient agar plates to obtain pure cultures. The viability of the isolated cultures was checked in Lauria Bretani (LB) (HiMedia) broth and those found to be viable were screened for biofilm formation. The isolates were screened for their ability to form biofilm by microtitre plate method.

The cultures were screened in three different media for biofilm formation, viz, i.e, Nutrient broth, LB broth and Basal media (HiMedia, India) supplemented with glucose. Isolates from fresh agar plates were inoculated in

respective media and incubated for 18 h at 37°C in static condition. Individual wells of sterile, polystyrene, 96 well-plate bottom tissue culture plates (Tarson, Kolkata, India) wells were filled with 200µl aliquots of the diluted over night cultures (100 times diluted) and broth without culture was used as control. The plates were incubated for 24 h, 48 h and 72 h at 37°C to observe biofilm formation. After respective incubation period content of each well was gently removed by slightly tapping the plates. The wells were then washed with phosphate buffer saline (PBS pH 7.3) to remove free-floating 'planktonic' bacteria. The plates were then stained with 0.1% (w/v) crystal violet solution. Excess stain was washed off thoroughly with 95% ethanol and plates were kept for drying. Optical density (OD) of the wells was determined with a micro ELISA auto reader (Perkin Elmer) at wavelength of 570 nm[3,4]. These OD values were considered as an index of attachment to surface and forming biofilms. The experiment was performed in triplicate and repeated three times and the mean OD value was considered.

DISCUSSION:

ISOLATION OF BACTERIA

In the present investigation five different colonies was observed in nutrient agar medium. Total viable count was determined from selected plates having 30 to 300 colonies (Plate – I). The isolated total numbers of colony were present in Table -1. The isolated colonies were named as S1, S2, S3, S4 and S5. The results were showed in Plate - II.

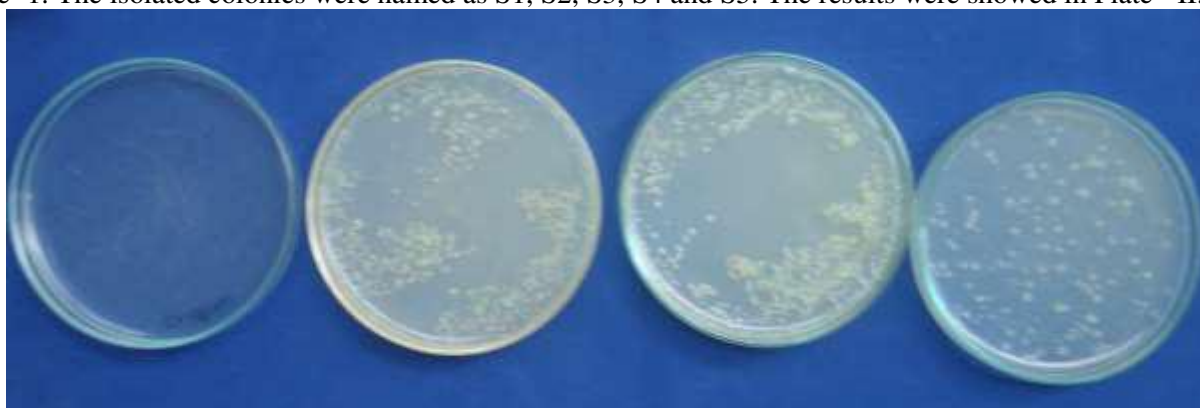


PLATE – I

ISOLATION OF BIOFILM FORMING BACTERIA

CONTROL

Dilution 10^{-5}

Dilution 10^{-6}

Dilution 10^{-7}

PLATE – II

A

B

C



D

E



PURE CULTURE OF ISOLATED BACTERIA

A- *Bacillus subtilis*, B- *Escherichia coli*, C- *Staphylococcus aureus*, D- *Streptococcus mitis* E & E-*Pseudomonas aeruginosa*

TABLE - 1
Isolation of Biofilm Bacteria from gingival inflammation sample

S. No.	Dilution Factor	No. of Colonies	CFU/ml
1	10 ⁵	268	2.68×10 ⁷
	10 ⁶	235	2.35×10 ⁸
	10 ⁷	180	1.8×10 ⁹

In the present study selected plates having 30 to 300 colonies. Five different bacterial colonies were observed. The isolated bacterial stains were identified such as *Bacillus subtilis*, *Streptococcus mitis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Similarly A report on the biological activity of bacterial symbionts of sponge *Pseudoceratina purpurea* shows that they actively inhibited the growth of fouling bacteria isolated from natural marine biofilm. The 3 active isolates belonged to genus *Bacillus* and 1 isolate belonged to *Virgibacillus* and were also found to show a broad spectrum biological activity against marine biofilm forming bacteria [5,6,7]. The higher number of biofilm forming bacteria inhibited by the extracts of active symbionts may indicate the chemical diversity of secondary metabolites produced by the bacterial symbionts. In addition, the fact that nonpolar bacterial extracts were found to be active, is a highly desirable in term of possible application of in the marine environment so that it won't be washed out easily [8,9,10]. The characteristic feature of biofilm forming bacteria is production of EPS. The biosynthesis of EPS is believed to serve many functions concerning promotion of the initial attachment of cells to solid surfaces, formation and maintenance of microcolony and mature biofilm structure and enhanced biofilm resistance to environmental stress and disinfectants. In some cases, EPS matrix also enables the bacteria to capture nutrients. The composition and structure of EPS is strongly determined by the amount of fermentable sugar present. The bacterial strains studied in the present project work show an interesting pattern of utilization of different energy sources influencing the pattern of film formation. The maximum film formation was observed in presence of glucose and this result is in agreement with earlier studies conducted on oral biofilm [11,12]. There was very poor biofilm formation in case of lactose which is in agreement with earlier studies [13,14]. The EPS characterization of four biofilm forming strains (*Staphylococcus aureus*) was done and the result indicated presence of a macromolecular complex constituting of carbohydrate and protein.

CONCLUSION:

Dental plaque is the primary etiology for chronic gingivitis, which typically develops within 10 to 21 days in the absence of plaque control. Approximately 50% of the population over the age of 30 has some form of gingivitis. Micro-organisms colonize a variety of surfaces of the human body, such as skin, the respiratory and digestive tracts and the oral cavity in the form of biofilms. Biofilms consisting of *Actinomyces*, *Candida albicans*, *Fusobacterium nucleatum*, *Streptococcus mutans* and *Veillonella dispar*. The role of dental plaque at the interfaces of tooth causes the main basis of gingival inflammation, which could lead eventually to periodontitis. In the present study five different bacterial colonies were observed. Isolates were identified by cultural, morphological and biochemical characteristics. The isolated bacterial stains were identified such as *Bacillus subtilis*, *Streptococcus mitis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

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Influence of Biosolarization in the Ecofriendly Cultivation of Cluster Onion

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Abstract: Onion is one of the most important bulb vegetable crops grown in India and around the globe. Organic vegetables fetch 20-30 % higher price value than the vegetables from conventional farming. Soil solarization involves covering moist soil with transparent polyethylene sheet during hot months for sufficient time to raise the soil temperature to the levels lethal for soil borne pests such as weeds, insects, disease pathogen, nematodes, etc. The experiment was laid out in a randomized block design with 14 treatments replicated thrice. The treatments include a combination of solarization for four weeks with three different amendments viz., Vermicompost, Farm Yard Manure (FYM), and neem cake along with non solarized control and solarization without amendment. At the end of treatment period, inoculation with Consortium Bio Fertilizers (CBF) was done for specific treatments. The results of the experiment revealed that the growth attributes of cluster onion were superior under treatment combination involving solarization for 4 weeks with vermicompost @ 5 t ha⁻¹, neem cake 1 t ha⁻¹, CBF 2 kg ha⁻¹ and foliar application of panchakavya @ 4 % sprayed 4 times. This was closely followed by the solarization with FYM @ 12.5 t ha⁻¹, neem cake @ 1 t ha⁻¹, CBF @ 2 kg ha⁻¹, foliar application of panchakavya @ 4 % sprayed 4 times.

Key Words: Biosolarization, cluster onion, growth attributes.

INTRODUCTION:

Onion is one of the most important bulb vegetable crops grown in India and around the globe. Organic vegetables fetch 20-30 % higher price value than the vegetables from conventional farming. Soil solarization involves covering moist soil with transparent polyethylene sheet during hot months for sufficient time to raise the soil temperature to the levels lethal for soil borne pests such as weeds, insects, disease pathogen, nematodes, etc. Artificial soil heating or soil solarization is the only non-chemical soil disinfestation method which has been tested on a large scale under farming conditions. Combining organic amendments with soil solarization is a developmental approach for the control of soil borne plant diseases (Jeffschalan, 2003). Hence an experiment was conducted to find out the biosolarization practices for augmenting the growth attributes of cluster onion by adopting organic practice.

LITERATURE REVIEW:

Higher soil temperatures may be obtained with dark-colored nature of organic amended soils since they absorb more solar radiation than light-colored soils. Although the major benefit of solarization is reduction of soil borne pathogens by soil-heating effects, there are many other possible additional beneficial effects that can result in an increased growth response (IGR) of plants. Such additional effects include control of weeds and insect pests and release of plant nutrients (Stapleton, 1997).

MATERIALS:

The experiment was laid out in a randomized block design with 14 treatments replicated thrice. This experiment was conducted by practicing solarization with various amendments and nutrient management through various bulky organic manures, concentrated oil cakes, biofertilizers and foliar organic nutrition.

METHOD:

The treatment included a combination of solarisation for four weeks with three different amendments viz.,

Vermicompost, Farm Yard Manure (FYM), and neem cake along with non solarized control and solarization without amendment. At the end of treatment period, inoculation with Consortium Bio Fertilizers (CBF) was done for specific treatments.

DISCUSSION:

Growth is a multidimensional web of many parameters. It is a phenotypic expression with respect to nutrient status, provided all other conditions are favourable. The plant height is the primary character which decides the vigour of the plant and in turn the dry matter production. Increase in the plant height before maturity contributes for manufacture of more carbohydrates to act as better physiological source. Any practice to alter the plant height would influence the yield ultimately. In the present study, the possible reason for the variations under different treatments in plant height might be due to differential amount of nutrients made available in the treated plots because of solarization and also differential level of uptake of nutrients, which reflected in differences in growth. Increased plant height might be due to increased uptake of nitrogen which is the constituent of protein and protoplasm and vigorously induced the vegetative development of the plants as reported by Flores *et al.* (2005). The increased plant height may also be due to the supplementation of organic nitrogen, phosphorus and potassium along with micro nutrients besides the growth promotory effect of vermicompost as suggested by Warner *et al.* (2004) in tomato. Application of vermicompost increases the activities of N fixing bacteria and the rate of humification.

Humic acid in vermicompost enhances the availability of both native and added micronutrients in the soil as reported by Sreenivas *et al.* (2000). Besides, application of organic manures would have helped in the plant metabolism through the supply of important micronutrients such as Zinc, iron, copper, manganese etc., in an optimum level, in the early growth phase which might have encouraged the vigorous growth. The favourable response in plant height obtained in the present study could be attributed to the catalytic action of vermicompost in the soil which might have improved the soil physical conditions facilitating better aeration leading to deeper penetration of roots and higher nutrient extraction. Regarding the highest number of tillers per plant in the same treatment, more availability and uptake of nutrients, particularly N, P, K, micronutrients, water and production of growth promoting substances through the integration of vermicompost and the ready availability of nutrients from the groundnut cake might have contributed for the highest number of branches. The findings are in consonance with Pandian (2005) in cashew. The next best result was obtained in the treatment which receiving FYM, Neem cake, CBF and panchakavya along with solarization. This might be due to the nutrient content of neem cake along with the assured effect of vermicompost in increasing the growth characters. In line with the findings of Subha Rao and Ravi Shankar (1998), stimulatory effect of neem products might have attributed to the improvement in growth characters. Beneficial effect of *Azospirillum* and Phosphobacteria combined with the growth promoting effect of panchakavya. Improved availability of nitrogen in the rhizosphere by the inoculation of *Azospirillum* facilitates better uptake of nitrogen. Also, pectinolytic activity of *Azospirillum* might be involved in the hydrolysis of middle lamellae of *Azospirillum* colonized cortical cells and subsequent acceleration of uptake of water and mineral viz., PO_4 and NH_4 (Okon and Kapulnik, 1986). Moreover the inoculation of *Azospirillum* enhanced root branching and root formation which might have resulted in the higher nutrient uptake.

According to Hayman (1975), phosphobacteria solubilise and increase the availability of organic phosphorus to the plants and its uptake by the way of production of organic acids. Another mechanism by which phosphobacteria augment the plant growth is due to the biosynthesis of growth promoting substances like vitamin B_{12} and auxin. The present finding also go in agreement with the earlier reports of more nutrient uptake by the crop due to the inoculation of *Azospirillum* and phosphobacteria. This is in confirmatory with Velmurugan (2002) in turmeric. Sundarraman *et al.* (2001) stated that panchakavya acts as a growth promoter and immunity booster. The stock solution of panchakavya creates a depression, which facilitates a cosmic ray link which is the basic element for refreshing the growth process. Besides, panchakavya also carries considerable amount of nitrogen which helps in protein synthesis essential for the formation of protoplasm leading to cell division and cell enlargement. This also would have stimulated plant growth. Further, nitrogen is an important component of amino acids and co-enzymes which have considerable biological importance. Beneficial and proven biofertilizers such as azotobacter, *Azospirillum* and phosphobacteria and plant protection substances like *Pseudomonas* and saprophytic yeasts detected in panchakavya can be attributed to its efficacy as organic foliar nutrient that might have in turn, stimulated the growth, resulting in increased plant height and number of branches as reported by Somasundaram *et al.* (2004).

The induction of early flower bud formation might have been influenced by triggering of such metabolic processes and narrowing of carbon: nitrogen ratio by the significant accumulation of carbohydrates. Furthermore, foliar spray of panchakavya facilitates greater uptake of nutrients which leads to the effective conversion of vegetative phase to flowering phase. The present findings on improved growth parameters of onion due to application of panchakavya are in line with Archana (2008) in bitter gourd.

The destruction of harmful micro organisms like the spores of fungi, bacteria, actinomycetes and nematodes by the production of bio toxic volatiles might have also helped in the better growth of plants. This might be attributed

to the reduction in the competition between the seedlings and the weeds that have been destroyed. This is supported by Stevens *et al.* (2003) in nursery vegetable crops. The better physiological status of the seedlings was also identified by the higher level of chlorophyll content and the number of leaves in the plants. While development of favourable environment facilitated the plants to absorb more nutrients, the elimination of unfavourable factors also counts a lot in the growth and development of crop plants. The major deleterious factors are weeds which compete significantly with the crop for mineral nutrients, water, soil, space, as well as photo energy from the sun. This is followed by parasitic plant pathogens as well as nematodes, which are of destructive nature. Elimination of such factors in nursery environment due to solarization is in accordance with Abdallah *et al.* (2004) .

ANALYSIS:

Table 1. Effect of solarization in bio amended soil on plant height(cm), Number of tillers , number of leaves, leaf area(cm²)and Chlorophyll content (mg g⁻¹) in cluster onion

Treatments	Plant height (cm)	Number of tillers	Number of leaves Plant ⁻¹	Leaf area (cm ²) at peak flowering	Chlorophyll content (mg g ⁻¹)
T ₁	38.51	1.93	10.61	117.61	0.27
T ₂	39.67	2.08	11.12	119.76	0.33
T ₃	40.84	2.23	11.62	121.90	0.38
T ₄	43.15	2.57	12.64	126.15	0.49
T ₅	42.00	2.41	12.12	124.02	0.44
T ₆	44.30	2.74	13.16	128.29	0.55
T ₇	45.46	2.89	13.67	130.43	0.60
T ₈	50.10	3.52	15.71	138.96	0.82
T ₉	46.62	3.05	14.19	132.56	0.66
T ₁₀	51.26	3.69	16.22	141.10	0.89
T ₁₁	47.77	3.20	14.69	134.69	0.71
T ₁₂	52.44	3.86	16.74	143.24	0.94
T ₁₃	48.93	3.37	15.20	136.83	0.76
T ₁₄	53.89	4.02	17.25	145.36	0.99
S.Ed.	0.58	0.08	0.25	1.06	0.03
CD(p=0.05)*	1.15	0.15	0.50	2.12	0.05

FINDINGS:

The results of the experiment revealed that the growth attributes of cluster onion were superior under treatment combination involving solarization for 4 weeks with vermicompost @ 5 t ha⁻¹, neem cake 1 t ha⁻¹, CBF 2 kg ha⁻¹ and foliar application of panchakavya @ 4 % sprayed 4 times. This was closely followed by the solarization with FYM @ 12.5 t ha⁻¹, neem cake @ 1 t ha⁻¹, CBF @ 2 kg ha⁻¹, foliar application of panchakavya @ 4 % sprayed 4 times(Table 1).

CONCLUSION:

It was concluded that the treatment combination of solarization for 4 weeks with vermicompost @ 5 t ha⁻¹, neem cake 1 t ha⁻¹, CBF 2 kg ha⁻¹ and foliar application of panchakavya @ 4 % sprayed 4 times showed the best performance in improving the growth attributes such as plant height and number of tillers, number of leaves, leaf area, chlorophyll content.

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Utilization of process residual yeast for product making

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Abstract: The fermentation process using yeast cells expels tonnes and tonnes of residual yeast. These residual yeasts would be the best starting material for the production of yeast extract. More number of cells present in the residue was in the inactive form and they have viability. They were broken down to produce the yeast extract which is mainly composed of the inner cell contents of yeast. Yeast extract have a variety of applications such as in bread baking, in the media preparations for both bacterial and fungal culture, liquid yeast extract used in the cosmetic products and so on. In this work, a simple protocol is designed where the expenditure of time and energy is less to produce the yeast extract and the produced yeast extract was compared qualitatively with the commercially available yeast extracts. Although the composition of yeast extract may vary when compared with original yeast extract, the industries should understand the bio-utility of yeast by reusing the process residual yeast to convert into various other products.

Key Words: Viable cells, Yeast extract, Inner cell contents, Bio-Utility, and Media.

INTRODUCTION:

Yeast is always single celled microorganisms which are classified under the fungus kingdom in the *Ascomycota* and *Basidiomycota* phylum. At present there are about 39 genera and more than 350 types of yeast are present (1). They have a different type of habitats in the nature. Yeast is mainly found on the soils and sugary environment mostly on the leaves, flowers, plant resins, etc. Yeast is commonly used in the production of bread, alcohols, non-alcoholic drinks, probiotics, antibiotics, yeast extract, and so on. The main application of yeast is in the field of fermentation where they convert the sugar molecules into alcoholic compounds under the absence of oxygen.

Spent yeast is the residual yeast found inside the fermenter after the fermentation process had finished (2). Most of the food product-based industries, antibiotic producing industries use tonnes and tonnes of yeast cells for the production process. Once the fermentation is completed, they are meant to be waste and are discarded to the compost yard. But the studies on the spent yeast have revealed that some of them are still viable and some in inactive form which have the capability to ferment strongly and also, they have high nutritional values (3). Spent yeast have about 7% ash, 6% fat, 32 % carbohydrate and a good amount of vitamin B, calcium, phosphorous, magnesium, iron and other micronutrients (2). Generally, this residual yeast from the fermenter is available cheaply in the markets. The discarding of spent yeast is not economically sensible and reuse of it to produce other products would be nice for industries (4). These residual yeasts can be converted into many types of products as nucleosidase, invertase, 1,3 β glucan etc. Residual yeast is one of the main sources of yeast extract. Yeast extracts from the spent or residual yeast from the fermenter acts as a nutrient rich raw material and is one of the most economically low-cost methods (5). Yeast extract is the cell components of yeast without their intact cell wall. The residual yeast is treated and washed in rotators so as to expel the leftover sugar. Yeast extract is created with no chemical additives and with the help of enzymes that divide the proteins in yeast into the taste-providing components. These are separated from the insoluble residual constituents of the cell and dried. The result of this method is yeast extract which is a mixture of various amino acids, carbohydrates, vitamins and minerals. The outcome is soppy, rich yeast mass. They are used as food additives, flavourings, and as a nutritional media for both bacterial and fungal cultures (5). Other than yeast residues, *Saccharomyces cerevisiae*, *Candida utilis* and *Kluyveromyces marxianus* can also be used for the production of yeast extract. The yeast extracts are available in different forms as liquid form, powder form, and fluid form (6). In this

work the process residual yeast was collected from the fermentation industry. They were pre-treated for purification and were converted to yeast extract using the process steps described below.

LITERATURE REVIEW:

Ok Kyung Lee *et al.*, (2013) highlights the residual biomass of *Dunaliella tertiolecta* after lipid extraction and used for bioethanol fermentation. Here pre-treatment procedure was not required for enzymatic saccharification of the residual biomass and finally the waste residual biomass generated during microalgal biodiesel production were used for the production of bioethanol (7). Hasan Tanguler et al 2008 utilized the spent yeast for the yeast extract production by incubating the cell suspension at different temperature for autolysis (9). Byung-Gon Rye *et al.*, (2013) studied about the waste spent yeast from brewery industry as a growth substrate to grow the yeast *Cryptococcus curvatus* for biodiesel production. Many types of yeast can be isolated and extracted by this method (10). Vieira F et al., (2016) developed a simple method where the number of required steps in the extraction process was decreased which speeds up the process compared to the other available tedious and time consuming methods (11)

MATERIALS:

The availability of solid residual yeast in the distillery bag and the complete understanding of the fermentation process are studied by visiting the Bannari Amman Distillery Limited, Erode, Tamilnadu, India. Later 5 litres of sample was collected and bought to the laboratory for experimentation. Chemicals such as Luria Bertani medium, Agar- Agar, Yeast extract and sodium hydroxide for the experiment were bought from HiMedia laboratories private limited, Mumbai.

METHOD:

CONFIRMATION OF THE PRESENCE OF YEAST:

The presence of yeast cells in the sample was confirmed by the microscopic view of the sample. Initially the sample was diluted with the distilled water of 1:10 dilution ratio. It was then simple stained with the safranin dye and it was analysed through the optical microscope at 40X magnification.

PURIFICATION OF THE SAMPLE:

The solid yeast residue was cleaned using 0.9% saline for about ten times to remove all the wort components. For each purification process the sample was diluted with water and saline and was centrifuged for about 10 minutes at 2500 rpm in 500 ml centrifuge tube. After centrifugation the supernatant was discarded and the pellet contains the yeast cells which was again resuspended in saline for about 2 to 3 minutes and the process was repeated for about ten times. Finally, after the purification process the presence of yeast in the residue was again confirmed by the staining and microscopic view of the sample.

VIABILITY AND NON - VIABILITY FRACTION:

The Viable and non-viable cells present in the process residual yeast is determined using the haemocytometer. The sample yeast after purification was diluted in the ratio 1:10 with the distilled water. A drop of sample is kept on the haemocytometer and then a drop of methylene blue dye was added to it. It was then examined under the optical microscope. The percentage of viable cells and the non-viable cells were determined by the following formula,

$$\text{VIABILITY \%} = \frac{\text{No. of live cells}}{\text{Total no. of cells}} \times 100$$

CELL DISRUPTION:

The purified sample was then subjected to thermal cell degradation to get the starting material for yeast extract production. The sample was autoclaved at 121°C for 15 minutes followed by rapid cooling in an ice bath. It was then centrifuged at 3000 rpm for 10 minutes. The pellet was discarded and the supernatant was subjected to the above-mentioned procedure for two times. Finally, the collected supernatant was stored at -20°C.

FILTRATION AND CONCENTRATION:

The supernatant obtained from the centrifugation process was filtered using the 0.22µm micro filter paper to remove the unwanted biological substances. The filtrate was then concentrated by the evaporation process. Then it was made into powder form by drying process. The concentrated filtrate was then kept in the hot air oven at the temperature 120°C for about 2 hours. The resultant paste is spread over the petri plate and dried using the hot air oven. After the drying process the dried product was scraped off from the petri plates using spatula and ground into fine powder using mortar and pestle.

MICROBIAL GROWTH ASSESSMENT:

The growth of microbes such as bacteria (both gram positive and gram negative), yeast and fungi was examined in order to assess the quality of the produced yeast extract in both solid and liquid media. For bacteria, both

gram positive (*Bacillus subtilis*) and gram negative (*E.coli DH5a*) species, the growth pattern was observed and compared with both produced and commercially available yeast extract (Hi-Media). In first approach the bacteria was grown only with the yeast extract. For this Liquid medium, 20g of yeast extract was added in 1000ml of deionized water and deionized water was the negative control whereas for solid medium, 8g of yeast extract and 15g of agar was dissolved in 1000ml of distilled water. In second approach the bacteria was grown in the combination of yeast extract and Luria Bertani (LB) medium. For this 5g of yeast extract and 25g of LB medium was added along with 1000ml of deionized water. Similarly, the growth curve was analysed for a yeast culture (*Saccharomyces cerevisiae*). For liquid media, 15g yeast extract was dissolved in 1000ml of distilled water and the distilled water was taken as negative control. For solid medium 8g of yeast extract and 15g of agar was added with 1000ml of distilled water. For all the solid media, agar is kept as control. The growth pattern was analysed by taking OD at 600nm in UV-spectrophotometer (Systronics 2202) for every 2-hour interval till the culture reaches the death phase. For fungi, *Trichoderma spp* was used to determine the quality of Produced yeast extract. 2% of Potato starch and 1% Yeast extract and 2% agar were used to analyse the growth pattern in the solid medium.

RESULTS AND DISCUSSION:

COLLECTION OF YEAST SAMPLE:

Fig 1 represents the sandal yellowish raw yeast residual sample collected from the industry. The presence of yeast in the sample was confirmed by staining the sample and analysing it through the optical microscope. Fig 2 indicates the microscopic view of yeast cells present in the collected sample.



Figure 1. Residual yeast sample

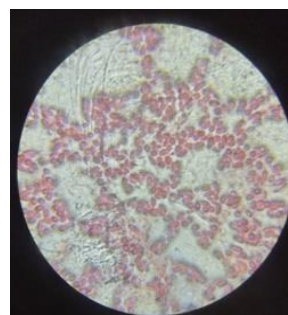


Figure 2. Microscopic view of yeast cell present in the sample

PURIFICATION OF THE SAMPLE:

The purification or the pre-treatment was mainly done to remove all the wort components and the bitter odour present in the sample. The residual yeast sample may also contain some salts in the form of crystals. The 0.9% saline converts the salt into oxide form and it is removed. During centrifugation, the salts get dissolved in the supernatant and the cell mass got deposited at the bottom in the form of pellet. By repeating the procedure, the bitter odour of the residual yeast was also removed. The presence of yeast in the pellet after purification process was again confirmed by staining the pellet and analysing through the optical microscope. Fig 3: signifies the microscopic view of the yeast cells present after the centrifugation.

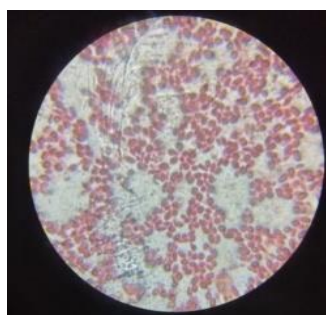


Figure 3. Microscopic view of yeast cell after purification

FRACTION OF VIABLE AND NON-VIABLE CELLS:

The viability of the cells was determined by using methylene blue dye. The cell membrane of dead yeast cells is permeable to the methylene blue dye whereas, the live cells not. By viewing the sample in the haemocytometer

through the microscope, the live cells appeared in white colour and the dead cells appeared as dark blue colour. The number of live cells observed on a single square was 2 and dead cells were 3. The cell density was calculated as 200,000 cells /ml and the viability percentage was about 36.36%.

No of live cells: 4

No of dead cells: 7

No of squares counted: 4 (corner big squares)

Initial volume: 0.1 μ l

Dilution factor: 20

Cell density: 200,000 cells/ml

Viability: 36.36 %

Non-Viability: 63.64 %

CELL DISRUPTION:

1 litre of purified sample was then allowed for the thermal cell disruption at different temperatures. This temperature fluctuation causes the cell wall to break and release the inner cell contents such as proteins, cell organelles, etc. The centrifugation allows the cell debris to settled down which could be removed easily and the cell contents were collected which were present in the supernatant. For 1 litre of sample, 500 mL of supernatant was collected after the cell disruption.

FILTRATION AND CONCENTRATION:

The supernatant was filtered through the microfilter paper to remove the wastes. The produced product is known as yeast extract. It was then evaporated and concentrated by placing it in the hot air oven. The 500 mL of supernatant is concentrated to 300 mL of yeast extract paste. The liquid supernatant is concentrated into paste form. Fig 4 represents the picture of yeast extract in the paste form. The final produced yeast extract was then stored at -20° C for further use.



Figure 4. Yeast extract in the paste form.

MICROBIAL GROWTH ASSESSMENT:

BACTERIAL GROWTH ASSESSMENT:

E. coli DH5 α and *Bacillus subtilis* as gram negative and gram-positive bacterial types respectively were used to systematically analyse the bacterial growth pattern in both solid and liquid media formulated by the produced yeast extract. The values showed that both the bacteria can grow in the solid and liquid medium containing the produced yeast extract as the sole source of nutritional support as well as the in the combination with the Luria Bertani medium. Figure 5a and 5b represents the growth of *E. coli DH5 α* and *Bacillus subtilis* on the petri plates respectively containing only yeast extract and agar. In control plates no colonies were detected (Figure 5c). Figure 6a and 6b represents the growth of *E. coli DH5 α* and *Bacillus subtilis* respectively in the liquid broth containing only yeast extract. The results show that both the bacteria can grow in the produced yeast extract as a sole source of energy. The growth pattern was compared with the commercially available yeast extract. The graphs show that the produced yeast extract solely can be used as medium for the growth of both gram-positive and gram-negative bacteria. The results of the growth pattern observed with the produced yeast extract determined along with the combination of Luria Bertani medium shows that both the bacteria can grow efficiently and there is no significant difference in the growth curve of LB medium with commercial yeast extract and LB medium with produced yeast extract. Figure 7a and 7b gives the growth curve of *E. coli DH5 α* and *Bacillus subtilis* in both commercial yeast extract and the produced yeast extract along with the LB medium. The graph shows that the produced yeast extract is also capable to act as a nutritional medium for the growth of both gram positive and gram negative bacteria.



Figure 5a.

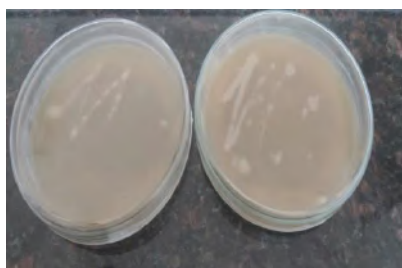


Figure 5b.



Figure 5c

Fig 5a and 5b represents the growth of *E. coli DH5α* and *Bacillus subtilis* on the petri plates respectively containing only yeast extract and agar. Fig 5c represents the control (agar only)

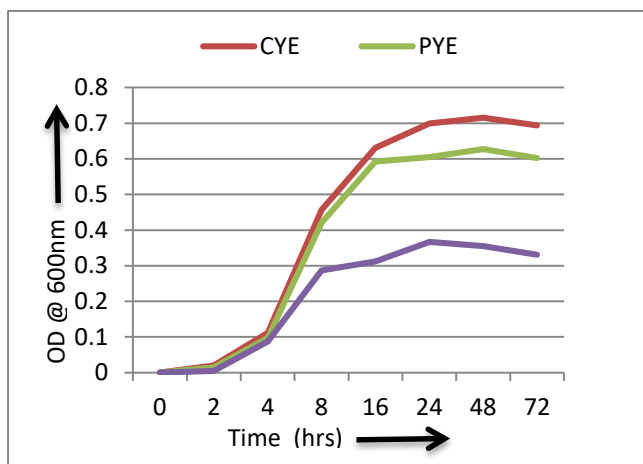


Figure 6a

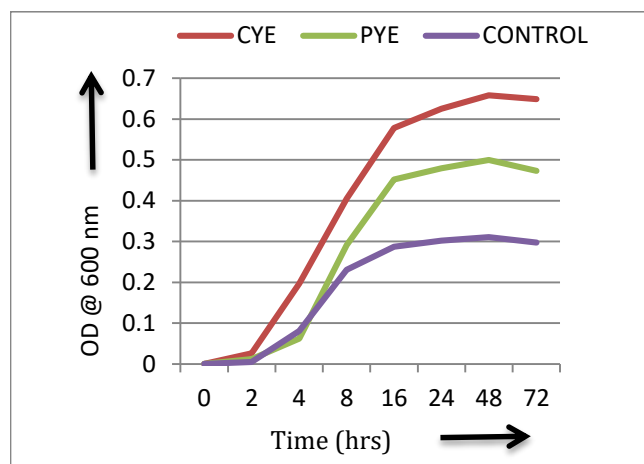


Figure 6b

Figure 6a: Growth of *E. coli DH5α* in the liquid broth containing only yeast extract

Figure 6b: Growth of *Bacillus subtilis* in the liquid broth containing only yeast extract.

CYE – Commercial Yeast extract; PYE – Produced Yeast Extract; Control – Deionized water

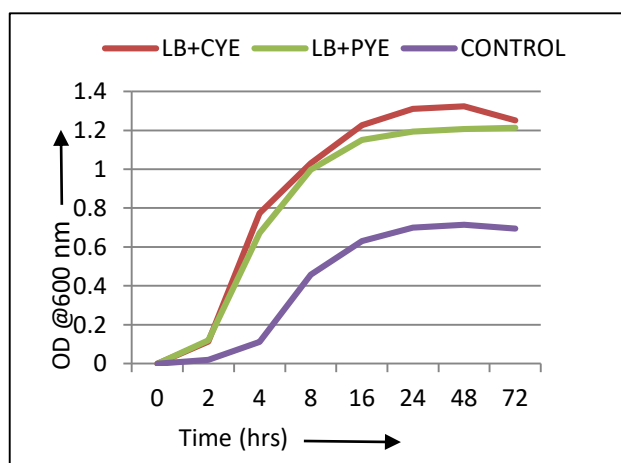


Figure 7a

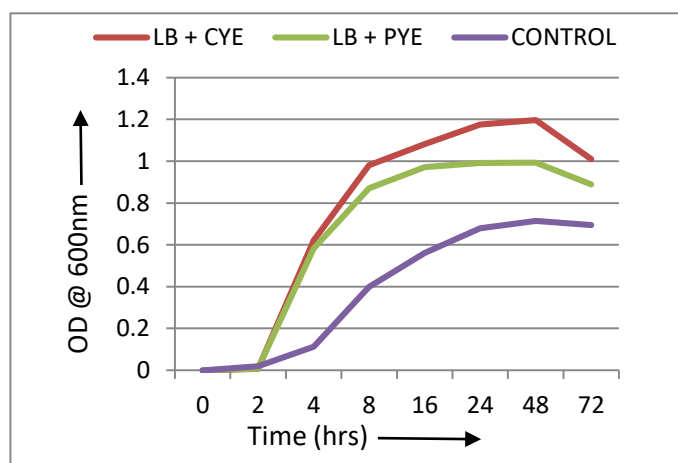


Figure 7b

Figure 7a: Growth curve of *E. coli DH5α* in both commercial yeast extract and the produced yeast extract

Figure 7b: Growth curve of *Bacillus subtilis* in both commercial yeast extract and the produced yeast extract

CYE – Commercial Yeast extract; PYE – Produced Yeast Extract; Control – Deionized water

YEAST GROWTH ASSESSMENT:

The growth of yeast cell in the produced yeast extract was supported by the *Saccharomyces cerevisiae* species in both liquid and solid media containing only yeast extract. Figure 8a represents the growth *Saccharomyces*

cerevisiae on the petri plates containing only yeast extract and agar. In liquid broth containing only yeast extract, the *Saccharomyces cerevisiae* had grown well and is in accordance with the growth pattern produced by the commercial yeast extracts. Figure 9 gives the comparison chart of the growth curve of the *Saccharomyces cerevisiae* in the liquid medium containing commercial yeast extract and the produced yeast extract. The outcomes from both the liquid and solid media had concluded that the produced yeast extract can be used as a nutrient medium for the growth of yeast cells.



Figure 8a

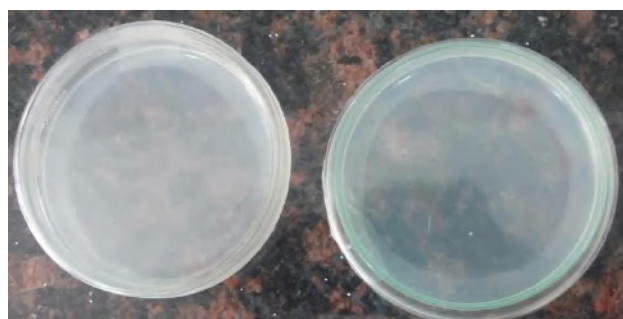


Figure 8b

Figure 8a: Growth of *Saccharomyces cerevisiae* on the petri plates containing only yeast extract and agar.
Figure 8b: Control which contains only Agar.

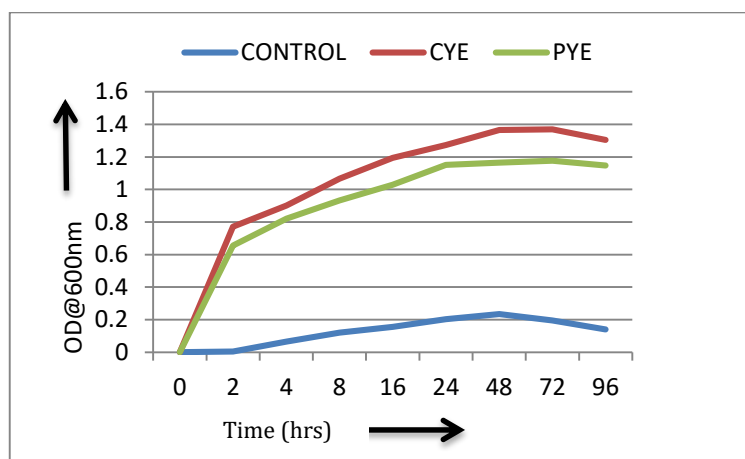


Figure 9

Figure 9: Comparison chart of the growth curve of the *Saccharomyces cerevisiae* in the liquid medium containing commercial yeast extract and the produced yeast extract.

CYE – Commercial Yeast extract; PYE – Produced Yeast Extract; Control – Deionized water

FUNGAL GROWTH ASSESSMENT:

The yeast extract can also support to serve as a component of nitrogen source in nutritional medium for the growth of fungi. *Trichoderma spp* was used for the determination of the quality of the produced yeast extract for the growth of fungal cultures. Figure 10a represents the growth *Trichoderma spp* on the petri plates having yeast extract along with the potato starch. The complete growth of *Trichoderma spp* takes about five days. The results showed that the produced Yeast extract can be able to serve as a nitrogen source along with the potato starch as a carbon source. So the produced yeast extract can be also be used for the fungal culture in solid medium.



Figure 10a



Figure 10b

Figure 10a: Growth of *Trichoderma spp* on the petri plates containing Potato starch , yeast extract and agar.

Figure 10b: Control which contains only Agar.

Fermentation is almost used in all the food product and Pharmaceutical industries. Fermentation process using yeast cells expels a huge amount of residual yeast which has a lot product value. The developed protocol for the production of yeast extract is very simple and the growth results also shows that the produced yeast extract is capable to use as a nutritional medium for the growth of various microbes. The advantage of this protocol is that there is no use of chemicals or enzymes in the production process.

CONCLUSION:

This study shows the conversion of residual processed yeast in the fermenter into a usable yeast extract which can be used in the nutritional media for bacterial and fungal growth. Thus we had developed a simple, cost efficient and time saving quick process for the production of yeast extract from the residual yeast. The scale up of this process should be considered by the industries for the effective way of reusing the residual yeast.

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DEGRADATION OF PLASTIC WASTES BY MICROBES

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Abstract: Plastic wastes degradation creates various adverse effects on humans, animals, plants and environment. Among the available degradation methods of plastic wastes, microbial degradation is the cheapest and environmental friendly method. This study deals with the isolation characterization and identification of the plastic degrading bacteria and also study the degradation of plastic using individual bacterial isolates and by the consortium of bacterial isolates. Out of 20 isolates obtained from soil and water sources, four of the bacterial isolates namely *Bacillus cereus*, *Serratia marcescens*, *Pseudomonas auruginosa* and *streptomyces* sp were selected based on the plastic degrading ability. The effect of pH, temperature, inoculum concentration, agitation speed and initial substrate concentration on the reduction of organic matters were found. The optimum conditions for biodegradation were found to be pH – 7, temperature - 36°C, inoculums concentration - 2% and agitation speed - 150rpm. The degradation efficiencies were found to be 7.4%, 18.73%, 15.38% and 16.58% for *Bacillus cereus*, *Serratia marcescens*, *Pseudomonas auruginosa* and *streptomyces* sp respectively. Consortium of the bacteria showed 48.75% degradation efficiency. This study also revealed that the microbial degradation by these bacterial strains were eco-friendly and sustainable approach for the degradation of plastics.

Key Words: Plastic degradation, microbial degradation, eco-friendly and sustainable.

INTRODUCTION:

Polymer material that can be moulded in to different shapes by heating is called as a plastic. It is made by carbon, hydrogen, silicon, oxygen. Chloride and nitrogen by chemical bonds for the linking of monomers. Generally natural gas, coal and oil are used for the extraction of plastic material (1). Higher tensile strength, less weight and its ability to water and microbial attack plastics replaced paper and other cellulose-based products. LDPE, MDPE, LLDPE, PP, PS and PVC are the common plastic materials used. Higher molecular weight, complex three-dimensional structure and hydrophobic in nature make plastic material recalcitrant in nature that hampers its availability to microorganisms (2).

Every year around 0.15 tons of plastics are produced, with a growth rate of 12% per annum (3) which causes the accumulation of plastic waste considered as a serious environmental danger (4) of about 25 million tons/year (5). Plastic takes 1000 years for efficient decomposition. The various health hazards such as lung disease and cancer are produced by global warming, problem of waste disposal and CO₂ release and dioxins due to the burning of plastics (6). Land filling, incineration and recycling are found to be inefficient in plastic waste management, and hence the use of microbes for the efficient degradation of recalcitrant plastic is of growing concern (7). Parafin consumption by microbes as a carbon source created a curiosity among the researchers for the degradation of plastics.

LITERATURE REVIEW:

Biodegradation is the complex process involving decomposition or destruction of contaminants such as organic and inorganic materials by microbes (8). Generally, either aerobic biodegradation in which organic compounds are simplified in to carbon dioxide and water or anaerobic biodegradation in which carbon dioxide, water and methane are produced. The biodegradation of organic compounds either into carbon dioxide or water in the presence of oxygen (aerobic biodegradation) or in the absence of oxygen (anaerobic biodegradation) by living organisms, includes the catalytic activity of microbial enzymes. In this context, different organisms are needed some of which are capable of degrading long polymers into their simple form and some are capable of utilizing simple monomers subsequently releasing simple waste products while others have the ability to deteriorate the simple excreta.

Rhizosphere soil of mangroves, polythene buried in the soil, marine water, plastic and soil at the dumping sites are the rich source of plastic degrading microbes (9). Low density polythene are degraded by *Mucor circinilloides* and *Aspergillus flavus* isolated from municipal landfill area, showed by analyzing CO₂ evolution test, scanning electron microscopy (SEM) and colonization studies (10). LDPEs biodegradation are highly implicated by *Pseudomonas* species with 17.8 % weight reduction. Degradation of polythene sheets with *Aspergillus niger* shows the highest weight reduction of 4, 32%. The research work on microbial degradation of plastics begin since 1970. Although aliphatic polyesters are amenable for degradation by microorganisms available in an ecological system, there are several plastics which strongly opposes the microbial attack. This type of behavior is exhibited by almost all conventional plastics like PE, PP, PS and PVC etc.. Several microorganisms including bacteria, actinomycetes, and fungi have the striking potential of biodegradation of plastics under changing biotic and abiotic factors. Up to this time, several bacterial and fungal strains have been identified. Recently a new fungal species have been identified which feeds on plastics and is ubiquitous in the soil. The fungus named *Aspergillus tubingensis* breaks down large polymers by releasing certain enzymes. A new discovery related to the natural evolution of a bacterial species capable of breaking down the bonds between the polymers of PET (polyester) was reported in 2016. Later, in 2018, while studying the enzyme structure of *Ideonella sakaiensis* 201-F6, scientists found that they had unintentionally transformed the enzyme having capability of degrading PET plastics in few days rather than taking centuries to break it down. Moreover, mutant enzyme (PETase) also evolved with the ability to break down polyethylene-2,5furandicarboxylate (PEF, an alternative form of PET).

Some fungi including *Fusarium oxysporum* and *F. solani* were studied previously for their ability to eat up PET but bacteria are relatively easier to exploit on large scale level. A study conducted in 2013 showed the isolation of new bacterial species *Roslateles depolymerans* strain TB-87 with the potential of degrading several biodegradable aliphatic polyesters including PBS, PES, PCL with the exception of PLA and poly (3hydroxybutyrate-co-hydroxyvalerate (PHBV)). Another study conducted by Urbanek et al. isolated 113 bacterial and 8 fungal species from Arctic environment which could degrade poly (butylene succinate-co-adipate) (PBSA), PBS, PCL or PLA. *Pseudomonas* sp. and *Rhodococcus* sp. were found to have the highest degradation ability. The study further reported *Colonostachys roseas* as efficient degrader causing 100% deterioration of starch films within 16 days and 52.91% degradation of PCL film within 30 days at 28°C (11).

The microorganisms involved in biodegradation process differ from each other in their mode of action and their optimal growth conditions in soil depending upon their properties (12). Heterotrophic microorganisms can effectively colonize plastics as their substrate (12). Moreover, different factors such as polymer properties, the nature of organisms and pretreatment methods generally act upon the biodegradation of polymers. Properties of polymers like ductility and friability, molecular weight, melting temperature, glass transition temperature, modulus of elasticity, nature of functional groups and substituents attached, and type of additives added affect polymeric biodegradation.

MATERIALS:

The plastic waste materials were collected from the garbage store and sample of soil were collected from the local area of Chidambaram. The soil sample was kept at refrigerator at 5°C.

Enrichment, Isolation and Purification of Plastic Degrading Bacteria

The collected soil sample was diluted with 99 ml of sterile saline and the sample was diluted by serial dilution method. An aliquot of about 0.1 ml of each dilution was inoculated separately in to nutrient agar media which was used for the enrichment of plastic degrading bacteria. The incubation of the media was carried out for 37°C for 36 hours. The clear vision indicates the degradation ability of the microbes. The positive isolates were sub cultured on Nutrient agar medium for purification, after purification these isolates were maintained in separate slants at 4°C respectively.

Plastic degrading bacteria characterization and identification

Morphological, physiological and biochemical characterization studies were carried out by Bergey's manual of Determinative Bacteriology for the characterization and identification of plastic degrading bacteria.

METHOD:

Microbial degradation of Plastics

Strips of 3 X 3 cm size were weighed and transferred in to the conical flask containing 50 ml of nutrient broth (NB) medium and inoculated with culture suspension (0.1 ml with cell density 1×10^7 cells/ml). The conical flasks containing 50 ml of nutrient broth with plastic cups without inoculation were served as control.

The consortium was also prepared to check for the plastic degrading ability (0.1 ml/of each culture suspension). All the flasks were kept on a rotary shaker at 37°C for 30 days. the rectangular plastic cup strips were collected after 5, 10, 15, 20, 25 and 30 days respectively, washed thoroughly using distill water, shade dried and then weighed for final weight and percentage weight loss were calculated using the following formula

$$\text{Weight loss (\%)} = \left\{ \frac{\text{Initial weight (mg)} - \text{Final weight (mg)}}{\text{Initial weight (mg)}} \right\} \times 100.$$

DISCUSSION:

Effect of pH:

pH is one of the important factor in the degradation of plastics by microbes. Batch studies were conducted to find out the optimum pH, in the range of 6 – 9 by keeping the sludge composition in the range of 2 % (v/v).

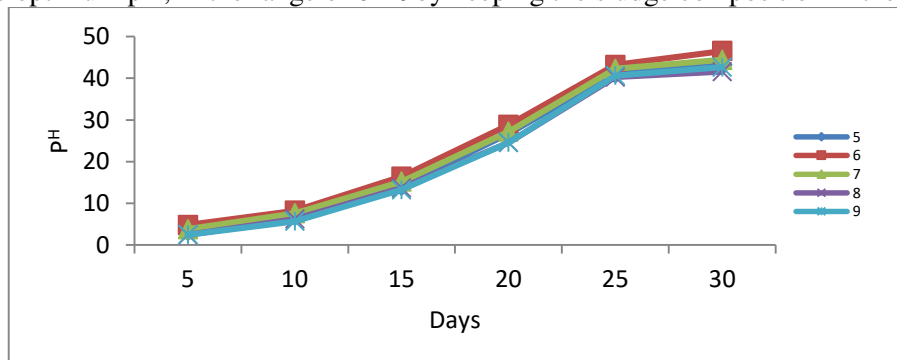


Figure 1. The effect of pH on the degradation of plastics using mixed microbes

Fig. 1 shows the effect of pH on the degradation of plastics using mixed microbes. In the acidic conditions, the plastic degradation was found to be low. From the figure, pH of 7 was found to be optimum for the maximum plastic degradation.

Effect of Temperature:

In general, the rate of biochemical reactions and of substrate transfer process increases with temperature. However, the solubility of oxygen decreases as temperature increase, resulting in poor biodegradation conditions for aerobic microbes. Thus, increase in temperature generates two reciprocal effects on biochemical reactions. Experiments were conducted at different temperatures viz, 30°C, 32°C, 34°C, 36°C and 38°C at a pH of 7.

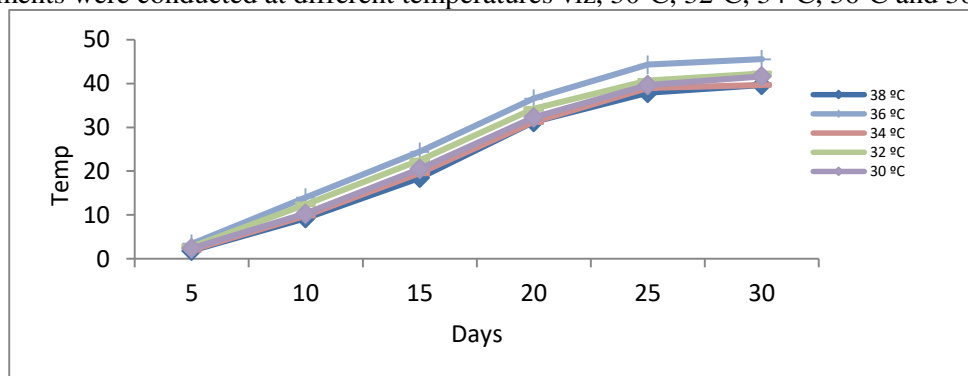


Figure 2. Effect of temperature on the degradation of plastics using mixed microbes

From Fig. 2, it was observed that a temperature of 36°C was found to be optimum for the maximum degradation of plastic materials and hence it was maintained for further studies.

Effect of Inoculum's Concentration:

Inoculum's concentration was varied from 1% - 5 % (v/v) for plastic degradation, keeping pH (7) and temperature (36°C) constant.

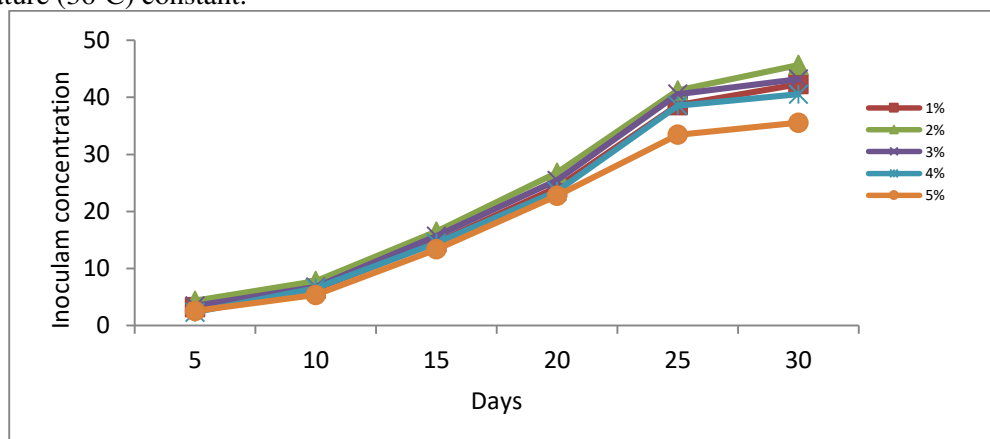


Figure 3. The effect of inoculum's concentration on the degradation of plastics using mixed microbes

From the results it was found that 2 % inoculum's concentration was optimum and hence it was maintained for further studies (Fig.3)

Effect of Agitation Speed:

Optimization of agitation speed for the plastic degradation was carried out at different speeds viz., 100 rpm, 125 rpm, 150 rpm, 175 rpm and 200 rpm. This difference in agitation speed has significant variation in plastic degradation.

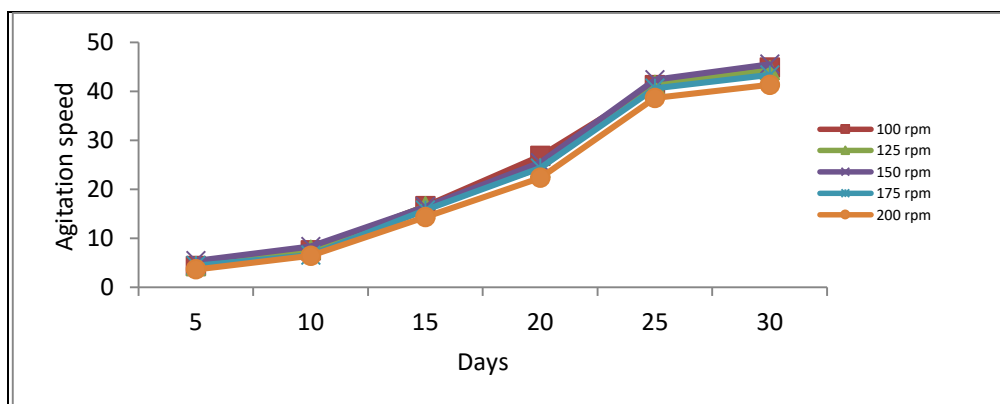


Figure 4. The effect of agitation speed on the degradation of plastics using mixed microbes

These mixed consortia have maximum plastic degradation of 45.54% at 150 rpm and further increase in agitation rate caused observable fall in plastic degradation. This is clearly shown in Fig.4. This could be attributed to the effect of shear rate on the cell wall, resulting in cell damage.

RESULT:

Microbes play an important role for the decomposition of the plastics biologically. The degradation leads to breaking down of polymers to monomers creating an ease of accumulation by the microbial cells for further degradation. Among these microbes *Streptomyces* sp showed highest degradation efficiency of 21%, followed by *Pseudomonas aeruginosa*, *Bacillus cereus* and *Serratia marcescens* showed the degradation efficiency of 20%, 18% and 17% respectively. Consortium of the bacteria showed 76% of plastic degradation after 30 days of degradation studies. According to this study, *Streptomyces* sp showed high plastic degrading potential of 21%. There is a report where *Streptomyces* sp. was able to degrade the plastic by 9.16% and polythene by 21% within one month incubation anaerobically. Various reports are available on *Pseudomonas* and *Bacillus* sp. on indigenous beach sand bacteria for plastic degradation for 16 weeks incubation period which increased with increase in time⁷. Study has been done where *Pseudomonas* sp. showed high degrading capacity with 42.5 % which was incubated for the period of 40 days.

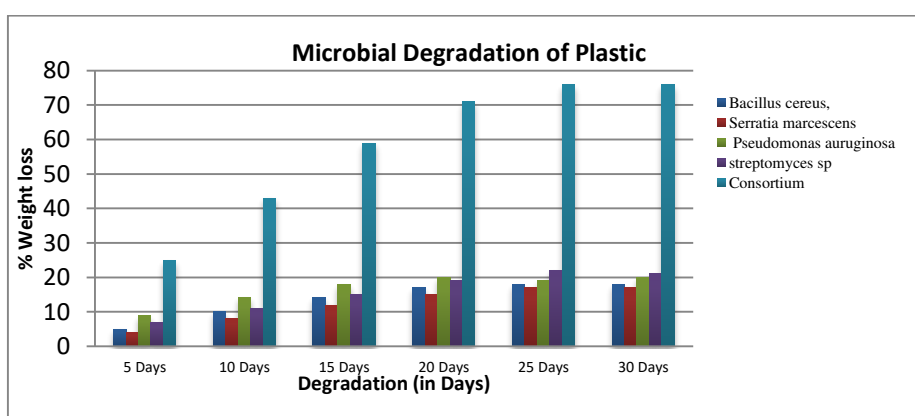


Figure 5. Degradation of plastics using mixed microbes

Determination of polymer reduction rate:

First order kinetic model was used to determine the rate constant of plastic reduction based on the initial and final weights along the specific intervals.

Equation is as follows

$$K = - \frac{1}{t} \left\{ \ln \frac{W}{W_0} \right\}$$

K = First order rate constant for plastic uptake per day.

t = time in days

W = weight of residual plastic material (g)

W_0 = Initial weight of plastic material (g)

Since this model gives a constant fraction per unit time present/removed within the plastics, it was adopted for the study. The Kinetic parameters are shown in the table 1.

Bacillus cereus			Serratia marcescens			Pseudomonas auruginosa			streptomyces sp			Consortium		
a	b	R ²	a	b	R ²	a	b	R ²	a	b	R ²	a	b	R ²
5.45	0.07	0.90	18.79	0.04	0.97	9.50	0.05	0.95	11.52	0.05	0.95	9.85	0.02	0.93
6.08	0.01	0.92	15.12	0.04	0.94	9.54	0.05	0.96	11.26	0.05	0.96	8.21	0.02	0.93
5.67	0.07	0.95	11.31	0.05	0.96	9.53	0.05	0.95	11.75	0.04	0.95	11.14	0.02	0.92
8.07	0.05	0.91	15.2	0.04	0.99	9.94	0.05	0.98	11.26	0.05	0.95	8.99	0.03	0.95
9.68	0.05	0.95	11.88	0.04	0.96	9.87	0.05	0.97	11.1	0.05	0.95	4.78	0.02	0.90
5.45	0.07	0.90	18.79	0.04	0.97	9.50	0.05	0.95	11.52	0.05	0.95	9.85	0.02	0.93

Table1. The Kinetic parameters of first order Model

RECOMMENDATIONS:

Biodegradable materials in certain industrial applications needs to be improved followed by the development of optimized industrial degradation facilities and littering control strategies in order to ensure environmental safety and sustainability.

CONCLUSION:

The demand for plastics is an ever increasing trend. The need of the time is to use bio based biodegradable plastics to maintain the health of environment. For the disposal of plastics, biodegradation was an effective method. Various microbial strains have been detected that convert plastic polymers to monomers. Exploitation of microbes for degradation of plastics was eco-friendly method. Various properties of plastics affect its rate of biological degradation. The weak forces (hydrogen bond), the covalent forces, affect the physical and chemical properties of plastics and thus affect the rate of degradation.

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Anti diabetic Activity of Halodule Uninervis in the Coastal of Mandapam

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Abstract: Diabetes mellitus is a global health problem and the incidence of DM is increasing at alarming rate all over the world. It is a chronic degenerative metabolic disease with high morbidity and mortality rates caused by its complications. In recent years, there has been a growing interest in looking for new bioactive compounds to treat this disease, including metabolites of marine origin. Several aquatic organisms have been screened to evaluate their possible anti-diabetes activities, such as bacteria, microalgae, macroalgae, sea grasses, sponges, corals, sea anemones, fish, salmon skin, a shark fusion protein as well as fish and shellfish wastes. Both in vitro and in vivo screenings have been used to test anti-hyperglycemic and anti-diabetic activities of marine organisms. So the researchers are done alot of experiments which are abundant to human consumption for beneficial of health and also which is cheapest in nature. So that recent studies shows sea grass act as a neutraceutical in nature to treat some disease conditions. The present study reveals Sea grass is high in protein, fibre, iron, zinc, magnesium, potassium, vitamin C and low in calories and less in colony forming unit. So that results shows Sea grass act as a anti oxidant, anti bacterial and anti inflammatory in nature. These properties helpful in to treat diabetes for controlling blood sugar levels. Not only DM it will be treat the chronic diseases like cancer and skin diseases mainly leprosy. And also good for muscle pains, wounds, fevers (malaria), stomach problems and rheumatism.

Key words: Sea grass, Halodule uninervis, Anti diabetic activity.

INTRODUCTION:

The population of the world will be increasing day by day. At the same time they are suffering with non communicable diseases especially Diabetes mellitus which is related to life style practices and faulty dietary habits. Diabetes is a chronic metabolic disorder, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. It leads to hyperglycaemia. If left untreated, can cause many complications include diabetic ketoacidosis, cardiovascular diseases, stroke, chronic kidney disease, foot ulcers and retinopathy leads to high mortality and morbidity rates. The average age at onset of diabetes seen in middle or elderly people. But according to WHO, 8.7 percent of population in the age group of 20 and 70 years suffering with diabetes. Currently, one in every four people are suffering with adult-onset of diabetes (type-2) under the age group of 25 years and 2.5 percent in the urban and 1.8 percent in the rural population above the age of 15 years, according to ICMR. India currently represents 49 percent of the world is diabetes burden, with an estimated 72 million cases in 2017, and almost double to 134 million by 2025⁶. The most commonly diabetes is Type-2 Diabetes mellitus (NIDDM), which primarily occurs and seen 90 percent of cases other than 10 percent of Type-1 Diabetes mellitus (IDDM) and Gestational diabetes mellitus. Obesity and overweight are the most important risk factors responsible for diabetes compare to other factors. Type 2 diabetes has increased markedly since 1960 in parallel with obese. During 2017, we were conducted a study on, Obesity is a predisposing factor of diabetes mellitus, the results shows majority of the subjects (88%) are obese because of their sedentary lifestyles⁵.

Keeping this in view, the researchers had taken alot of experiments which are abundant to human consumption, which are inturn to control the blood sugar levels. In recent years, there has been as growing interest in looking for new bioactive compounds to treat this disease, including metabolites of marine origin. Several aquatic organisms have been screened to evaluate their possible anti-diabetes activities, such as bacteria, microalgae, macroalgae, sea grasses, sponges, corals, sea anemones, fish, salmon skin, a shark fusion protein as well as fish and shellfish wastes. Both in vitro and in vivo screenings have been used to test anti-hyper glycaemic and anti-diabetic activities of marine organisms. In that, sea grass is the one of the hypoglycaemic food, to control blood sugar levels.¹ Sea grass is not only used for physical purposes for humans and act as a neutraceutical in nature to treat diabetes mellitus. And also sea grass is a good food supplement used in variety of recipes. Sea grass contain macro and micro

nutrients like vitamins and minerals calcium, magnesium, iron, zinc, sodium, potassium, manganese, phosphorous and copper. And also had anti bacterial, anti oxidant, anti spasmodic and anti inflammatory effects, to treat chronic disease conditions like diabetes, cancer, skin diseases mainly leprosy, good for muscle pains, wounds, fevers (malaria) and stomach problems. Some of the studies existing about sea grass as a relief for rheumatism. These studies demonstrated sea grass could be used as a potential source for natural health product⁷.

REVIEW OF LITERATURE:

Chiara lauritano et al (2016) Diabetes is a chronic degenerative metabolic disease with high morbidity and mortality rates caused by its complications. In recent years, there has been a growing interest in looking for new bioactive compounds to treat this disease, including metabolites of marine origin. Several aquatic organisms have been screened to evaluate their possible anti-diabetes activities, such as bacteria, microalgae, macroalgae, sea grasses, sponges, corals, sea anemones, fish, salmon skin, a shark fusion protein as well as fish and shellfish wastes. Both in vitro and in vivo screenings have been used to test anti-hyper glycaemic and anti-diabetic activities of marine organisms. This review summarizes recent discoveries in anti-diabetes properties of several marine organisms as well as marine wastes, existing patents and possible future research directions in this field. Pushpa Bharati et al (2016) Marine plants are known to produce a large number of structurally diverse secondary metabolites. They are found to be economically important in the field of food additives, nutraceutical and drugs. Sea grasses are a paraphyletic group of marine hydrophilus angiosperms, which evolved three to four times from land plants back to the sea. It lives in an estuarine or in the marine environment, and nowhere else. Sea grasses contain several compounds in their secondary metabolism in which they differ from terrestrial plants. They produce novel chemicals to withstand extreme variations in pressure, salinity, temperature, and so forth, prevailing in their environment, and the chemicals produced are unique in diversity, structural, and functional. The phytochemical present in sea grasses exhibit antibacterial, antioxidant, antitumor activity. New inclination in the field of drug discovery from natural sources highlights on the investigation of the marine ecosystem to survey numerous complex and novel chemical entities. These entities are the source of new lead form treatment of many diseases such as cancer, AIDS, inflammatory condition, arthritis, malaria and large variety of viral, bacterial, fungal diseases. Several species of sea grasses are used as human food or as raw material for the production of compounds of nutritional interest. Compared to algae, sea grasses remain less exploited despite the fact that they offer tremendous opportunities to find new commercially valuable phytochemicals. Therefore, their metabolite contents constitute another treasure of the ocean which is hidden. This paper gives an overall view on their nutraceutical activity and their potential as anticancer agent so that the hidden facts will be explored to provide a new cheap source for therapeutically and nutraceutical application. Thus, it is evident from various literature that sea grasses could be used as potential source for natural health product.

METHODOLOGY:

Sea grass assigned six families and encompassing 12 genera of angiosperms. Sea grasses have evolved into 12 genera spreading to the Pacific and Atlantic continents. In India, sea grass meadows can be found on the eastern and western coast, in Lakshadweep islands as well as in the Andaman and Nicobar Islands and entire Palk Bay and Gulf of Mannar and Mandapam group of islands in Tamilnadu.

In Mandapam only five species are available, these are

- Syringodium,
- Cymodocea rotundata
- Cymodocea serrulata
- Halodule uninervis
- Enhalus koenigi.

Three are used for human consumption, that is Cymodocea serrulata, Halodule and Enhalus. These are hypoglycaemic foods.

PREPARATION OF SEA GRASS POWDER:



Figure 1.

Halodule uninervis (Fig-1), having 2-4 leaves in each branch. Leaves are the edible portion, to consume only after processing. Compare to this three, Halodule uninervis is the best one to control blood sugar levels. Used biodegradable packaging method to transport Halodule uninervis, by sprinkling of sea water until starting the drying method. Before drying, washed it under running tap water and removed waste particles and kept it at room temperature (Fig-2) for five days and sundry thoroughly for two days especially in the early morning, later Powdered it.



Figure 2.

After drying (Fig-3), the non edible portions are removed and weighed the edible portion and ground to powder form in a grinder. Two kgs of Halodule uninervis makes only 500 grams of powder.



Figure 3.

ANALYSIS:

The prepared sample were selected and standardized by checking the anti diabetic properties through the nutrient analysis was carried out in lab through standardized procedures.

RESULT AND DISCUSSION:

The results of 100gram of sea grass powder (Table-1)

S.NO	TEST	RESULT	UNIT
1	Carbohydrate	44.85	g/100g
2	Protein	9.9	g/100g
3	Fat	0.4	g/100g
4	Dietary fibre	37.98	g/100g
5	Iron	47.6	mg/100g
6	Zinc	3.814	mg/100g
7	Magnesium	1.6	g/100g
8	Potassium	1.46	g/100g
9	Sodium	3.87	g/100g
10	PH	8.34	g/100g
11	Vitamin C	3.53	µg/g

Table-1

It is rich in dietary fiber, magnesium, zinc, iron, and polyphenols. Carbohydrates are low in nature. The limit range for microbial growth on food sample is 20-25 colonies. If the number of colonies is more than that, the food sample is not consumable. In the sample the microbial growth was seen, but it was less than limit range, which implies that it is consumable Zinc plays an important role in antioxidant defense in type 2 diabetic patients by improves the

oxidative stress in these patients by reducing chronic hyperglycemia. Magnesium is an important regulator in insulin and glucose metabolism to control glycaemic profiles. Polyphenols prevent the development of long-term complications in diabetes. High fiber diet is a healthy way to control blood glucose levels. Because fiber does not require insulin to digest. So these nutrients are shows improvement in biochemical parameters like glucose levels and lipid profile, which are valuable in diabetes treatment. And also fiber used for weight loss for obese.

We have done the animal experiment to see the effect of toxicity and anti diabetic effects to streptozocin- induced rats. The results showed there is no toxicity and reduction of blood glucose levels are seen.

CONCLUSION:

In the present study, Halodule uninervis was selected for anti diabetic. Because it is rich in fibre, zinc, magnesium which is helpful for controlling blood glucose levels.

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Biofortification with Spirulina to Elevate the Nutrient Quality of Greens

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Abstract: India is one of the countries having problem of malnutrition. Food availability is not problem to our country but it less in nutrition. The goal of biofortification is to contribute to reduce the high prevalence of specific nutritional deficiencies which commonly occur in low income populations. Biofortification is an intervention strategy currently being researched and developed for increasing the bio-absorbable content of micronutrients and macronutrients in the edible portion of staple food crops. Developing biofortification crops also improves their efficiency of growth in soils with depleted or unavailable mineral composition. Spirulina is a food source that consists of more than 60% protein and natural antioxidants like beta-carotene, vitamin E, vitamin B1, B5, & B6, zinc, manganese, copper and selenium & amino acid methionine. Present study selected the green leafy vegetables, adding with Spirulina, because green leafy vegetables (GLV) are rich in minerals and Spirulina rich in protein. By using biofortification method Protein are increased at GLV. In that we have selected Gogu (1.7/100gm), Amaranthus (3.5/100gm), spinach (2.29/100gm), Coriander (3.3/100gm) and Mint (4.6/100gm) adding with Spirulina in the form of cultivated water, sprinkled powder and powder mixed with different concentrations of Spirulina in 1litre of water. Results of present study reveals majority of protein increased in double quantity at – Gogu (3.9/100gm), Amaranthus (6.89/100gm), Spinach (6.19/100gm), Coriander (6.13/100gm) and Mint (6.38/100gm) seen in powder mixed in different concentrations of Spirulina in 1litre of water compared to other methods.

Key words: Biofortification, Spirulina, Protein, Malnutrition, Green leafy Vegetables.

INTRODUCTION:

Food fortification is the process of adding micronutrients to foods. As defined by the World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations, fortification refers to ‘the practice of deliberately increasing the content of an essential micronutrient, that is, vitamins or minerals in a food, irrespective of whether the nutrients were originally in the food before processing or not, so as to provide a health benefit with minimal risk to health. Certain types of fortification are more accurately called enrichment in which micronutrients added to food are those that are lost during processing (World Health Organization, 2006) ¹ Micronutrient malnutrition is frequent and severe in the developing world nevertheless, it can also represent a public health problem in more industrialized countries. Biofortification or biological fortification refers to nutritionally enhanced food crops with increased bioavailability to the human population that are developed and grown using modern biotechnology techniques, conventional plant breeding, and agronomic practices (McGuire S. FAO. Adv Nutr 2015) ². Apart from this, around two billion people across the world suffer from another type of hunger known as “hidden hunger,” which is caused by an inadequate intake of essential micronutrients in the daily diet (Hodge J, Muthayya A et al., 2016) ³ despite increased food crop production. Spirulina platensis has been used as biofortifying agent to enhance the nutrients quality of green leafy vegetables. Iron is an important dietary component and the bioavailable iron form should be high to overcome iron deficiency anaemia.

S. platensis is rich in proteins, carbohydrates, lipids, minerals, vitamins, essential fatty acids, phenolic acids and tocopherol. From Mayan Civilization onwards S. platensis is consumed as a food. In addition to its nutritional significance, S. platensis is well known for its antioxidant, anticancer and immuno modulatory activities (R. Deng, and T. J Chow, 2010) ⁴. In spite of its nutritional and medicinal significance, the consumption is very low because of its unpleasant unique odor. Biofortification of a leafy vegetable with the nutrients from S. platensis is an attractive solution, as the quantity of leaf consumed is more and bioaccumulation in leaf is greater than other parts of the plant.

REVIEW OF LITERATURE:

Natalia Palacios Rojas et.al., (2012)⁵ Biofortification or the breeding of staple food crops to increase their micronutrient density, is widely viewed as a valuable strategy for sustainably improving the nutritional status of some malnourished populations

Devendra Kumar Yadava et.al., (2018)⁶ Malnutrition has emerged as one of the most serious health issues worldwide. The consumption of unbalanced diet poor in nutritional quality causes malnutrition which is more prevalent in the underdeveloped and developing countries. Deficiency of proteins, essential amino acids, vitamins and minerals leads to poor health and increased susceptibility to various diseases, biofortification of crop varieties is considered as the most sustainable and cost-effective approach where the nutrients reach the target people in natural form.

Reihaneh Zeinalian et.al., (2017)⁷ *Spirulina platensis* as a source of potential valuable nutrients for prevention and treatment of chronic diseases. The effects of *Spirulina platensis* on anthropometric parameters, serum lipids, appetite and serum Vascular Endothelial Growth Factor (VEGF) in obese individuals. *Spirulina* supplementation at a dose of 1 g/d for 12 weeks is effective in modulating body weight and appetite and partly modifies serum lipids. This can further confirm the efficacy of this herbal supplement in control and prevention of obesity and obesity-related disorders.

METHODOLOGY:

Spirulina Cultivation and Biofortification with green leafy vegetables

This *Spirulina* culture along with required minerals should be released into the small size tank.

The minerals requires

- 8 grams of sodium bi carbonate
- 5 grams of sodium chloride , 0.2 grams of urea
- 0.5 grams of potassium sulphate
- 0.16 grams of magnesium sulphate
- 0.052 ml of phosphoric acid and
- 0.05 ml of ferrous sulphate.

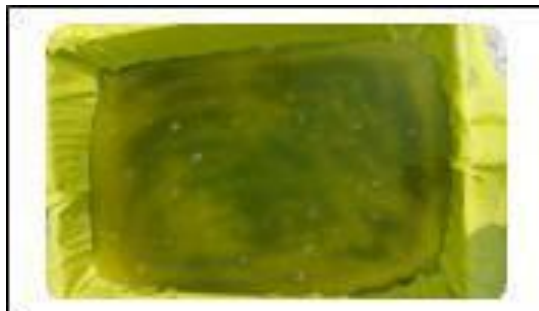


Figure 1

These measurements should be considered for 1 litre of water. After releasing the mother culture and minerals into the tank, the water should be agitated every day for 1 week using a long stick for 25 to 30 minutes. Cultivation of Green Leafy vegetables by using biofortification method with *Spirulina* mother culture and dry powder. After Harvest the leaves kept in solar food dryer and make into powder for nutrient and phytochemical analysis.



Figure 2

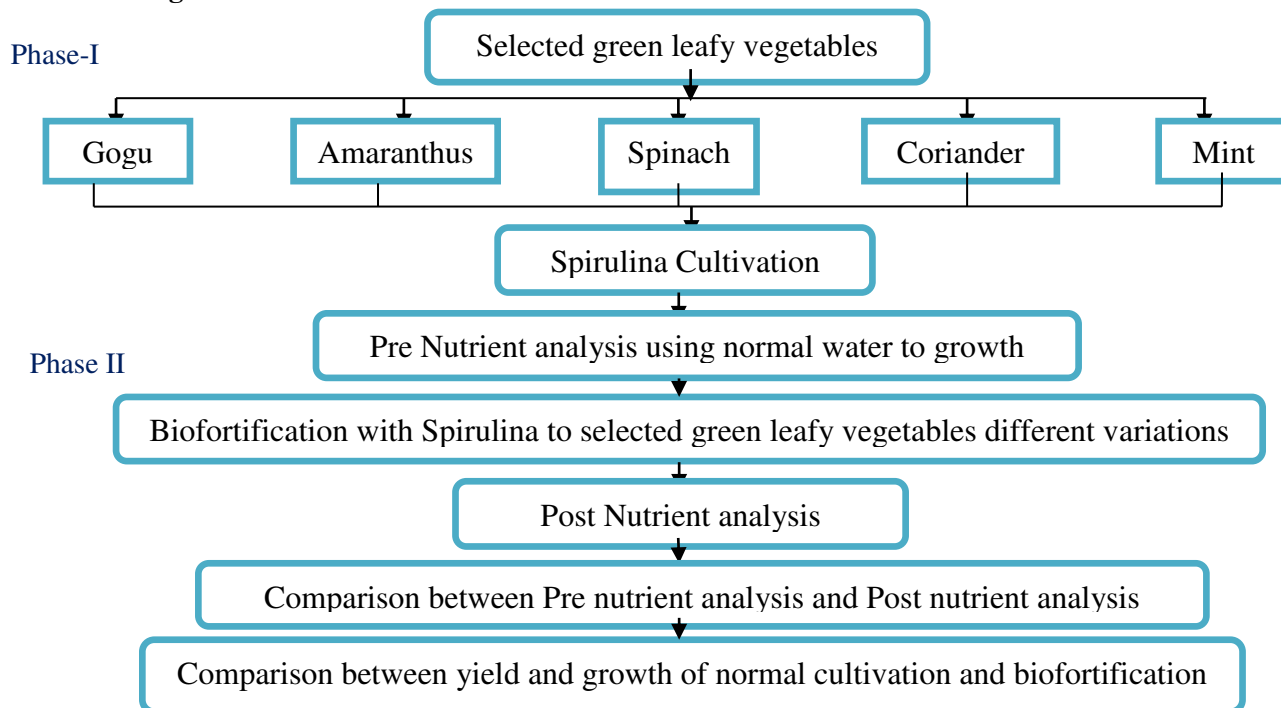


Figure 3



Figure 4

Research Design:



RESULTS AND DISCUSSION:

Cultivation of selected green leafy vegetables by using Spirulina powder with different concentrations and time period (Table 1)

Table 1)

S. No	Name of the set up		Variations										Control
1	Seed soaking in different concentrations of Spirulina powder (1litre of water)		25gm			50gm			75gm			----	
2	Time Period of Soaking		4hr	8hr	overnight	4hrs	8hrs	overnight	4hrs	8hrs	overnight	---	
3	No. of Plants Germinated (25seeds)	Amaranthus	22	22	23	21	23	24	23	23	24	23	
		Spinach	22	212	24	22	23	22	24	24	25	23	
		Gogu	23	24	23	23	24	24	24	24	24	22	
		Coriander	20	20	21	23	23	25	23	22	25	23	
		Mint	19	20	20	20	22	22	19	21	23	20	
4	Height of the Plants (cm)	Amaranthus	Day3	6.7	6.5	7.5cm	6.5	7.3	7.9cm	15	16.5	17.4 cm	10.5
			Day6	12.4	12.8	13 cm	12.6	15	15.5cm	21	22.8	24 cm	18.4
			Day9	18.5	19	20.5cm	20.9	20.9	21.5cm	6.5	6.8	7.9cm	5.6
		Spinach	Day3	5.3	5.5	6cm	5.9	6.2	6.8cm	11.2	13.5	15.4 cm	11.3
			Day6	9.5	10	10cm	10.8	10.8	13.6 cm	17	18.5	22 cm	18.9
			Day9	15	15.5	15.9cm	17.2	17.5	19.5 cm	6.5	7	7.2 cm	6.5
		Gogu	Day3	6	6.2	6.5cm	6.2	6.5	6.9 cm	14.5	16.3	17.5 cm	12.5
			Day6	13.8	14	14cm	14.2	14	14.6 cm	21.2	22.9	24 cm	20.6
			Day9	20.3	20	20.5cm	21.5	21.	23.2cm	3.2	3.8	4 cm	4.2

									</				

The above table 1 experiment shown that 25 Spinach and Coriander seeds germinated at 75gm of Spirulina powder (overnight soaking) and height can also change at different time period and concentration.

Comparison of Nutrient Components - Overall experiment (Table 2)

Nutrient Component	AMARANTHUS Nutritional values Per 100gm					
	Control	Spirulina cultivation water	Spirulina a dry powder	25gms of Spirulina powder and green seeds soaked at overnight	50gms of Spirulina powder and green seeds soaked at overnight	75gms of Spirulina powder and green seeds soaked at overnight
Protein (gm)	3.59	3.95	3.89	5.29	5.69	6.89
Fat (gm)	0.86	0.97	0.95	0.92	0.98	1.08
Vitamin-A (IU)	4126	4896	4596	4816	4826	5126
Vitamin-C (mg)	34	44.3	39	53.2	52.9	64.3
Iron (mg)	31.8	34.7	32.9	39.8	41.83	46.8
Calcium (mg)	296	309	463	494	493.9	498
Phosphorus (mg)	72	78	73.2	72.7	73.9	79.2

The above table 2 shown that Nutrient quality of Amaranthus increased at 75gm of Spirulina powder and green seeds soaked at overnight compare to overall experiment

SPINACH Nutritional values Per 100gm (Table 2.1)

Nutrient Component	Control	Spirulina cultivation water	Spirulina a dry powder	25gms of Spirulina powder and green seeds soaked at overnight	50gms of Spirulina powder and green seeds soaked at overnight	75gms of Spirulina powder and green seeds soaked at overnight
Protein (gm)	2.29	3.32	3.19	5.16	5.19	6.19
Fat (gm)	0.99	1.06	1.02	1.32	1.09	1.08
Vitamin-A (IU)	4923	5580	5168	5693	5796	5980
Vitamin-C (mg)	26.2	29	26.8	59	61.2	65
Iron (mg)	3.2	3.82	3.17	16.8	16.12	19.6

Calcium (mg)	74	78.6	78	87.8	88.2	98
Phosphorus (mg)	139	142	141	133	136	142.3

The above table 2.1 shown that Nutrient quality of Spinach increased at 75gm of Spirulina powder and green seeds soaked at overnight compare to overall experiment

GOGU Nutritional values Per 100gm (Table 2.2)

Nutrient Component	Control	Spirulina cultivation water	Spirulina dry powder	25gms of Spirulina powder and green seeds soaked at overnight	50gms of Spirulina powder and green seeds soaked at overnight	75gms of Spirulina powder and green seeds soaked at overnight
Protein (gm)	2.9	3.7	2.3	3.67	4.16	6.19
Fat (gm)	1.26	1.07	1.08	1.09	1.26	1.08
Vitamin-A (IU)	2986	2784	2663	2986	3.19	5980
Vitamin-C (mg)	34	32	29	46	48	65
Iron (mg)	3.97	4.61	3.98	10.24	10.94	19.6
Calcium (mg)	198.5	163.8	137.4	213.5	211.98	98
Phosphorus (mg)	152	159	155	159.6	161.2	142.3

The above table 2.2 shown that Nutrient quality of Spinach increased at 75gm of Spirulina powder and green seeds soaked at overnight compare to overall experiment

ANALYSIS:

In this Study shown that done the nutrient analysis by using standard procedures at our university food chemistry lab and also check the phytochemical analysis and Microbial analysis in that there is no bacterial growth in selected biofortified green leafy vegetables

Phyto constituents	Selected green leafy vegetables														
	Amaranthus			Spinach			Gogu			Coriander			Mint		
	H2o	Ethanol	Ethyl acetate	H2o	Ethanol	Ethyl acetate	H2o	Ethanol	Ethyl acetate	H2o	Ethanol	Ethyl acetate	H2o	Ethanol	Ethyl acetate
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	-	+	-	-	+	+	+	+	-	+	-	-	+	+	-
Saponin	+	-	+	+	-	+	+	-	+	+	-	-	+	+	-
Flavonoid	+	-	+	+	-	+	+	-	+	-	+	+	-	+	-
Glycoside	-	+	-	-	+	-	-	+	+	-	-	-	+	+	-

(+) Presence, (-) Absence

CONCLUSION:

- Spirulina powder gets potent and stimulate to growth the desire plant and high nutrient quality, when its soak overnight.

- Spirulina cultivation water gives moderate growth and nutrient quality to the desired plant (Selected green leafy vegetables)
- The selected green leafy vegetable (Amaranthus leaves) which was normally grown with Protein content 3.59gm per 100gms, surprisingly Amaranthus seeds in 75gms of Spirulina powder soaked overnight gives the more Protein content 6.89gm per 100gms.
- Thus biofortification method of Spirulina powder towards selected green leafy vegetables give beneficiary yield and more nutrient quality, when compared to the normal grown plants.

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Studies on development of snack product from sea foods

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Abstract: Shrimp (*Litopenaeus vannamei*) is a marine animal which is highly nutritious and consumed all over the world. It is good source of protein and has lot of calories when compared to other sea foods. Snack product from sea foods are not much available commercially and hence chips incorporated with shrimp will act as a value added product. The aim of our project work is to develop fried product using shrimp blended with wheat flour and to make a snack product. The dough is prepared by blending the flour (30g-50g) with shrimp (3-5 %) and it is dried in hot air oven at 60 °C (for about 20-40 min). Seasonings are added to enhance the taste of the final product. Individual and interaction effects of factors upon moisture content, shrinkage, oil uptake and overall acceptability of the final product. The responses for optimized shrimp chips are 5.382%, 2.121%, 16.22 %, 7.511 of moisture content, oil uptake, shrinkage, overall acceptability and desirability values were found to be 0.871 respectively.

Key Words: Shrimp, pre-processing, ultrasonicator, Snack product, interaction effects.

INTRODUCTION:

A snack is a crispy and crunchy food and it is smaller than a regular meal. Generally the snack food is eaten between meals. The snack products are of five categories and they are baked snack foods and fried snack foods or salted snacks, confectionery snacks, specialty snacks, natural snacks, traditional snacks. The baked snack foods are biscuits, cookies, wafers, puffed snack, sweet snacks, pies, snack bars, toaster pastries etc., and the fried snack foods are potato chips, sweet potato chips and tortilla chips, pretzels, corn chips extruded snacks. The specialty snack foods are dried snacks, meat snacks, pork snacks, health food bars (power bar) rice or corn cakes. The confectionery snack foods are different types of candies. The natural snacks are nuts, fruits and vegetables (prosise et al 2004). The prawn contains proteins and amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, valine, serine, glycine, alanine, and tyrosine. The prawn can give about 2-66.2% Recommended Daily Allowance of protein (osamu deshmaru 1978). With such high level of protein, it can help to treat marasmus and kwashiorkor caused by protein deficiency. Wheat contains 82.5% of endosperm. It contains protein, pantothenic acid, riboflavin, niacin, and thiamine. The wheat is milled by (roller miller) mechanical machine. The milled flour is slightly yellowish colour, and it is bleached by bleaching agents. The bleached flour is creamish white in colour. The flour contains soluble protein (albumin and globulin) and insoluble protein (gliadin and glutenin). The water absorption power of the flour mainly depends upon the damaged starch.

LITERATURE REVIEW:

Shaikhmahmud et al (1957) reported the muscle tissue biochemical composition of commercially important penaeid prawns in relation to their maturation. Four different species of prawns were prepared and analyzed. It was observed that protein was the significantly dominant component and carbohydrate was present in negligible quantity. Deepa.D et al (2017) attempted to use the tiny shrimps (*Solenocera crassicornis*) for developing ready to eat fried masala shrimp as snack food. The proximate composition of dried shrimp and fried masala shrimp was estimated. It was noted that the protein concentration of fried shrimp was comparatively greater than the dried shrimp. Olatunji et al (1978) studied breadfruit flour as a component of composite flour. Comparative nutritive studies were made for Prawn crackers made from the flakes of breadfruit, potato and plantain. The result showed that the energy content of the of the breadfruit chips was higher than that of plantain and potato chips. The crude fibre content and protein was also observed to be appreciable in breadfruit chips. T Mai Tran et al (2019) experimented reducing oil content of potato crisps by using a sweet pre-treatment technique. In this study, potato crisps were blanched, pre-dried and dipped in

the solution of sugar 2s before frying at 180°C. It was observed that pre-drying and dipping in sugar solution reduced the oil content of the fried chips of about 30% with increased sweetness. Also, the pre treated chips had some changes in colour and seemed to shrink more after frying.

MATERIALS:

Sample Preparation:

Prawns were bought in commercial fish market near cuddalore and is eviscerated (removal of internal organs and inedible parts) and washed with warm water and stored at refrigerated condition 6°C

Pre processing:

The eviscerated sample were treated with 2% concentration of turmeric extracts were treated in room temperature 37°C Then, the samples were autoclaved at 121°C .The sample filled flask was placed in Ultrasonicator for 45 min at 30°C to deteriorate the microbial content in shrimp . This is to reduce the microbial load such as *Escherichia coli* and *Staphylococcus aureus* agents (Lawhavinit, Kongkathip et al. 2010).The shrimp from UltraSonicator were processed with 3 stage heat treatment process. First stage heat with water at 50°C. The second stage was heat with chlorinated water with <0.01ppm ,Then the shrimps were filtered and repeatedly washed , Third stage heat with water again and potassium meta bisulfate (0.1%) was added which enhances the drying of the sample (Liu, Cheng et al. 2010). The 3 stage heat treated shrimp undergone drying in tray dryer at 50°C for about 8 hours. Drying changed the textural and structural properties of shrimp.The color change occur due to drying. It is found to that low temperature leads to the lower shrinkage which is due to expansion of vapor inside the sample during rapid drying which leads due to the high porous structure and ensures the complete drying of the product. Moisture gets evaporates and final dried shrimp was obtained (Namsanguan, Tia et al. 2004). The dried shrimps were size reduced in ball mill and they were subjected into sieve analysis to determine average particle size.

METHOD:

Figure 4.1 shows the process flow diagram used for manufacturing chips from shrimp .The raw materials (Shrimp, wheat flour, chilli powder, salt) were added to the flour and it is then fried at 140°C for about 40 seconds. The flour composition is optimized by carrying out various trials. After frying seasonings were dusted on the surface of chips. The prepared chips were packed and stored at ambient condition 37°C for analysis and the sensory test were carried

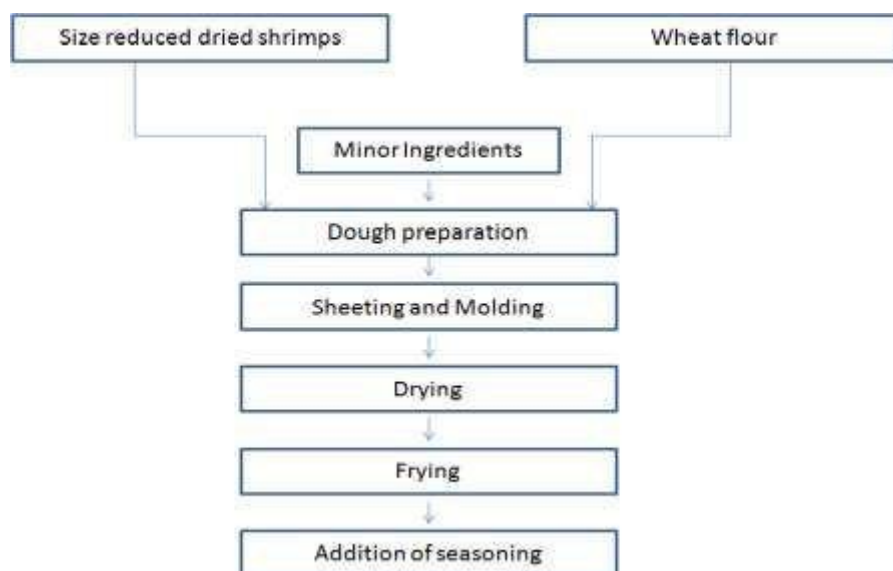


Figure 4.1. Flow diagram of prawn chips manufacturing

ANALYSIS:

DETERMINATION OF COMPOSITIONAL ATTRIBUTES OF CHIPS:

Moisture content

The moisture content of samples was measured by drying the sample 5g in a hot air oven at 105°C until constant mass weighed (AACC, 1986). Moisture loss was expressed on a dry basis and calculated using the difference the original moisture content and the moisture content at time t (V. Dueik et al., 2009).

$$\text{Moisture content} = \frac{W_0 - W_t}{W_0} \times 100$$

W_0 – Initial weight of the sample (g)

W_t – Final weight of the sample (g)

Shrinkage:

Shrinkage is calculated after the chips are fried. The volume of the sample at any given time can be calculated by πr^2 where r is the radius of the diameter. Ten samples were taken to determine the shrinkage for each frying condition at equilibrium condition (V. Dueik et al., 2009).

$$\text{Degree of Shrinkage } S_v = \frac{V_0 - V(t)}{V_0} \times 100$$

Where,

V_0 - The original volume of the sample

$V(t)$ - The volume of the sample at time t .

Sensory evaluation:

To evaluate the quality of chips in terms of colour, taste, texture, crispiness and overall acceptability using a nine point hedonic scale for likeness by the panellists. The scores are designed to 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 disliked. Each sample was presented to the panellists for identification. The water was provided between samples for mouth rinsing of each panellists. All groups received scores of over 4 (color, texture, crispiness and taste) on a 1-9 point hedonic scale

OIL CHARACTERISTICS:

Acid value:

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution. 50 ml of ethyl alcohol and 1 ml of phenolphthalein indicator solution was added. The solution turns to light pink from colorless. Alkali blue indicator was used as an end point will disappearance of blue color which developed during addition of indicator.

$$\text{Acid value (mg KOH/g)} = \frac{56.1 \times V \times N}{W}$$

Where,

V = Volume in ml of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution

W = Weight in gm of the sample

Peroxide value:

The peroxide value is a measure of the peroxide oxygen present in the oil. *The peroxides present are determined by titration against thiosulphate. Starch is used as indicator.* 4g of oil is weighed into a clean dry boiling tube and 1g of powdered potassium iodide and 20ml of solvent mixture is added. The tube was placed in boiling water for about 30 seconds. Then it was transferred to the conical flask containing 20ml of 5% potassium iodide solution. The test tube was washed with 25ml water each time and collected in the conical flask. The solution is titrated against sodium thiosulphate solution until yellow color is almost disappeared. 0.5 ml of starch is added and titrated till the blue color disappears. A blank also be set at the same time.

$$\text{Peroxide value (meq/kg)} = \frac{S \times N \times 1000}{\text{weight of the sample}}$$

Where,

S – Sodium thiosulphate (test – blank)

N – Normality of sodium thiosulphate

Saponification value:

The saponification value is the number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat. 1 g of oil was weighed in a small beaker and dissolves it in about 3ml of the fat solvent. Transfer the oil into the beaker of 250 ml conical flask by rinsing the beaker. 25ml of alcoholic KOH was added. Flasks are heated on a boiling water bath for 30 min. It is cooled to room temperature and titrated with 0.5 mol/lit HCl with phenolphthalein as indicator, until the pink colour disappears. (William Odoom et al (2015).

$$\text{Saponification value (mg KOH/ 1g)} = \frac{(B-T) \times 28.08}{\text{weight of the oil}}$$

Where,

B – Blank of the sample ml

T – Titrate value of the sample ml

Iodine value:

Iodine number is used to determine the amount of unsaturated fatty acids in the oil. 4ml of oil is taken in the flask. 20ml of Iodine Mono chloride reagent is added in to the flask. Then the flask is allowed to stand for half an hour. Set up a blank in another iodination flask by adding 10ml Chloroform to the flask. 20ml of Iodine Mono chloride reagent is added to the blank and mix the contents in the flask. 10 ml of potassium iodide solution was added into the flask. Titrate the test against standardized sodium thiosulphate solution until a pale straw colour is observed. 1ml starch indicator is added into the flask, a purple color is observed. The titration was continued until the color of the solution in the flask turns colorless. The disappearance of the blue color is the end point of the titration then it is repeated for the Blank sample (William Odoo et al., 2015).

$$\text{Iodine value} = \frac{\text{Equivalent weight of iodine} \times B - A \times \text{normality of sodium thiosulphate} \times 1000}{\text{weight of the sample} \times 10000}$$

Where,

B – Blank of the sample (g)

A – Titrate value of the sample (g)

FINDINGS:

Preliminary studies:

Preliminary studies were carried out to mask the aroma of prawn to the acceptable levels. First trial were done based on the addition of prawn in chips either of powder or coarse forms. It was observed that powdered form of prawn creates more smell to the product compared to coarse form. Then the composition of flours were determined by trail method, Wheat composition varies from 40% to 60 % and maida flour 20% to 40% and the prawn composition varies from 30% to 70% and other minor ingredients are added. Out of these variations, based on sensory property of the final chips product, optimised will be selected and analysed

Particle size distribution:

Particle size distribution of dried prawn were analysed in sieve analyser for different particle sizes. After 20 minutes, particles retained at different sieves were collected and weighed accurately. The final particle size distribution of the sample were found to be 0.1424mm respectively

Sieve size	Particles retained (g)	Cumulative distribution size
2.8 mm	15.9	0.4129
2 mm	5.2	0.1350
1.40 mm	5.6	0.1454
850µm	3.2	0.0831
355µm	4.4	0.1142
212µm	2.6	0.0675
Pan	1.5	0.0389
Total	38.4 g	0.1424 mm

Table 6.2 : Average particle size distribution of shrimp (mm)

Oil analysis for fried oil:

William Odoo et al., (2015) has studied that saponification value, iodine value and insoluble impurities are some important parameters are considered in the determination of sunflower oil quality. The saponification value of sunflower oil 25% met the Codex Alimentarius Standard. The iodine value 75% of the sunflower oils from the processing centers met the APCC and the Codex standards.

Chemical analysis	Refined sunflower oil	
	Before frying	After frying
Acid value	0.28 mg KOH/g	1.12 mg KOH/g
Peroxide value	2.5 meq/kg	10 meq/kg
Saponification value	190.4 mg KOH/g	280mg KOH/g

Iodine value	5.7 mg KOH/g	7.1 mg KOH/g
Moisture content	2.8%	27.2%
Specific gravity	0.893	0.915

Table 6.3 Chemical analysis of before and after frying of oils for refined sunflower oil

In fried oil, acid value, peroxide value, saponification value and iodine value are increased when comparing to fresh oils because of the chemical reaction during frying. When the oil is react with oxygen it produce free radicals ie., Hydro peroxides is the secondary metabolites. It indicates the quality of oil. In fresh oil, the reaction is not possible to occur so the acid, peroxide, saponification and iodine values are less.

Water absorption capacity of the flour:

The final optimized flour were observed for amount of water taken up by the flour to achieve the desired consistency during dough preparation were compared with the control sample wheat flour of 10 g and another sample of addition of prawn with wheat flour were compared

Material	Volume of absorption (ml)
Wheat flour	5.4
Wheat flour + Prawn	8

Table 6.4 : water absorption capacity of flour

RESULT:

When comparing with the fresh prawn and the fried product, the drying process component percentage varies with the raw material because of reduction in the moisture level. Due to this, there was slight increase in total lipids, proteins and ash contents. After drying, moisture level in the final product was found to be 6% by (Castro and Pagani 2004). The ash content of a shrimp was found by 24.97% .The study on total lipids by (Bragagnolo and Rodriguez-amaya 1997) was found to be 0.92% which was dried at 50°C which was found to be low in the raw shrimp. The lower value is absorbed because the shrimp was affected by several factors such as age, feeding, growth rate and degree of maturation. Protein content in the raw shrimp was lower than that of (de Lima, Rabello et al. 2007, Sriket, Benjakul et al. 2007) which was 21.5 % for the final product and 14% for control sample This shows that shrimp can be used as alternative source of protein in comparison with other sea foods.

CONCLUSION:

According to (Ravichandran, Rameshkumar et al. 2009) protein concentrate obtain from the shrimp by products which can be used as an supplement for human consumption and can be used for different food formulation. Thus the high protein value leads to the purpose of utilization in the enrichment of various food products.

Fibre content in the raw shrimp is composed of chitin in addition to drying 17% in the final product which shows in the present research. According to that result obtain in the proximate analysis infers that the people from different ages who suffers from nutritional health problems to compensate the fight against hunger for the future sustainable development.

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STUDIES ON DEVELOPMENT OF JAGGERY BASED HARD BOILED CANDY

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Abstract: Jaggery is the sugarcane based traditional Indian sweetener. Jaggery has more nutritious content like minerals. Normally refined sugar is used in the hard boiled candy process. Jaggery has more health benefits than refined sugar. Jaggery is said to cure arthritis. The investigations were carried out to study the effect of different levels of Jaggery and sugar on hard boiled candy products, the samples prepared were subjected to sensory analysis. Under the sensory analysis sample with the jaggery level of 70 % has more acceptance. The shortlisted samples were analyzed for moisture, ash and reducing sugar etc. Results were compared to control sample. Jaggery candy has more ash than the sugar candy which implies more minerals content. Reducing sugar results are also interpreted.

Key Words: jaggery, hard boiled candy, minerals, sensory analysis.

INTRODUCTION:

Sugar boiled candy is also known as hard boiled candy which is consumed world widely consist of refined sugar. But in India jaggery is preferred. Jaggery, a product of sugarcane, is such a product which is rich in important minerals like calcium, potassium, magnesium [1]. Magnesium present in jaggery strengthens our nervous system, helps to relax our muscles, gives relief from fatigue and takes care of our blood vessels. Along with selenium it also acts as an antioxidant and scavenge free radicals from our body. The potassium and low amount of sodium present in it maintain the acid balance in the body cells and also combat acids and acetone and control our blood pressure. It is rich in iron, and helps to prevent anaemia [1]. Replacing refined sugar with jaggery in hard boiled candy increases the nutritional value of hard boiled candy. Jaggery (also known as gur) is a traditional unrefined non-centrifugal sugar consumed in Asia, Africa, Latin America and the Caribbean [2]. Refined sugar is typically sold as granulated sugar, which has been dried to prevent clumping. Raw sugar is comprised of yellow to brown sugars made from clarified cane juice boiled down to a crystalline solid with minimal chemical processing, which helps in retaining more mineral salts and phytochemicals[3]. In India 40 to 50 % of sweetener is obtained from jaggery[2]. Tamil Nadu, Maharashtra, Andhra Pradesh, and Karnataka.[11] The increasing production trend and market value of jaggery has much significance to learn about peoples liking towards jaggery[1]. In terms of sucrose purity, refined sugar is more pure than brown sugar and jaggery sugars[3]. Usage of jaggery in hard boiled candy is not much explored so we optimised the candy using jaggery. The specific objectives were to optimise the initial concentration of jaggery and sugar, study on the texture, reducing sugar, solubility test, ash content, moisture analysis and sensory analysis.

MATERIALS:

Jaggery and sugar were procured from local market in Erode. Liquid glucose of 42 DE was used. The flavours used were brought from IFF. Induction cooker used is prestige's induction cooker picc 10.0.

METHOD:

Control:

Raw materials as per requirement were weighed. Sugar along with liquid glucose and water were taken in a sauce pan. The mixture was heated in the induction cooker with continuous stirring to initially dissolve the sugar completely (110-112°C), and cooking was continued for around 140 -142°C. Then cooking was stopped and weighed quantities of flavor, acidulant were added. The mass was mixed well and poured into Teflon coated moulds. The mould was cooled for 10-15 mins at 18-20°C. After cooling candies were demoulded and wrapped in laminated wraps.

Trail samples:

In a similar method as described for the control sample. The trails were made using different substituent level (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0) were conducted and sample were packed and stored under ambient conditions.

ANALYSIS:

Sensory analysis:

Semi trained panels of 10 healthy members were identified and samples were subjected to sensory analysis to short list the best sample. The samples were given to the panel members and were evaluated according to 9 point hedonic scale, with parameters of color, taste, chewiness, after taste, stickiness. [9]

Determination of moisture content:

The total moisture present in the final candy was determined by hot air oven method. Samples were accurately weighed 5 grams. Dried at 105°C for 6 hours until concordant value is attained.[6]

Determination of reducing sugar:

5 gram sample was taken crushed and dissolved in 200 ml of distilled water. And filtered with a dry filter paper, the solution is titrated against Fehling A and B solutions in the heated flask. Titration was done until the end point from blue to brick red is achieved. [5].

Total Ash analysis:

Each sample with weight of 5 grams where added in the crucible of porcelain. Placed in the muffle furnace at the temperature of 550°C. Water and other volatile materials are vaporized and organic substances are burned in the presence of O₂ in air to CO₂, H₂O and N₂. It is placed in the furnace till the appearance of gray whitish ash. (3 hr). [4] The sample is placed in desiccator and weighed.

Texture analysis:

Texture analysis is done by the sensory panel. The samples were given to the panel and were evaluated according to 9 point hedonic scale. [8].

RESULTS AND DISCUSSION:

The stability of hard boiled candy with different proportion i.e. 0% to 100% of refined sugar and jaggery respectively were analyzed by moisture content, reducing sugar, mineral content was determined by ash texture and overall acceptance were studied by sensory panel.

Moisture content:

Stability of the candy is highly influenced by moisture present in it. As we see in the fig. 1, If the percentage of jaggery increase the moisture content also increase. At 0% jaggery the moisture content is low (1.376%) and 100% jaggery the moisture content is high (3.626%). The stability decrease with increase in moisture content. As the moisture content increases the stickiness also increase. Its evident that jaggery contains more moisture than refined sugar and so we can see the increase in the moisture content.[6]

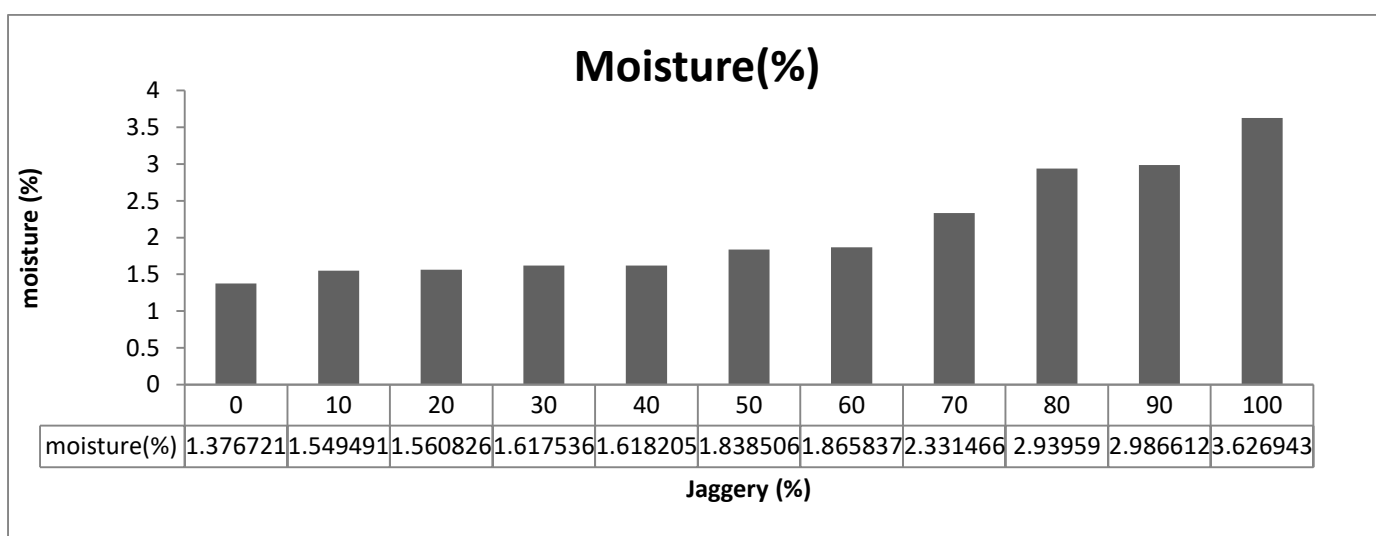


Figure 1. moisture content of hard boiled candy at different proportion.

Ash content

During the process of refining sugar the mineral content will be removed by the processes. As it is clearly shows that with 0% jaggery there is no ash, and with the 100% jaggery the mineral content is 0.4% which implies that mineral content is retained in the candy. And for the 60% jaggery the value is 0.18%, for 70% jaggery it is 0.22 which comes under the FSSAI standards (0.4%)[7].

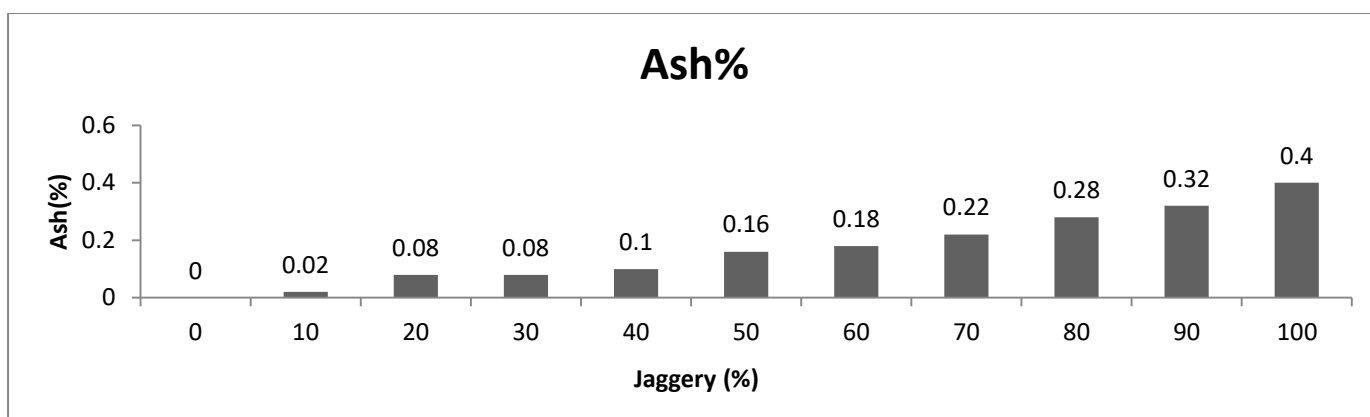


Figure 2.

Texture analysis:

From the fig. 3 its evident that control sample i.e 0% jaggery has the maximum value of 8.7 and followed by 8.5 for 60% jaggery and then 8.4 for 70% jaggery. and with the minimum value of 4.4 for 100% jaggery. This is because of the moisture and reducing sugar content present in it. In 100% jaggery the candy was so hard and it sticks to the mouth which was uncomfortable. In 10%, 20% jaggery although the moisture was low its very brittle and easily breaks so it got low marks. And 60%, 70% jaggery the texture was good and did not stick to the mouth.

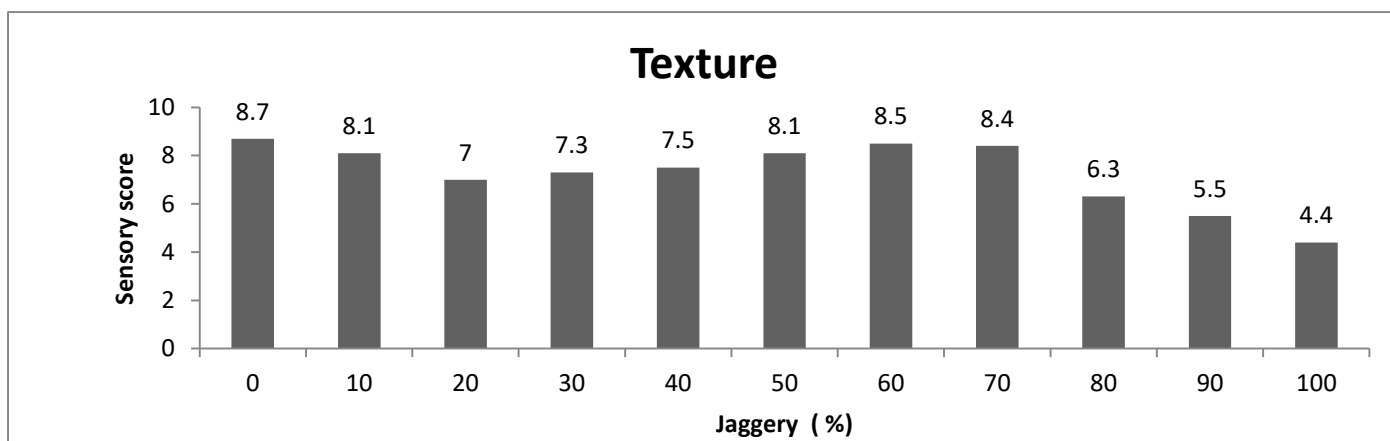


Figure 3

Overall acceptance:

From the fig. 4 control sample was liked by everyone with a score of 8.7 and with 10%, 20%, 30% jaggery there is a very little jaggery flavor which is odd so it scored 8, 7.4, 7.6 respectively with the increase in jaggery from 50% the taste is so good with a score of 8.2. And for the 100%, 90%, 80% although the taste is good there is a very poor texture so it got scores of 5.1, 6.1, 6.8 respectively. As for the 60%, 70% the taste as well as the texture is also good so it scored 8.6 and 8.5 respectively.

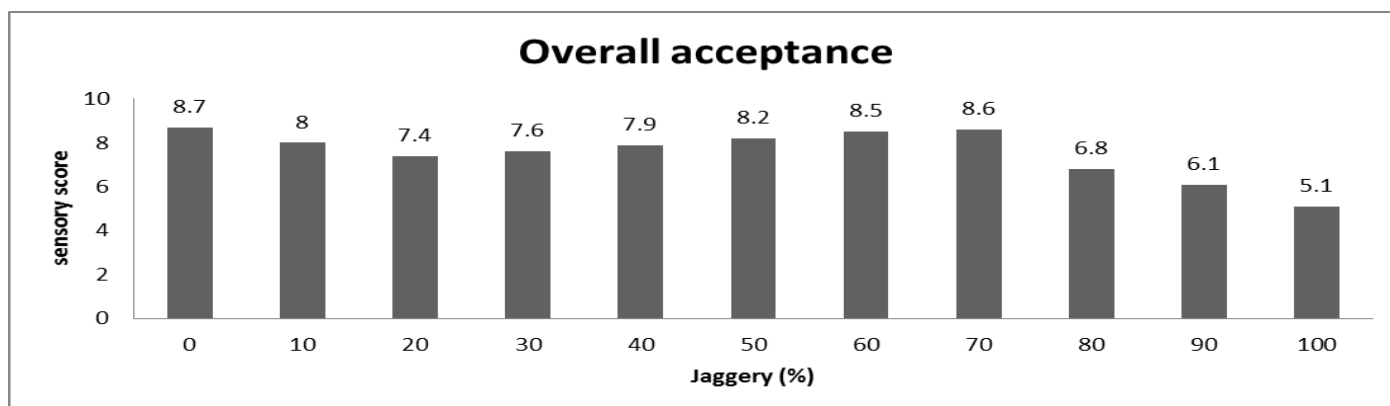


Figure 4.

Reducing sugar:

From the texture and overall acceptance its evident that 60%, 70% jaggery has a very good scores so control, 60%, 70%, 100% were undergone for the reducing sugar test. Stability is determined from this test as the reducing sugar is low the stability and stickiness is low. From the fig. 5 its evident that control sample has a value of 18.75% which is

low comparing with the other samples. Reason for higher value of the 100% jaggery is that inversion takes place during cooking, comparing with refined sugar the inversion is high in jaggery which is the reason for the stickiness of the mouth, for 60%, 70% jaggery the values are 19.06, 19.28 respectively which is near to the control value and so the texture of those candies were good.

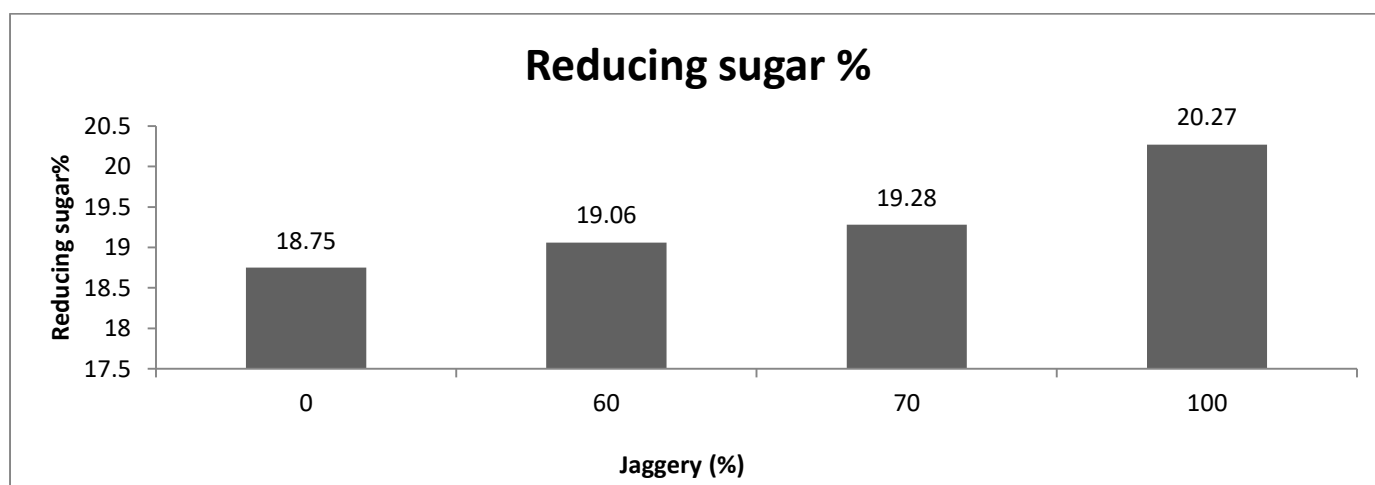


Figure. 5

CONCLUSION:

Now a days people prefers jaggery over refined sugar because for refining purpose many chemicals are used but in jaggery there is no refining process. Jaggery has more nutritional content than the refined sugar[1]. The use of 80-100% jaggery produces low texture as explained in 5.3 with some off flavor. While using 10-50% jaggery there will be more quantity of the refined sugar there will be no benefit. From the overall acceptance and texture, It is concluded that 60%, 70% jaggery is best for the mass production.

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11. Jaggery – A Traditional Indian Sweetener PVK Jagannadha Rao, Madhusweta Das & SK Das* Department of Agricultural and Food Engineering, Indian Institute of Technology, Kharagpur 721 302, West Bengal
12. Investigating the suitability of Isomalt and liquid glucose as sugar substitute in the formulation of Salbutamol sulfate hard candy lozenges Rajesh Kini, Mahalaxmi Rathnanand*, Deepak Kamath Department of

Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal University, Madhav Nagar, Manipal, Karnataka

13. Influence of product composition and operating conditions on the unsteady behavior of hard candy cooling process M. Agustina Reinheimer*, Sergio Mussati, Nicolas J. Scenna. INGAR-CONICET – Instituto de Desarrollo y Diseño – Avellaneda 3657, S3002GJC Santa Fe, Argentina
14. Flavor Release and Perception in Hard Candy: Influence of Flavor Compound–Flavor Solvent Interactions. AMANDA L. SCHOBBER AND DEVIN G. PETERSON*Department of Food Science, 215 Borland Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802-2504
15. Impurities in White Sugars: VIII. Effects of Some Impurities on Decomposition of Sucrose during the Barley Candy Test.J. A. AmblerS. Byall

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Department of Biotechnology, Kumaraguru College of Technology, Tamil Nadu, India

Physico chemical characterization of Chitosan-Gelatin based Banana fibrous scaffold with *Tridax procumbens* extract for wound healing applications

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Abstract: Tissue engineering is a multidisciplinary field in which scaffold acts as template for the body cells to grow in order to provide appropriate environment for tissue to regenerate. Tissue compatibility and biodegradability are the essential factors for scaffold to interact with the surrounding tissues and degrade after healing without the use of surgical removal. In order to provide biodegradable and biocompatible scaffold, the natural fibre such as banana fibre were used. Banana fibre is majorly composed of cellulose, hemicelluloses, lignin, pectin and ash. The Pseudo stem was taken for fibre extraction. Fibre sample was subjected to NaOH treatment in order to remove cellulose and lignin content. The extracted and dried fibre powder was used for scaffold preparation. Chitosan and Gelatin were dissolved in acetic acid and distilled water respectively. 1g of banana fibre and 1g plant extract of *Tridax procumbens* were added with 1ml of each Chitosan and Gelatin solutions. The solutions were blended and scaffold prepared by using freeze drying method. Scaffolds were subjected to FTIR analysis, where various peaks observed for amide I, II and III. Swellability analysis showed that the banana fibrous scaffold showed more swelling behaviour than the scaffold added with plant extract. DPPH assay and TGA analysis were done for antioxidant property and thermal degradation of the prepared scaffolds.

Key Words: Tissue compatibility, biodegradable, renewability, *Tridax procumbens*, tissue regeneration, scaffold.

1. INTRODUCTION:

The term 'tissue engineering' was officially coined at a National Science Foundation workshop in 1988. It is an interdisciplinary field that applies the principles of engineering and life science for the development of biological materials to improve the function of tissue or organs. It is a promising strategy for regeneration and replacement of repaired tissue or organ. An important goal in tissue engineering is the development of biomaterials that promote tissue and organ regenerative processes effectively. The objective is that the biomaterials must preferably degrade at a certain rate from the site of implantation. Cartilage scaffolds, bone scaffolds are the major examples of scaffold in tissue engineering while in soft tissues that will render in the making of structural component of the whole organ. Thus, scaffolds can help to make up the whole organ by maintaining a suitable environment for it. Three dimensional scaffolds made up of natural polymers or polymer blends is typically used as temporary medium for the cells to grow. Scaffold should support process such as cell inoculation, migration, differentiation, development for cell regeneration and healing. For this, the scaffold should be developed in such a way that, it must allow cell movement and attachment by providing enough mechanical strength to the cells. Finally, the scaffold material should degrade in such a way that it does not affect the body. So the scaffold should possess in toxicity effect [1].

Mostly natural polymers, scaffold, hydrogel are used as biomaterials in tissue engineering. The biodegradable and biocompatible scaffold with excellent surface interaction for addition, migration, proliferation, differentiation and infiltration to the cells is used in tissue engineering. Due to their admirable biocompatibility, soft properties, stability, water retention allows breathable for oxygen, nutrients and other metabolites in scaffold. Chitosan is a natural organic polymer with cationic charge which is biodegradable, biocompatible, good interaction with cell membranes and non-toxic. It is obtained by the deacetylation of chitin from linear heteropolymer of glycosamine and N-acetyl glycosamine residues. Chitosan is a natural polymer and it is a hydrolyzed form of Chitin [2]. It possess amine and hydroxyl groups which will increase the osteoblast activity and in bone formation [3]. It has the structure similar to glycosaminoglycans, which is a major composition of bone and cartilage [4].

Gelatin is derived by the hydrolysis of collagen. It has numerous benefits in biomedical engineering because of its biodegradability and biocompatibility nature. It contains Arginine-Glycine-Aspartine structure in it; which helps in cell adhesion and migration. It is blended with Chitosan to make it an effective composite scaffold [5]. Glutaraldehyde (GA), is widely used as cross linker to crosslink the biopolymers. Here in scaffold preparation GA is used to polymerize the monomer units of Chitosan and Gelatin and it aids in the formation of polymerized chain. At high pH (more than 7.5), polymerization occurs rapidly. For example at PH 8.5, polymerization occurs rapidly within 4 hours even at 4°C. By adjusting the PH of the Chitosan-Gelatin solution with the help of buffer we can polymerize the proteins present in the solution very rapidly. Also at high temperature GA gives high polymerization. At high temperature and pH in the presence of GA, gives efficient polymerization. The polymerized chain gives high mechanical strength, high porosity, and also we can alter the rate of degradation rate [6]. Biomass (pseudostem) waste, a rich source of natural fibres can be utilized for numerous application and preparation of various products. Natural fibres have important advantages such as low density, stiffness, mechanical properties, high disposability, renewability and they are recyclable and biodegradable. Cellulose is the major composition of the banana fiber. Banana fiber has high mechanical strength, good strength properties, lower density, light weight, smaller elongation, temperature resistant, highly moisture absorbing, and has high biocompatibility, biodegradability [7]. The current study made an attempt to develop scaffolds by using natural source for the soft tissues that make up the organ. By using this scaffold and organ culturing, tissue engineers made to overcome the need of stem cells.

MATERIALS AND METHODS:-

FIBER EXTRACTION:-

Banana pseudo stems were collected from local market in Sathyamangalam and are cleaned and washed. The fresh Pseudo stem (100g) was finely cut and dissolved in 1litre of 0.1M NaOH. NaOH is used to remove the cellulose and cell wall contents. The mixture was kept in room temperature for 2 to 3 days with continuous stirring. After that, the mixture was filtered by using filter paper or 0.5mm sized mesh. The extract was dried at 50°C-70°C for about 12hours in the hot air oven and grind finely for further process. The treatment of banana fiber includes, separating the cellulose fiber by acid treatment for processing. Ultrasonication is used to break the fibers into nano-fibers. These nano-fibers when incorporated in to the Chitosan-Gelatin scaffolds provide high mechanical strength. It plays vital role in the scaffold preparation and application in tissue engineering.



Figure 2.1: Powdered Banana fiber.

The prepared banana fiber extract was treated with different solutions at different concentrations. The 5g of extract was treated with 0.1M of NaOH followed by magnetic stirring at 90°C for 30minutes. The NaOH treated banana fiber solution is taken for further process. The solution is treated with 0.1M of H₂SO₄ for 10minutes in room temperature and filtered with filter paper. Then the filtrate is disposed and residue is taken. The residues are subjected to 0.05N HCl for 10minutes in room temperature and again filter the solution. The residual fiber is kept in hot air oven at 70°C for overnight to remove the moisture content and the sample is prepared and stored at room temperature.

PREPARATION OF PLANT EXTRACT:-

Leaves of *Tridax procumbens* plants was washed, chopped and dried at room temperature for about 15 days until the moisture content of the leaves is completely dried. The well dried materials were ground into fine powder and kept in air tighter zip bags separately. 300g of air dried, powdered material (leaves) was macerated by water successively for 48 hours with occasional stirring. The mixture was then filtered after 48 hours. The filtrates were evaporated to dryness using a rotary evaporator at 45°C under reduced pressure. This maceration process is subsequently carried out for about three to four times and finally the solvent extract is obtained. The obtained aqueous plant extract is thus lyophilized to obtain *Tridax procumbens* powder form.

PREPARATION OF CHITOSAN-GELATIN (CG) SOLUTION:-

1% Chitosan powder is mixed in 1% Glacial Acetic acid solution (1ml acetic acid in 100 ml distilled water) and the solution is maintained at constant magnetic stirring at 60°C for 3 hours to obtain homogenized mixture of the particles. 1% Gelatin (crystals) is mixed in 100 ml of distilled water and the solution is maintained at a constant magnetic stirring at room temperature for 24 hours to obtain homogenized mixture of the particles. The prepared solutions were blended with the help of magnetic stirrer by maintaining a constant stirring. The blended solutions were kept for 24 hours to obtain a homogenous solution.

FABRICATION OF CG BASED FIBROUS SCAFFOLD:-

Chitosan and Gelatin was used as base material for the preparation of fibrous scaffold. 1 ml of each Chitosan and Gelatin solutions were taken and blended to create 3 scaffolds: a. Chitosan and Gelatin (CG) without any extract. b. CG with Banana fiber (CGBF). c. CG with Banana fiber and plant extract *Tridax procumbens* (CGBFPE). The blended solution was mould in to mould structure. The solution was mould into 96 well plate and freeze at very low temperature for 24 to 48 hours. The 96 well plates filled with blended solution are lyophilized for about 48 to 76 hours to obtain spongy scaffold.

PHYSICO CHEMICAL CHARACTERIZATION OF FIBROUS SCAFFOLD:-

Surface morphology of the fibrous scaffold was analyzed by subjecting scaffold to the compound microscope under 40x magnification. The porosity and arrangement of fibers were analyzed. The porosity of the fibrous scaffold is the important in ensuring the cell proliferation and migration into the temporary mimic medium for the cells. More the porosity of the fibrous scaffold more the cells will migrate into the scaffold. The fiber should be arranged in uniform direction so that the cells will easily migrate through the uniform direction and starts proliferation. The uniform arrangement of the fibers and CG solution is done by freeze drying of the scaffold as freeze drying stimulate the drying process evenly.

FTIR ANALYSIS:-

The infrared spectra of the fibrous scaffold were determined by Fourier transform infrared spectroscopy and the spectra had a resolution of 4 cm⁻¹. The FTIR spectra were recorded within the range of 4000 - 500 cm⁻¹. With FTIR results, the functional groups and chemical bonds were analyzed.

SWELLING BEHAVIOR:-

To study the swelling activity of the fibrous scaffold, the fibrous scaffolds were weighed and the dry weight was noted. The weighed dry scaffold is immersed in PBS solution with pH of 7.4 at room temperature. The saturation of the scaffold was noted at a defined set periods. The equilibrated measurements are kept as constant for noting the swelling behavior of the scaffolds. The swelling behavior is measured with the following equation,

$$\text{swelling ratio\%} = \frac{(\text{weight of swelled scaffold} - \text{weight of dried scaffold})}{\text{weight of dried scaffold}} * 100$$

DETERMINATION OF ANTI OXIDANT ACTIVITY BY USING DPPH (2, 2-DIPHENYL-2-PICRYLHYDRAZYL) METHOD:-

DPPH reagent was used to determine the anti oxidant activity of the banana fiber and plant extract incorporated fibrous scaffold. DPPH, a free radical is used to measure the free radical scavenging activity and it is used to evaluate anti oxidant activity of the scaffold. 0.1 mM DPPH was dissolved in ethanol solution and the CG based blended solutions were added. The mixed solutions were allowed to incubate at dark for 1 hour at 25°C. The incubated solutions were measured using UV spectroscopy at 520 nm.

THERMOGRAVIMETRIC ANALYSIS:-

The scaffolds produced were subjected to TGA analysis to check the thermal degradation study. Thermal stability of the scaffolds is important in studying the degradation nature of the scaffold. The rate of degradation is the time that the scaffold stays inside the body. The scaffold provides the temporary mimic medium where the cells migrate into it and starts proliferating inside the scaffold. Hence rate of degradation is important in studying the characteristics of the scaffold.

RESULT AND DISCUSSION:

PREPARATION OF CHITOSAN-GELATIN SOLUTION

The Chitosan and Gelatin each of 1 % solution was prepared and blended with each other.



Figure 3.1: Chitosan blended with Gelatin solution.

FABRICATION OF CG BASED FIBROUS SCAFFOLD:

The blended Chitosan-gelatin solutions along with banana fiber and plant extract were mould into 96 well plates and it was freeze. The refrigerated plate was subjected to lyophilization, where it was vacuum dried.



Figure 3.2: Fibrous scaffolds (CG, CG with banana fiber, CG with plant extract, CG with banana fiber and plant extract)

PHYSICO-CHEMICAL CHARACTERIZATION OF THE FIBROUS SCAFFOLD – POROSITY STUDY:

The produced scaffolds were subjected to porosity check and surface arrangement. The scaffolds porosity is checked with compound microscope with 40x magnification. The results showed that the scaffold is arranged uniformly with spatial arrangement. The increased porosity of scaffold was viewed by this magnification. The porosity determines the cell proliferation and cell migration. The scaffold having more pores will allow the cells to enter in to the temporary mimic environment which allows the cells to proliferate easily.

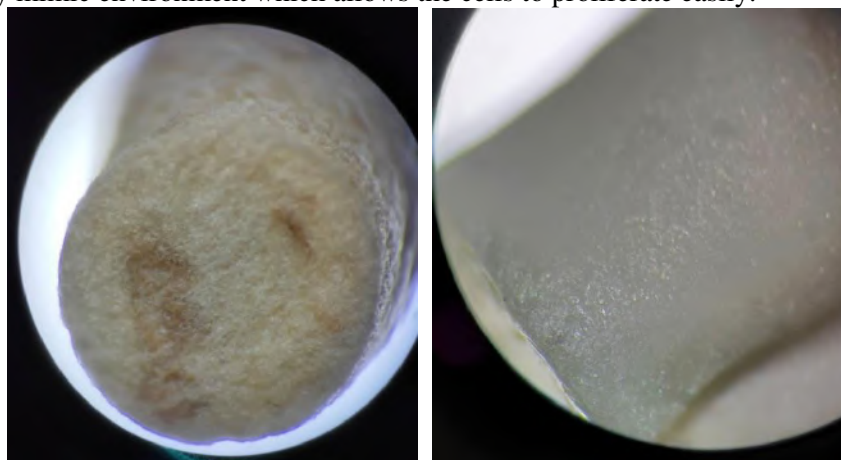
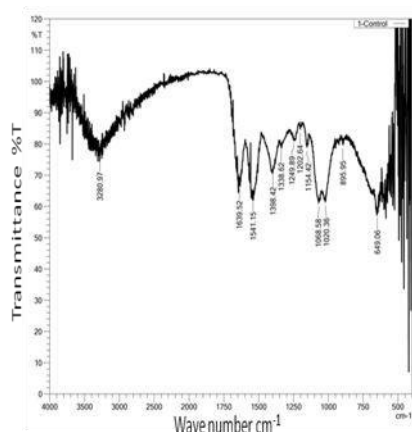


Figure 3.3: Porosity and uniform arrangement of CG based fibrous scaffold.

FOURIER-TRANSFORM INFRARED SPECTROSCOPY ANALYSIS OF THE FIBROUS SCAFFOLD

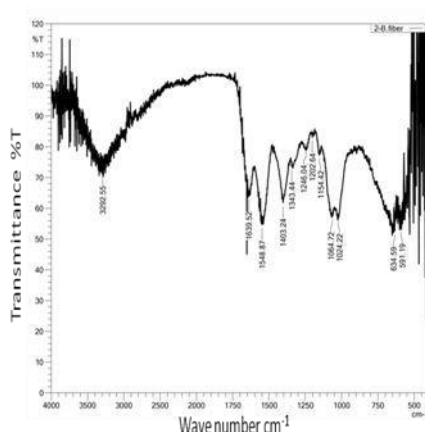
The infrared spectra for the scaffold were obtained. The chemical bonds and functional groups present in the different scaffolds were examined. The examination is done for conformation study. Characteristic bands were obtained and it is observed at $1600-1685\text{ cm}^{-1}$ which shows CO stretch for amide I, $1503-1595\text{ cm}^{-1}$ which shows

NH stretch for amide II, 1350-1375 cm^{-1} which shows CH stretch for amide III and 3115-3250 cm^{-1} in the CG scaffold.



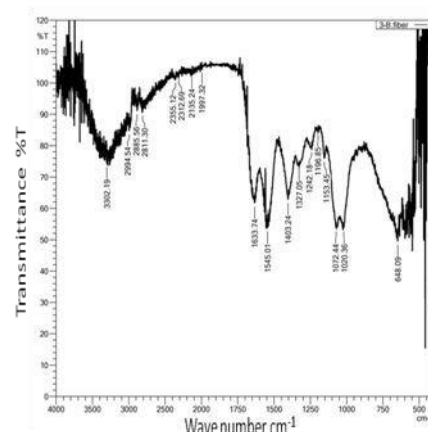
Characteristic bands were obtained and it is observed at 1600-1650 cm^{-1} which shows CO stretch for amide I, 1530-1590 cm^{-1} which shows NH stretch for amide II, 1350-1420 cm^{-1} which shows CH stretch for amide III in the CG based fibrous scaffold with banana fiber.

Figure 3.4.a: Peaks of CG scaffold



Characteristic bands were obtained and it is observed at 1600-1650 cm^{-1} which shows CO stretch for amide I, 1530-1590 cm^{-1} which shows NH stretch for amide II, 1350-1420 cm^{-1} which shows CH stretch for amide III in the CG based fibrous scaffold with banana fiber.

Figure 3.4.b: Peaks of CG with Banana fiber scaffold



Characteristic bands were obtained and it is observed at 1630-1640 cm^{-1} which shows CO stretch for amide I, 1503-1565 cm^{-1} which shows NH stretch for amide II, 1380-1425 cm^{-1} which shows CH stretch for amide III in the CG based fibrous scaffold with banana fiber and plant extract.

Figure 3.4.c: Peaks of CG with Banana fiber and plant extract scaffold

SWELLING BEHAVIOR:

The swelling behavior for the different composition of the scaffolds were examined till equilibrium and weight percentage is calculated based on the dry weight and swell weight of the fibrous scaffolds. All different composition scaffolds showed increased swell ability. The base material Chitosan gelatin blended with banana fiber showed increased retaining of PBS than all other different composition.

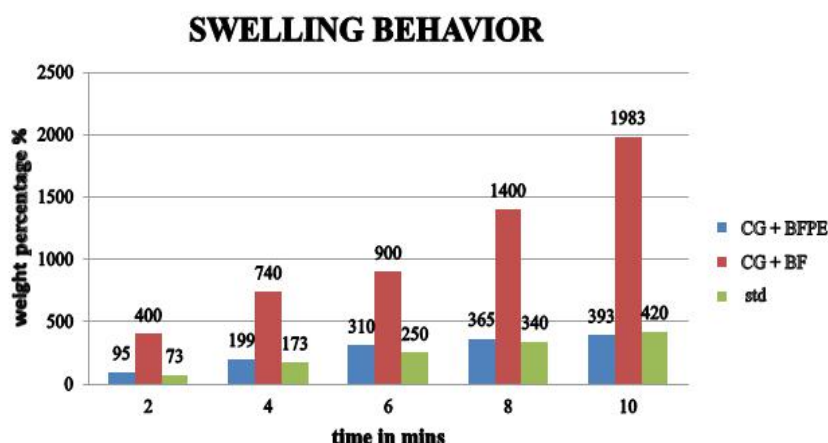


Figure 3.5: Swelling behavior of the scaffolds for different time interval

DETERMINATION OF ANTI OXIDANT ACTIVITY BY USING DPPH (2, 2-DIPHENYL-2-PICRYLHYDRAZYL) METHOD:

The free radical scavenging activity is determined for CG scaffolds, CG scaffolds with banana fiber and also for CG scaffolds with banana fiber ad plant extract. It is determined with ascorbic acid solution as standard.

The anti oxidant activity for CG with banana fiber gets decreased for 0.2 to 1 mg/ml. The activity for the CG with banana fiber and plant extract showed increased scavenging activity of the free radical. The free radical scavenging activity depends on the increased concentration. The CG based scaffold along with banana fiber and plant extract showed increased scavenging activity of 0.36 at 1 mg/ml.

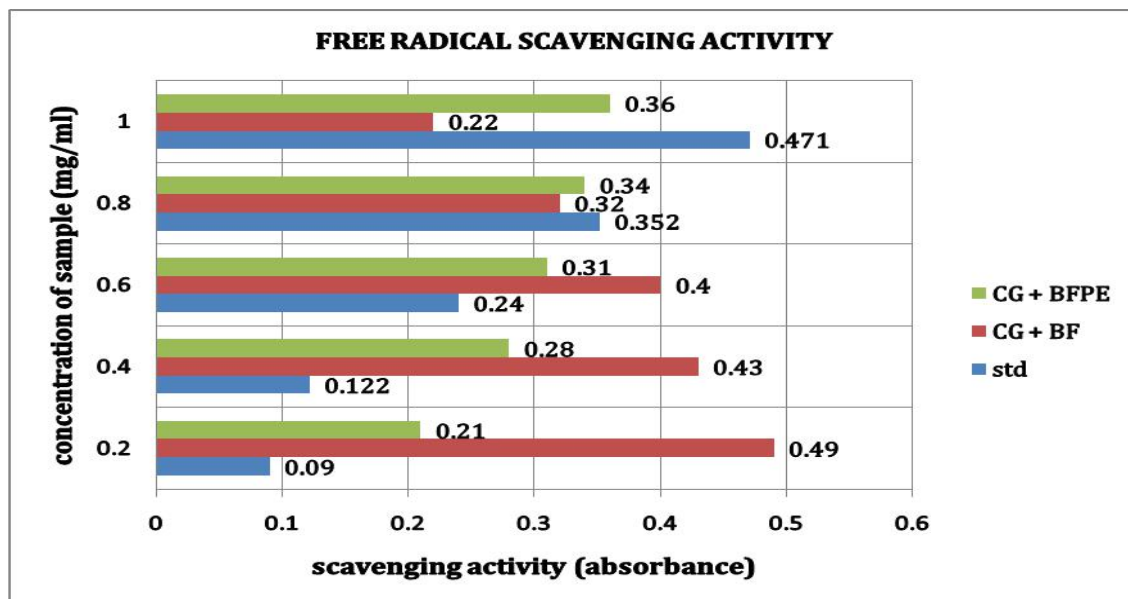


Figure 3.6: DPPH scavenging activity for CG, CGBF, CGBFPE (CG with banana fiber and plant extract of *Tridax procumbens*)

THERMOGRAVIMETRIC ANALYSIS OF THE FIBROUS SCAFFOLDS:

The scaffolds that were subjected to the TGA study showed excellent rate of degradation and showed very good thermal stability even under high temperature. Figure 3.7.b and c indicates that the scaffolds can withstand slightly higher temperature than compared to control. This shows that the scaffolds will stay inside for the body for a certain period of time from months to years which shows that the scaffolds provide time for the cells to stay inside and proliferate to form permanent medium.

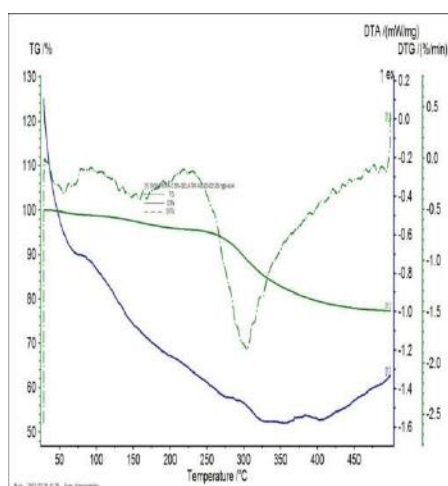


Figure 3.7.a: TGA analysis of CG scaffold as control



Figure 3.7.b: TGA analysis of CG scaffold with banana fiber.

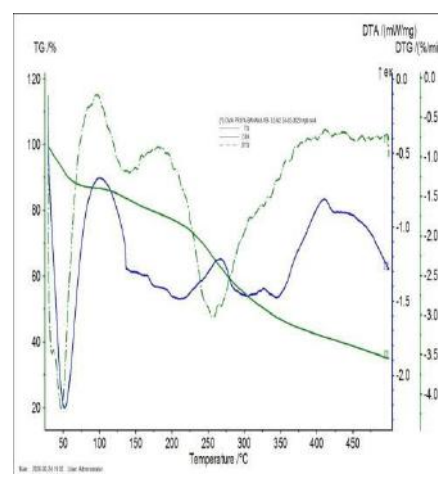


Figure 3.7.c: TGA analysis of CG scaffold with banana fiber and plant extract

CONCLUSION:

CG based fibrous spongy scaffolds with banana fiber and plant extract of *Tridax procumbens* were prepared and analyzed for their physicochemical properties. By using the freeze drying method the highly porous uniform structured scaffolds were produced. The physicochemical characterization showed increased porosity, highly swelling activity of scaffold when banana fiber is incorporated. FTIR study proved the presence of essential bonds

which makes scaffold highly efficient, DPPH activity proved that scaffolds free radical scavenging activity increases with increase in concentration, TGA showed that the scaffolds have high thermal stability nature. Together with all properties, the CG based banana fiber scaffold with plant extract will be a promising novel scaffold for wound dressing applications in tissue engineering.

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Detection of blanket worm with tea leaf using sensor

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Abstract: Tea is a popular beverage all around the world. Processing of tea plays a vital role. There is a chance of many diseases and allergic to because due to the presence of *Lymantria dispar* Linnaeus (blanket worm) along with tea leaf during the process. To solve this problem sensor is the best option to detect and remove the blanket worm. The main goal of this research is to develop a sensor that is more sensitive, so that when the hairy blanket worms pricks the sensor during the process it could sense and remove the blanket worm. The latest trends of research in agriculture are toward the use of sensors to develop disease resistant and to increase food quality and productivity of the plant with reduced expenditure. However research into diseases of tea leaves is one area that has not yet seen any significant efforts

Key Words: Tea plant, Blanket worm, sensitive sensor.

INTRODUCTION:

India is one of the largest producers of tea in the world, second only to China. Although approximately 75% to 80% of the tea produce is consumed within the country. Assam Tea, Darjeeling Tea, Kangra Tea and Nilgiri Tea are registered as Geographical Indications which means these names can be used exclusively by tea producers of the respective areas [1-3]. *Lymantria dispar* Linnaeus: General anatomical features of the *Lymantria dispar* (Linnaeus) reveal a well-developed caterpillar. The head capsule shows the frons as being the antennal segment; the epistomal sulcus invaginated internally to form a connection with the tentorium, and the absence of an ecdysial cleavage line [4,5]. The head capsule does not reveal anterior tentorial pits externally. Anatomy of the thoracic and abdominal regions shows a total of twelve segments circumvented with setae arranged in tufts. Segments one, four, five, six, seven, eight, nine, ten, and eleven have paired spiracles. Here, the response of *Lymantria dispar* Linnaeus to the content of tea leaf is worst. They do a great deal of damage to tea production and consuming quite a bit of crop during the 2 to 3 weeks. Very often food of their choice is also the food of our choice. The latest trends of research in agriculture are toward the use of gene technology to develop worm resistant variant of the plant, and to increase food quality and productivity of the plant with reduced expenditure [6,7]. Numerous technological improvements are responsible for the progress in crop management techniques in recent times; including advances in and deploy an system for detection, identification in tea plant at processing unit with minimal manual input. However, research into diseases of tea leaves due to blanket worm is one area that has not yet seen any significant efforts.



Figure 1

LITERATURE REVIEW:

- A. Matthew Wright:

In particular, a worm in a sensor network requires along sequence of packets propagating hop-by-hop to each new infected node in turn. We thus have detectors that observe communication patterns in the network, a worm

spreading hop-by-hop will quickly create chains of connections that would not be seen in normal traffic. Once detector nodes identify the worm propagation pattern, they initiate remote software attestations to detect infected nodes

- *Katie Pratt:*

Two non invasive sensing methods, acoustic emission and hyper spectral imaging, to greatly increase the rapid detection of worm-infected. This will increase detection accuracy, assurance and effectiveness without physically cutting the apple.

- *Clare Liptak and Dr.Timothy Motis:*

Walking through a field to observe pest problems is an important part of any monitoring approach. However, many worms are active at night when it would not be practical for a farmer to be in the field or garden. There are also days when the farmer is away. These limitations are overcome, at least in part, by using any type of container or device that traps enough worms to give the farmer an indication of what species are present. Monitoring traps function day and night and can be made with local materials, such as plastic water or juice bottles. Traps can be placed to target various types of worms.

- *BizIntellia:*

Detecting bugs and rodents through sound detection is another effective way to ensure plants quality. Wireless acoustic sensors situated at random locations in a field can pick up sound waves of insects. Locations with high sound waves indicate a higher concentration of bugs. A farmer can thus spray pesticides on these locations to ensure the quality of crops.

- *E.SaeedAzfar, Adnan Nadeem, and Abdul Basit:*

Genetic control is the method of pest management where the crops are genetically altered so that they are resistant to pest and diseases caused by pests. Crops can be genetically altered in ways that produce chemical or physical barriers to prevent harm from pest. Genetic control has made it possible to reduce the harm done by pests. Genetic modification has so many benefits including longer shelf life, enhanced agronomic traits, insect resistance and tolerance to various environmental agitations.

SPECIFICATION:

Piezoelectricity is the charge created across certain materials when a mechanical stress is applied. Piezoelectric pressure sensors exploit this effect by measuring the voltage across a piezoelectric element generated by the applied pressure. They are very robust and are used in a wide range of industrial application. There are several ways in which piezoelectric sensors function. Piezoelectric material consists of polarized ions within the crystal. A piezoelectric sensor applies pressure on the piezoelectric crystal in proportion to the charge output. The resultant displacement in the ions within the crystal position is measured and recorded using piezoelectric vibration sensors. A piezoelectric accelerometer has a charge frequency response capacity ranging from 20Hz to 10KHz. A piezoelectric accelerometer can have electromagnetic sensitivity of 0.0009 equiv.gm/gm. and base strain sensitivity 0.008 equiv.gm/micro strain. Piezoelectric force sensors should display a 5-volt full scale signal. Piezoelectric force sensors should have an operating temperature range from -50 to 350°C and should have Sensitivity of approximately 105pC/N. Piezoelectric pressure sensors should have rise time less than 2.0 micro seconds. The maximum pressure applied by piezoelectric sensors can be 1,000 psi and the voltage measurement range can be up to 5volts. Piezoelectric sensors are designed and manufactured to meet most industry specifications. Two main groups of materials are used for piezoelectric sensors: piezoelectric ceramics and single crystal materials. The ceramic materials (such as PZT ceramic) have a piezoelectric constant/sensitivity that is roughly two orders of magnitude higher than those of the natural single crystal materials and can be produced by inexpensive intermingling processes. The piezo effect in piezo ceramics "trained", so their high sensitivity degrades overtime. This degradation is highly correlated with increased temperature. The less-sensitive, natural, single crystal materials (gallium phosphate, quartz, and tourmaline) have a higher—when carefully handled, almost unlimited—long term stability. There are also new single-crystal materials commercially available such as Lead Magnesium Niobate-Lead Titanate (PMN-PT). These materials offer improved sensitivity over PZT but have a lower maximum operating temperature and are currently more expensive to manufacture.

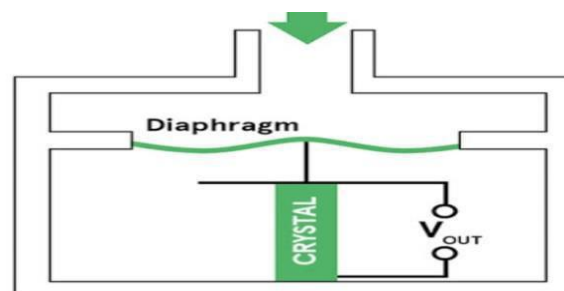


Figure 2

RESEARCH METHODOLOGY:

This paper proposes a system which is based on a sensor that detects the presence of blanket worm in tea leaf. A pain sensing device includes a sensor array including a plurality of sensors that sense pressure generated due to a contact of an object; and a signal process or configured to recognize the shape of the object, and to generate a pain signal according to the recognized shape of the object. One or more Embodiments relate to methods and devices for sensing a pain, and more particularly, to a method and device for sensing a pain generated due to a contact of an object by using a piezoelectric material. For human beings, psychological feeling such as softness, roughness or pain is important for interactions with other humans and objects. Furthermore, pain is an essential feeling that protects the human body from sharp objects such as a knife, a needle, or a nail. However, existing artificial tactile sensors so far simply detect pressure without generation of psychological feelings.

- *Sensing pressure generated due to a contact of an object and outputting electrical piezoelectric signals, where in the sensing of the pressure and the outputting of the electrical piezoelectric signals are performed by a plurality of sensors included in a sensor array.*
- *Recognizing a shape of the object, based on a number of sensors that output the electrical piezoelectric signals among the plurality of sensors, where in the recognizing is performed by a signal processor.*
- *Generating a pain signal according to a recognized shape of the object, where in the generating is performed by the signal processor.*
- *When the shape of the object is recognized, the generating the pain signal comprises:*
 - ✓ *Comparing a voltage value of the electrical piezoelectric signals with a critical voltage value in order to recognize the shape of the object as a pointy or sharp shape; and*
 - ✓ *Generating the pain signal when the voltage value of the electrical piezoelectric signals is equal to or greater than the critical voltage value.*

RESULT AND DISCUSSION:

As a result, piezoelectric sensors are not normally suitable for measuring static pressure. The output signal will gradually drop to zero, even in the presence of constant pressure. They are, however sensitive to dynamic changes in pressure across a wide range of frequencies and pressures. A piezoelectric accelerometer is widely used for OEM applications and is suitable for working at a lower power consumption and wider frequency range. Piezoelectric force sensors are low impedance voltage force sensors designed for generating analog voltage signals when a force is applied on the piezoelectric crystal and are widely used in machines for measuring force. A piezoelectric pressure sensor is also known as a piezoelectric sensor pressure. Piezoelectric pressure sensors are used for measuring change in liquid and gas pressure. Other piezoelectric sensors are commonly available.

CONCLUSION:

In this research, an automated system has been developed for detecting worm in tea leaves using piezoelectric pressure sensor with less number of features. The proposed method is able to detect the worms more accurately (93%) compared to the traditional methods. This method can also be extended to reduce and extract the features that are required there by reducing the required processing time.

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Optimization of Chlorophenol Degradation using *Aspergillusniger*

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Abstract: In the present investigation, *Aspergillusniger* which can utilize chlorophenol as a sole source of carbon and energy was selected for the degradation of chlorophenol. The central composite design (CCD) of RSM was employed to optimize four process parameters namely Initial phenol concentration, pH, and temperature and inoculum concentration for the removal of chlorophenol. The four independent variables were studied at five different levels and a set of 30 experiments were carried out and the results were analysed by ANOVA. It was shown that a second order polynomial regression model could properly interpret the experimental data with an R^2 value of 0.9319 and an F -value of 19.03 based on which the maximum degradation of phenol was estimated up to 93% within the range examined.

Keywords: Biodegradation, chlorophenol, *Aspergillusniger*, Central Composite Design.

INTRODUCTION:

Phenolic compounds are used for various purposes in many areas, for example chlorinated phenolic compounds are specifically utilized as preservatives of paint, leather, textile goods and as antimicrobial agents. They are produced in petrochemical plants and processes of chlorine bleaching of excessive pulp [1]. Because of improper treatment of these materials, they have widely contaminated soil and groundwater and their toxicity seriously affects living organisms. They are suspected carcinogens and well-known as precursors of dioxin [2]. To treat phenolic compounds, biological methods are preferable because this is economical, and there is a low possibility of the production of byproducts. Many papers support the biological treatment of waste or ground water. The microorganisms used are usually aerobes, including *Pseudomonas* sp. [4–6], *Alcaligenes* sp. [3,7], *Azotobacter* sp. [8], *Rhodococcus* sp. [9,10], *Phanerochaete* sp. [11,12], and *Cryptococcus* sp. [13]. These aerobes are more efficient at degrading toxic compounds because they grow faster than anaerobes and usually transform organic compounds to inorganic compounds (CO₂, H₂O). The difference of structure and toxicity among phenolic compounds requires that various bacteria have specific qualities to degrade each compound and a mixed culture may be applied. Mixed culture means that several kinds of microorganisms are cultured simultaneously. Mixed culture is divided into undefined and defined types. The activated sludge process is one example of an undefined mixed culture. Activated sludge is a complex group of microorganisms that can oxidize wastewater under aerobic conditions. To treat special wastewater, including very toxic and recalcitrant compounds by activated sludge, adaptation is generally needed. If microorganisms that can degrade these materials are added, the adaptation time might be reduced and the efficiency may increase. To do this, a study of pure cultures or defined mixed cultures is important. A defined mixed culture is a miniature of complex microorganisms in nature. Through the study of this, various interactions between microorganisms in nature may be analyzed. In fact, many papers concerned with mixed culture have been reported. Morsen and Rehm [13] studied a mixed culture of *Pseudomonas putida* and *Cryptococcus elinii* to degrade phenol, and Oh et al. [14] reported a mixed culture of known bacteria (*Flaobacterium* sp. BEN2, *Acinetobacter* sp. GEM63 and GEM2) and sludge to treat industrial wastewater. Also, Wiesel et al. [15] used five kinds of bacteria to treat polycyclic aromatic hydrocarbons. In the present study paper, statistical optimisation was applied to investigate significant variables between Initial phenol concentration, pH, temperature and inoculum concentration to optimise growth and phenol degradation capability of sp. *Aspergillus niger* using RSM.

MATERIALS AND METHODS:

MICROORGANISM AND CULTURE CONDITIONS:

Aspergillusniger employed in the present study was used for biodegradation of chlorophenol. The microorganism was maintained in Czapek Yeast Extract Agar (CYA) agar slant at (4 ± 1)°C and subcultured monthly. Inoculum was prepared by transferring two loopfuls of the microorganism from CYA agar slant into a 250- mL

Erlenmeyer flask containing 100 mL CYA liquid medium. The flask was incubated in a shaking incubator at 30°C and 150 r/min for 24 hr. After repeated centrifugation at 10,000 r/min for 20 min at 4°C and washing with sterile water, harvested cells were diluted to 1.5×10^8 CFU/mL Basal medium used in chlorophenol degradation included (g/L): NH_4NO_3 , 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; K_2HPO_4 , 1.5; KH_2PO_4 , 0.5; CaCl_2 , 0.01; trace element solution, 1 mL. Trace element solution included (g/L): FeSO_4 , 0.1; ZnSO_4 , 0.1; NaMo_2O_4 , 0.01; CoCl_2 , 0.1; CuSO_4 , 0.01 and H_3BO_3 , 0.01. Batch mode shake flask experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL degradation media, which based on the basal medium.

BATCH BIODEGRADATION EXPERIMENTS:

The factors affecting the growth of chlorophenol degradation rate of growing *Aspergillus niger* were examined in 250 mL Erlenmeyer flask with 50 mL accumulation medium. The accumulation medium was prepared by mixing 25 mL of aqueous chlorophenol solution with 25 mL of Czapek Yeast Extract medium. The pH of the Czapek Yeast Extract medium was adjusted to the desired value by adding acid or alkali solutions. CYA medium was autoclaved separately at 1.1 kgf/cm² gauge pressure for 15 min. A known amount of microorganism suspension (10% (v/v)) was added to the accumulation medium and the cultures were grown at 30°C for 7 days on a rotary shaker at 100 rpm constant shaking rate. This shaking frequency supplied the culture with enough oxygen to attain logarithmic growth. For each concentration a non-inoculated media was served as blank. The dry samples were drawn at predetermined time intervals and analyzed for residual chlorophenol concentration and biomass concentration. The residual concentration in the medium was determined.

Experiments were conducted with 50 mL of Czapek Yeast Extract medium containing 30 g/L of glucose under no inhibition conditions. To study the type of inhibition, experiments were also conducted with 25 mL of Czapek Yeast Extract medium containing 30 g/L of dextrose and 25 mL of 400 mg/L aqueous chlorophenol. The cultures were adjusted with pH 7.3 and inoculated for 7 days at a temperature of 30°C under aerobic conditions. The samples were drawn at pre-determined time intervals to analyze for biomass concentration and residual glucose concentration. The glucose concentration was measured at 540 nm using dinitrosalicylic acid method.

RESPONSE SURFACE METHODOLOGY (RSM):

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. The CCD is used to study the effects of the variables towards their responses and subsequently in the optimization studies. This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interaction between the parameters. In order to determine the existence of a relationship between the factors and the response variables, the data collected are analyzed in a statistical manner, using regression. A regression design is normally employed to model a response as a mathematical function (either known or empirical) of a few continuous factors and good model parameter estimates are desired (Montgomery, 2001).

The coded values of the process parameters are determined by the following equation

$$x_i = \frac{X_i - X_0}{\Delta x}, \quad \dots (1)$$

where x_i – coded value of the i^{th} variable, X_i – uncoded value of the i^{th} test variable and X_0 – uncoded value of the i^{th} test variable at center point. The regression analysis is performed to estimate the response function as a second order polynomial

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j \quad \dots (2)$$

where Y is the predicted response, β_0 constant, β_i , β_j , β_{ij} are coefficients estimated from regression. They represent the linear, quadratic and cross products of X_i and X_j on response.

RESULTS AND DISCUSSIONS:

OPTIMIZATION OF PROCESS PARAMETERS FOR THE REMOVAL OF PHENOL USING ASPERGILLUSNIGER:

The central composite design (CCD) of RSM was employed to optimize four process parameters namely Initial phenol concentration, pH, and temperature and inoculum concentration for the removal of phenol. The four independent variables were studied at five different levels (Table 1) and a set of 30 experiments were carried out (Table 2) and the results were analyzed by ANOVA.

Table 1 Range and levels of process variables

Variables	Code				
	-2	-1	0	1	2

Temperature (°C)	25	28	31	33	35
pH	3	5	7	9	10
Innoculum size	3	5	7	9	11
Initial Concentration	100	200	300	400	500

The relationship and interrelationship of the variables were determined by fitting the second-order polynomial equation to data obtained from 31 experiments. The response values (Y_1 and Y_2) used in each trial was the average of the duplicates. The second-order regression equations provided the levels of phenol removal as a function of Initial phenol concentration, pH temperature and inoculum concentration which can be represented in terms of coded factors as in the following equations:

$$\text{Removal of Phenol} = 97.86 + 2.67 * A + 3.83 * B + 2.42 * C + 0.083 * D + 0.50 * A * B - 3.38 * A * C + 0.25 * A * D - 1.37 * B * C + 4.50 * B * D + 1.87 * C * D - 3.53 * A^2 - 4.2 * B^2 - 4.28 * C^2 - 3.65 * D^2$$

A , B , C and D are the coded values of Initial phenol concentration, pH temperature and inoculum concentration respectively. ANOVA for the response surface is shown in Table 3

Table 2. CCD design matrix with experimental and predicted values for removal of Chlorophenol using *Aspergillus niger*

Run No.	Initial Chlorophenol Concentration	pH	Temperature	Inoculum size	Chlorophenol removal efficiency	
					Experimental	Predicted
1	-1	-1	-1	-1	71	52.8674
2	1	-1	-1	-1	87	62.4813
3	0	0	0	0	95	74.7874
4	0	0	0	2	81	59.7943
5	0	2	0	0	89	65.4884
6	-1	1	-1	1	75	58.2822
7	0	0	0	0	95	74.7874
8	0	0	0	0	95	74.7874
9	-1	-1	-1	1	63	38.7739
10	1	-1	1	-1	83	60.6155
11	0	0	-2	0	71	52.2536
12	-1	-1	1	-1	84	63.0940
13	1	1	1	-1	75	57.2162
14	0	0	0	0	95	74.7874
15	1	-1	1	1	75	54.8999
16	0	0	0	0	95	74.7874
17	0	0	0	-2	79	60.3179
18	-1	1	1	1	87	70.5088
19	2	0	0	0	82	65.3795
20	-1	1	-1	-1	74	53.0902
21	-1	-1	1	1	77	57.9081
22	1	1	-1	-1	86	65.9896
23	1	1	-1	1	91	70.8960
24	0	0	2	0	84	62.8586
25	-1	1	1	-1	76	56.8977
26	0	-2	0	0	67	49.6238
27	-2	0	0	0	79	55.7327
28	1	1	1	1	93	71.0303
29	0	0	0	0	95	75.0316
30	0	0	0	0	94	75.0316
31	1	-1	-1	1	71	52.8674

To test the fit of the model equation, the regression-based determination coefficient R^2 was evaluated, which is the proportion of variation in the response attributed to the model rather than to random error. The closer the values of R^2 to 1, the better the model would explain the variability between the experimental and the model predicted values. The

coefficient of determination (R^2) for phenol removal are calculated as ($R^2=0.9319$) which are nearly equal to 1) 93.18 % variability of the response, and only about 5.24% of the total variation cannot be explained by the models. The predicted R^2 value of phenol removal was 63.45 % has a reasonable agreement with the adjusted R^2 value of 93.18 %. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Adeq Precision of 17.942 indicates an adequate signal. These models can be used to navigate the design space. The statistical significance of Eq. was checked by F - test, and the analysis of variance (ANOVA) for the response surface quadratic model is shown in Table. The results demonstrated that the model is highly significant and is evident from Fischer's F - test with a low probability value (P model $> F$ less than 0.05). Model coefficients estimated by regression analysis for each variable is shown in Table. The significance of each coefficient was determined by t -values and P -values. The larger the magnitude of t -test value and smaller the P -value indicates the high significance of the corresponding coefficient.

Table 3 Analysis of variance (ANOVA) for Chlorophenol removal

Source	Coefficient factor	Sum of squares	DF	F	P > F
Model	2618.08	14	187.01	20.68	< 0.0001
A	170.67	1	170.67	18.87	0.0005
B	352.67	1	352.67	39	< 0.0001
C	140.17	1	140.17	15.5	0.0012
D	0.17	1	0.17	0.018	0.8937
AB	4	1	4	0.44	0.5155
AC	182.25	1	182.25	20.15	0.0004
AD	1	1	1	0.11	0.7438
BC	30.25	1	30.25	3.35	0.0861
BD	324	1	324	35.83	< 0.0001
CD	56.25	1	56.25	6.22	0.024
A ²	355.68	1	355.68	39.33	< 0.0001
B ²	523.04	1	523.04	57.84	< 0.0001
C ²	523.04	1	523.04	57.84	< 0.0001
D ²	381.34	1	381.34	42.17	< 0.0001
Residual	144.69	16	9.04		
Lack of Fit	143.83	10	14.38	100.68	< 0.0001
Pure Error	0.86	6	0.14		
Cor Total	2762.77	30			

Std. Dev.-3.02, R-Squared- 0.9319, Mean- 85.68, Adj R-Squared- 0.9018, C.V. %- 3.51, Pred R-Squared- 0.6345, PRESS- 829.65, Adeq Precision-17.942

In the present work, the linear effects of A, B and C , the interactive effects of BD and squared effects of A^2, B^2, C^2 and D^2 are significant model terms for phenol removal. To test the fit of the model equation, the regression-based determination coefficient R^2 was evaluated. The nearer the values of R^2 to 1, the model would explain better for variability of experimental values to the predicted values. The above models can be used to predict the within the limits of the experimental factors. Fig. shows that the actual response values agree well with the predicted response values of phenol removal. The interaction effect of the variables on phenol removal was investigated by plotting the 3D response surfaces with the vertical (Z) axis representing removal efficiency (response) yield and two horizontal axes representing the coded levels of two explanatory factors, while maintaining other variables at their median levels are shown in Fig.

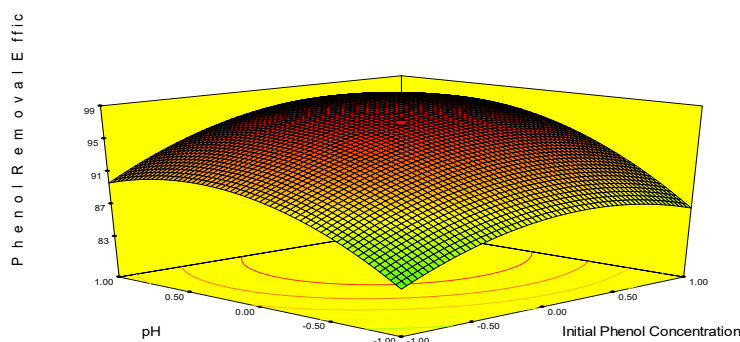


Figure 1 3D plot shoes the effect of pH and initial phenol concentration on removal efficiency of phenol using *Aspergillus niger*

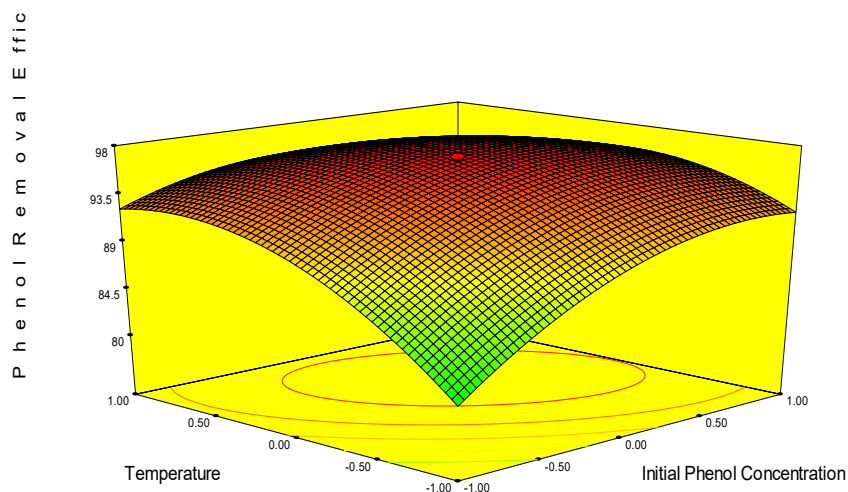


Figure 2. 3D plot shows the effect of temperature and initial phenol concentration on removal efficiency of phenol using *Aspergillus niger*

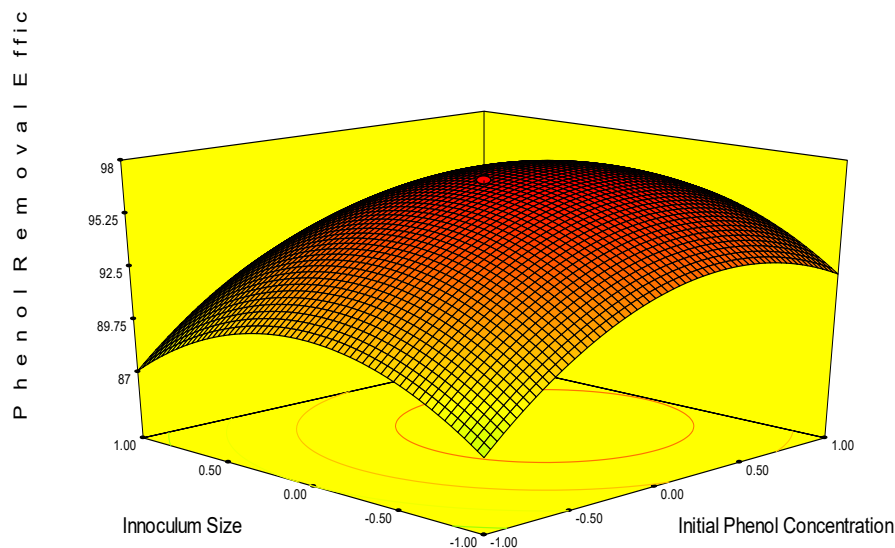


Figure 3 3D plot shows the effect of temperature and initial phenol concentration on removal efficiency of phenol using *Aspergillus niger*

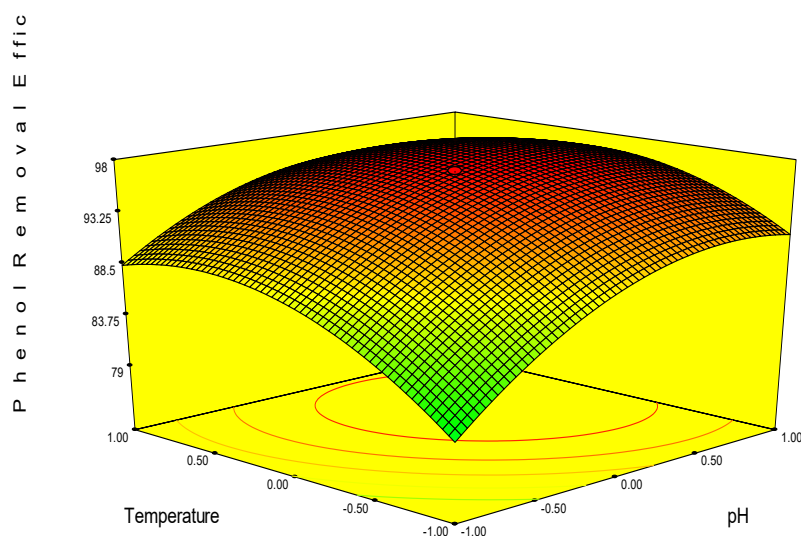


Figure 4. 3D plot shows the effect of temperature and pH on removal efficiency of phenol using *Aspergillus niger*

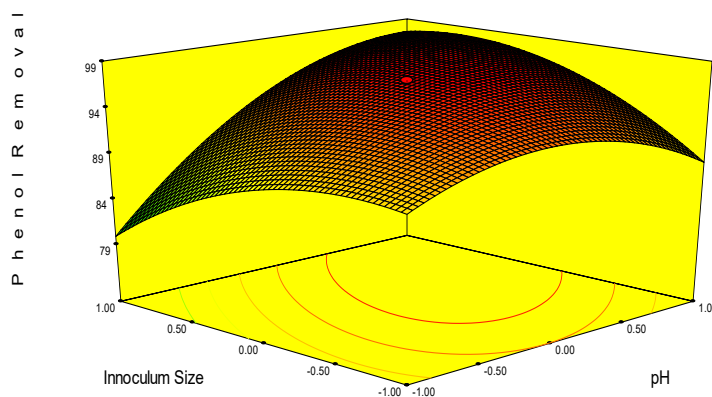


Figure 5. 3D plot shows the effect of inoculum size and pH on removal efficiency of phenol using *Aspergillus niger*

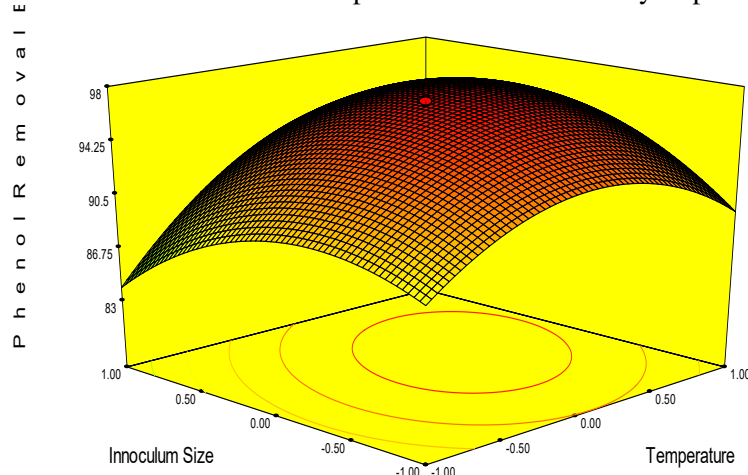


Figure 6. 3D plot shows the effect of temperature and inoculum size on removal efficiency of phenol using *Aspergillus niger*

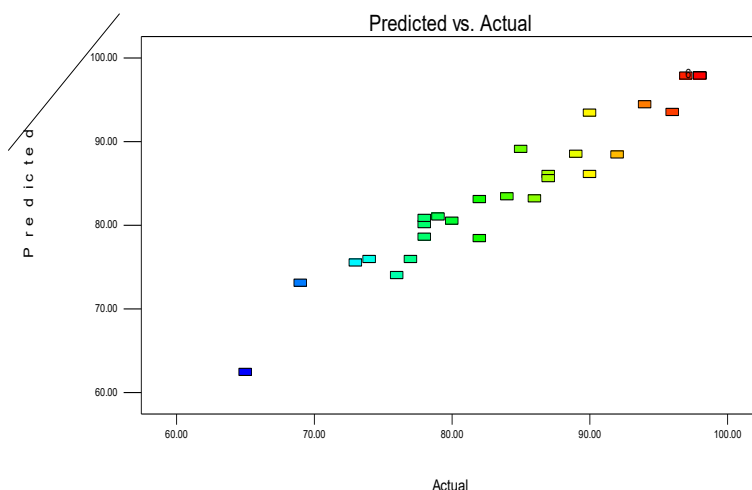


Figure.7 Parity plot showing the distribution of experimental vs. predicted values of percentage chlorophenol degradation.

The parity plot (Figure 7) showed a satisfactory correlation between the experimental and predicted values (obtained from Eq.3) of percentage chlorophenol degradation, wherein, the points cluster around the diagonal line which indicated the optimal fit of the model, since the deviation between the experimental and predicted values was minimal.

EFFECT OF INITIAL CHLOROPHENOL CONCENTRATION AND TEMPERATURE

The effect of initial chlorophenol concentration was vital role in the degradation process. It was observed from the Figs1-3. The removal efficiency of chlorophenol increases with increase in initial chlorophenol concentration up to

150mg/l and there after decreases with further increase in initial chlorophenol concentration. The rapid decrease of RE suggested that substrate inhibition might occur. Substrate inhibition is well known to predominate at a higher chlorophenol concentration owing to its toxicity to cell. The Fig4 and 6. shows the effect of temperature on removal efficiency of chlorophenol. Increase in temperature favors removal efficiency of chlorophenol till 30 °C and does not further. Incubation temperature plays an important role in the metabolic activities of microorganism. A higher temperature alters the cell membrane composition and stimulates protein catabolism, thus, causes the cell death. Optimum temperature recorded for maximum chlorophenol removal was at 30°C.

EFFECT OF INITIAL PH AND INOCULUM CONCENTRATION

The experiment was conducted at different initial pH (4- 8). It was observed in Fig.4 which shows the effect of initial pH on removal of chlorophenol. The removal efficiency of chlorophenol increases with increase in initial pH up to 6 and thereafter chlorophenol removal decreases with further increase in initial pH. Higher number of microorganism restrict microbial activity due to nutrient limitations whereas a lower amount of inoculation causes lower number of cells in the production medium thus consuming the lesser chlorophenol (Nagar et al., 2010). The Fig.5&6. shows the effect of inoculum concentration on chlorophenol removal. The removal efficiency of chlorophenol increases with increase in inoculum concentration up to 12 g/l and thereafter removal efficiency decreases with further increase in inoculum concentration.

CONCLUSION

From the above study it was concluded that as the chlorophenol is one of the major effluent of so many chemical industries and as it is causing lethal effect to the human system. So, it has to be treated to control its toxic effects. So we have chosen a simple, cost effective method known as biodegradation to degrade the chlorophenol and other effluents to protect the environment. In the present study we used one fungal culture *Aspergillus niger* as a biosorbant to degrade the chlorophenol and we fixed the optimum parameters for the maximum degradation of chlorophenol

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Biodegradable sanitary napkin preparation using *Ananas comosus* leaf fibers: an agricultural waste

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Abstract: Disposable absorbent hygiene products have evolved for superior performance, enhancing the convenience of daily lives. However, the use of disposable hygiene pads has brought safety concerns on chemical exposure and significant efforts have been made to assess the potential risk of health associated with the use of hygiene pads. By highlighting, the issues of non-biodegradability of personal hygiene product and how it has become a serious environmental concern all over the world.

Emphasis is given to use naturally available absorbent fibers which are widely available, biodegradable in nature and having low carbon footprint which makes it eco-friendly. This paper deals with the screening of natural fiber - pineapple leaf fiber (PALF) extracted from agricultural waste i.e., pineapple leaves. Absorption capacity of menstrual blood have been screened for the agro waste layered absorbent pad with altered suitable raw material of sanitary napkin's layer construction. Prepared napkin was validated by test methods of exposure assessment, risk characterization, patch test and "Behind the Knee" (BTK) clinical test. Biodegradation efficacy of prepared napkin shows better results. As the product is biodegradable, prevent non-biodegradable waste generation. In conclusion, it reviews that pineapple leaf fibers and altered raw materials preferred for sanitary napkin preparation shows more absorbing capacity and it overcomes the risk hazards caused by pre-hygiene pads. Sustainability of hygiene product can be attained by replacing petroleum based raw material with an eco-friendly one.

Keywords: Absorbent fibers, Agricultural waste, Retention of fluid, PALF.

INTRODUCTION

Women in the developing country lack awareness of basic sanitation during their menstrual days. On average, a woman uses approximately 15,000 pads over the course of her lifetime [1]. A packet of 7 pads costs Rs.80, which is significantly more than the daily income of the many poor families. Moreover, government, NGOs and other stakeholders have been providing free pads to some poor schoolgirls. However the provision may not be sustainable in the long run since to provide pads to post-pubescent poor schoolgirls would cost about 2000cr per year, increasing at nearly 5% yearly.

In India, women between the age 15-64 population rate is about 359,802,209 in that 54%-58% of the population live in abject poverty hence commercial pads are too expensive for most underprivileged schoolgirls and young women [1]. Girls who can't afford the sanitary pads opt for low cost reusable cloth pads which however, are unhygienic. Some even use crude low absorbent materials such as cotton

Currently, the world is facing a very big problem of carbon footprint of feminine hygiene product [2]. As there is a huge amount of non-biodegradable material dumped in landfill, which releases harmful gasses into the atmosphere. India being a developing country, with a population of 1.34 billion, out of which 323.6 million female between the age group of 15-49. If we consider that 10% of Indian women use disposable sanitary pad then each individual will generate at least half a kilo of waste a month.

1.1 Hygiene risk - Improvisation led to introducing SAP to increase absorbency, they began to be made of polypropylene (PP) based perforated top sheet and polyethylene sheet as barrier. This improved its functionality. These products seem innocuous but they may be laced with dioxins, petrochemicals, GMO (Genetically Modified Organisms) and fragrances [1]. When these chemicals come in contact with sensitive skin, tissue may get irritated. Dioxins are carcinogenic in nature hence the risk of cancer increases even at very low levels of exposure. Additives such as fragrances, deodorants, absorbency agents, urea and formaldehyde enhance the properties but can cause allergies and skin reactions.

1.2 Disposability - Polymers in sanitary pads are non-biodegradable material. This may create many serious problems. Sanitary pads are made of SAP [1]. When these pads are flushed, they block sewage lines as these chemicals absorb all the water in the sewage line. Disposed used sanitary pads cause occupational hazards for waste pickers who use their bare hands to sort out garbage. This is a health hazard. The common practice is to incinerate used sanitary pads. This releases dioxins and furans, creating an environmental hazard. In that way, 10% of the female population in India will generate 16180 tons of waste every month. In order to deal with it, we need to focus on developing a more sustainable product by choosing the raw material having low carbon footprint.

Therefore, this research evaluated the feasibility of developing highly absorbent, antibacterial biodegradable sanitary napkin preparation using pineapple leaf fiber and altered components for top sheet and barrier sheet.

LITERATURE REVIEW

Sanitary napkin, sanitary towel, sanitary pad, menstrual pad is an absorbent item worn in the underwear when menstruating, bleeding after giving birth, recovering from gynecologic surgery, experiencing a miscarriage or abortion, or in any other situation where it is necessary to absorb a flow of blood from the vagina [2].

Hygiene practices have effects on vulvo vaginal microbiota [3]. Specific products for intimate female hygiene are available in the market, such as the sanitary pads. Since these pads were introduced in the market, they became the focus of research that seek to improve their shape, manufacturing processes and the properties of materials used in order to provide more benefits to users [3].

The issues of non-biodegradability [2] of personal hygiene product and how it has become a serious environmental concern all over the world. Emphasis is given to use naturally available absorbent fibers such as organic cotton, banana fiber, jute, bamboo etc., [2] which are widely available and biodegradable in nature having low carbon footprint which not only makes it eco-friendly but also reduces the cost of sanitary pad. Sustainability of hygiene product can be attained by replacing petroleum based raw material with an eco-friendly.

Generally sanitary pad comprises of the multilayered structure in which each layer have specific function to perform. It consists of three main layers the top sheet, absorbent core and barrier sheet. Top sheet is designed to transfer fluid quickly from the top sheet to secondary layers. The top sheet contains thermoplastic fibers to prevent capillary collapse of this layer, and small amount of hydrophilic absorbent fiber to allow fluid to absorb. Commercially available top sheet are made up of polypropylene fiber. Absorbent core is interposed between top sheet and barrier layer main function is to absorb and retain the fluid. Moreover, to have comfort, absorbent core need to be thin, soft and pliable. The core was made up of wood pulp traditionally but there is constant effort to replace it by air laid wood pulp and SAP to improve its absorption efficiency. SAP [2] turns the absorbed liquid into a jelly-like state so that it would not retract back. Barrier sheet seals the fluid from staining or leakages. It is a breathable but fluid impermeable film made up of polyethylene. Few components of sanitary pad will disintegrate and be attacked by the bacteria in a public or private sewage disposal system but polyethylene or polymeric films used as a barrier sheet remain intact as this polymer are inert and are not broken down by bacteria and thus pollutes the environment [2].

Characterize the fabrics used in daily sanitary pads, focusing on the development of future products. The spectra generated by FTIR/ATR suggest that the samples were composed of polypropylene. The photomicrographs showed that the polymeric outer layer was made of nonwoven fabric manufactured by spun-bond and point bonding processes [3].

Natural fibers are one such proficient material which replaces the synthetic materials and its related products for the less weight and energy conservation applications. Natural fibers are also known for its low cost production yet, it have excellent mechanical properties and is environmental friendly [4]. Natural fibers can replace glass fibers in fiber-reinforced plastics. One of the natural fiber resource is pineapple leaf fiber (PALF) which is planted chiefly cultivated in coastal and tropical regions, widely as in Indonesia especially [4]. Before the fabrication, PALF has underwent alkaline treatment to increase the strength of fiber. Pineapple (*Ananas comosus*) is an significant fruit of India. Pineapple leaf contains only 2.5-3.5% fiber, covered by a hydrophobic waxy layer. Extraction of thousands of tons of pineapple leaf fiber can be done after the harvesting of the fruit [5]. The fiber extraction alone from the leaves is not economically viable, so the utilization of the residual sludge, remained after the process can be done in vermicomposting and other applications. Pineapple leaf fiber has been modified by alkali, acetylation and graft copolymerization. Grafting improved the thermal stability of pineapple leaf fiber modified fibers showed significant hydrophobicity, improved mechanical strength and chemical resistance [6].

PALF was prepared from raw pineapple leaf. It was then chemically treated to hinder water content. Scanning Electron Microscope (SEM) was used to investigate the miscibility between the fiber and matrix. It was found that PALF contain 87.56% holocellulose, 78.11% alpha cellulose, 9.45% hemi cellulose and 4.78 % lignin [7]. The chemical constituents obtained were in the range to data reported in literatures. It was also observed that the flexural modulus and strength of treated PALF reinforced PP composite increased linearly with increment of fiber loadings. SEM where fibers and matrix have shown better.

METHODOLOGY

Raw material: After consumption, non-edible parts like leaves are thrown. Agro-wasted pineapple leaves was collected (in kilos) from Erode district for fiber extraction.

Fiber Extraction: Using ceramic plate movement, fibers can extract from pineapple leaves [6].

Fiber Treatment: Extracted pineapple leaf fiber was treated with 5 % NaOH solution for one hour at 30°C [7].

Testing of chemical properties: Alpha cellulose, hemicelluloses, holocellulose, lignin enhances the water holding capacity of the natural fiber. Also, the rest moisture content decides the absorption rate. It can be determined by placing the sample in oven at 104o C for 4 hours and weighted in a electronic weighing machine. The percentage of moisture percent per unit weight of fiber is evaluated [7].

Design and development of sanitary napkin:

- ✓ Fully - auto sanitary napkin making machine.
- ✓ Raw materials for developing biodegradable sanitary napkin are organic cotton fabric, cotton non-woven sheet, extracted pineapple leaf fiber, bio-plastic.
- ✓ Product was designed by the size norms as per **INDIAN STANDARD: 5405 – 1980**

S. No	Component	Material
1	Top Sheet	100% Organic cotton non -woven fabric
2	Absorbent Core	Pineapple Leaf fiber (PALF)*
3	Barrier Sheet	Biodegradable plastic

Table 1: Biodegradable resources preferred for sanitary napkin preparation

Test methods for estimating performance quality of sanitary napkin: Testing was conducted to evaluate the performance of developed sanitary napkin.

- ✓ The synthetic blood is prepared to simulate menstrual blood to evaluate actual performance sanitary pad.
- ✓ Following ingredients were used to prepare synthetic blood [8]:
 - a) Plain Flour-10grams
 - b) Distilled Water-200ml
 - c) Food colour-2ml

Following were the test to be carried out on treated samples [8]:

Test Parameter	Standard	Instrument
Spray Test	AATCC (ISO 4920)	Spray Tester
Thickness	ISO9073-2:1995	Thickness test by SDL Atlas
Air permeability	EDANA 141.1	Textest Instruments AG
Strike Through	EDANA/INDA-Std WSP 70.3	Lister AC
Wet Back	EDANA/INDA-Std WSP 80.10	-
Vertical Wicking	AATCC TM 197-2011	-
Absorption Capacity	EAS 96;2008-Annex C	-

Table 2: Parameters/Standards for estimating performance level of sanitary napkin

Antimicrobial Activity Test: Antimicrobial test was carried out using Agar Diffusion test. Test was carried against gram positive (*Staphylococcus aureus*) and gram negative (*Pseudomonas aeruginosa*) bacteria. The treated samples were placed on the incubated agar plate with test bacteria for 24hrs at 37°C. After incubation the samples was assessed visually; the area of inhibition was the measure of antimicrobial efficiency.

Validation test for sanitary napkin: A step-wise process in assessing potential toxicological effects of raw materials, including hazard identification, hazard characterization, exposure assessment, and risk characterization. Additionally, clinical evaluation of product use and post-market surveillance can be conducted for thorough risk management of products. An overview of assessment approach is shown in Fig:1 [6]

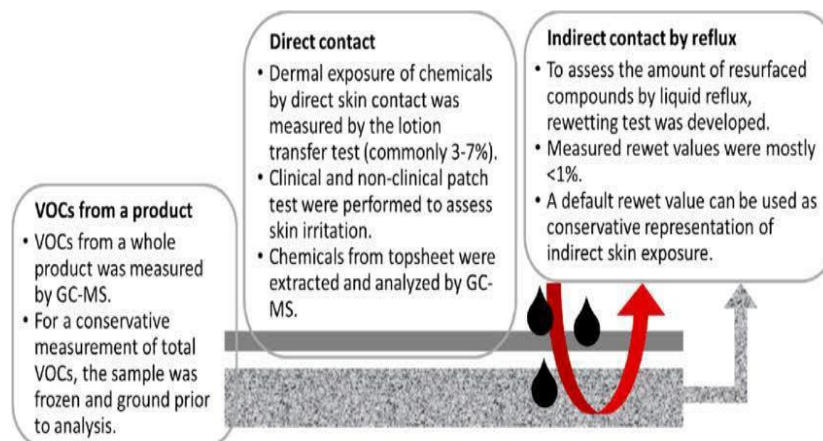


Fig:1 Exposure Assessment for different pathways

Clinical evaluation of prepared sanitary napkin: Clinical studies on sanitary pads by the “Behind-the-Knee (BTK) clinical test” [5] was performed by applying a test pad to the popliteal fossa of one leg under an elastic bandage for 6 h per day for four consecutive days. From the BTK test, skin irritation and dryness were scored after 30–60 min after removal of products, and the score of erythema and dryness for the test product was compared with those of reference product that had safe use history.

Biodegradation efficacy test for prepared sanitary napkin: The study of biodegradability of the pineapple leaf fiber can be done by burying the prepared sanitary napkin in the ground.(Asbanto *et al.*, 2018).

RESULTS & DISCUSSION

Fiber extraction

Pineapple leaf is one of the most available agro-waste after consumption. Extraction technique was performed by using ceramic plate movement (*i.e., manual scrapping*) for collected pineapple leaves (crown part). From the **socioeconomic prospective**, pineapple leaf fiber (PALF)* can be a new source of raw material extracted from waste products of pineapple cultivation and it can be potential replacement of the expensive and synthetic fibers. A detailed study of chemical, physical properties of the PALF brings out logical and reasonable utilization for various applications.

Fiber Treatment(Smoothering)

Extracted pineapple leaf fibers was treated with 5 % NaOH solution to smoothen it. Since PALF are susceptible to water content, the thoroughly drying and storing in a dry condition are crucial. Silica gel was placed in the desiccator together with PALF to prevent any water absorption.

Chemical Characterization of PALF

Pineapple leaf fiber is multi-cellular lingo cellulosic fiber. Percentage of chemical constituents present in PALF was as founded to be as follow:

Chemical constituent	Range in percentage - (Optimum) (Munirah <i>et al.</i> ,2007)	Range in percentage (Observed)
Alpha cellulose	78.11	64
Hemicellulose	9.45	9.25
Holocellulose	87.56	80
Klason lignin	4.78	4

Table 3: Chemical constituents present in PALF

Morphological Analysis

Scanning Electron Microscopy (SEM) analysis was shown in Fig:2,3. From this analysis, it was founded that PALF has ribbon like structure and is cemented together by lignin, pentosan – like materials, which contributes to the strength of the fiber PALF is multi-cellular and lignocelluloses material. Their study also found that the cells in this

fiber have average diameter of about 10 μm and mean length of 4.5 mm with aspect ratio of 450. The thickness of the cell wall (8.3 μm) lies between sisal (12.8 μm) and banana fiber (1.2 μm). PALF can serve as reinforcement fiber in most of the plastic matrix.

Pineapple leaf gives **high fiber content** because of the arrangement of fiber.

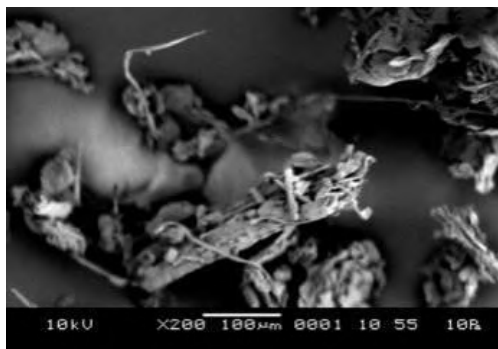


Fig:2 Surface area of pineapple

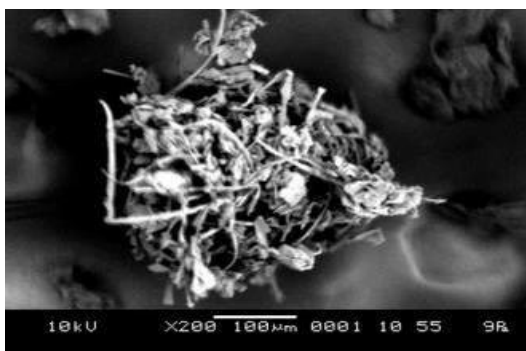


Fig:3 packing of pineapple leaf fiber

Determination of moisture content of PALF

Loss on drying method – Using expression, $MC = (w-d/d)*100$ {w = wet weight & d = dry weight}, absorption capacity of fibers was calculated. As like, PALF was compared with cotton fiber. And it was found that PALF contains **maximum** moisture content i.e., **10%**

Design and development of sanitary napkin

Sanitary napkin was developed by using biodegradable resources and it satisfies the size norms as per **INDIAN STANDARD: 5405 – 1980**. While the usage of the product it should not slip or move when it's worn, so wing type approach is preferred most (shown in below fig:4).

Essential properties of the sanitary napkin were found to be expected and satisfied such as: biodegradable, comfortable to wear, stay in place, hygienic and environmentally friendly, odourless.



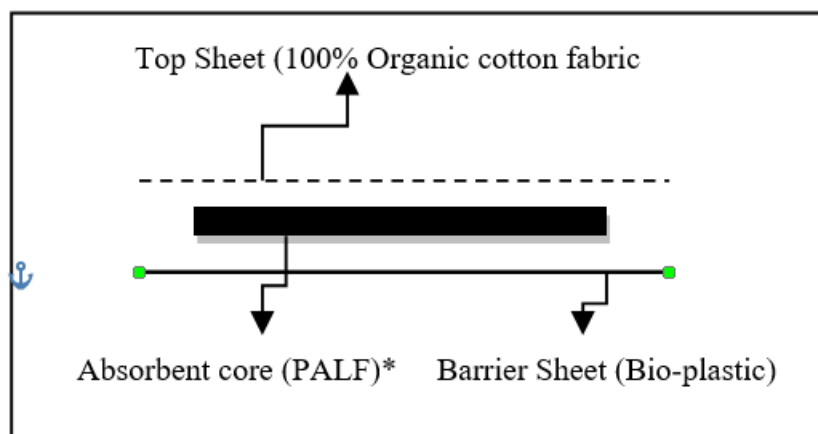


Fig:4 Prepared sanitary napkin(Winged) and its structure

Performance assessment of prepared napkin

As, by the S/N ratio of properties of sanitary napkin, the ranking factors of parameters to be considered in estimating [7] the quality of performance was shown the table-3

Ranking factors : Lower the better(1-2); Higher the better (2-3).

After the final assessment, it was proven that prepared napkin shows **higher the better** performance than commercialized one.

Therefore, A&B – Commercial pads; C – PALF Pad.

S.No	Parameters	A	B	C
1	Thickness	1	3	2
2	Air Permeability	1	3	2
3	Absorption Capacity	3	1	3
4	Vertical Wicking	1	3	2
5	Strike Through	3	1	2
6	Wet Back	1	2	3
7	Antibacterial	1	1	3

Table 4: Compared performance quality of the commercial and prepared sanitary napkin

Validation test for sanitary napkin

GC-MS analysis of prepared napkin proven that chemicals i.e., VOC's, DLC's, methyldibromo glutaronitrile, cinnamaldehyde, cinnamic alcohol, acrylic acid, phthalates, organic derivatives of tin, organically bounded chlorine etc., are 100% absent. Where, it shows reliable and versatile result of feasibility. It proven, prepared PALF napkin was "user's skin friendly". And, it was **highly safeguard to nature and women's health** comparing with commercial (already existing) napkins.

Clinical Evaluation of prepared napkin

It shows **irritation-free response without any itches, allergies, redness etc.**, under the observation, for 4 consecutive days (The Behind-the-Knee (BTK) clinical test). It also proven that prepared napkin free from dermatitis allergic inducing compounds.

Biodegradation Test

By soil dumping test, prepared sanitary napkin shows **better degradation rate. 3-6 months**, was the time period taken by prepared napkin, maximally.

CONCLUSION

The absorption capacity, wicking rate of PALF core was found to be high in comparison with cotton core. Top Sheet (Organic Cotton) shows good absorption capacity and skin-friendly (analyzed by GC-MS). Low density biodegradable plastic shows good fluid impermeability and act as excellent barrier layer-"No Leakage".

Developed sanitary napkin shows good result in air permeability, which means product will permit body heat to flow & will feel comfortable. And, developed sanitary napkin shows satisfactory absorption capacity. In general., it

also shows that PALF pad have highest wicking rate, followed by commercial pads. And it's strike through result shows positive response i.e., improved skin friendliness and dryness. The wet back of developed sanitary napkin was high, which is not desirable.

PALF pad's biodegradation efficacy was found to be minimal time period of 3-6 months, with decrease in their rate of tenacity.

Finally, prepared napkin resolved all the impacts created by pre-existing/commercialized one.

"Nature has encompassed every solution within itself." With more and more use of natural fiber in hygiene product will make it eco-friendly. Use of natural fiber in sanitary pad will reduce the cost of the product will lower accessible to low income group women. As the product is biodegradable, prevent non-biodegradable waste generation. We as a technologist have to find a sustainable way so that we endow a better world for next generation.

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Value addition of amla (*Phyllanthus emblica*) fruit bar with amla seeds

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Abstract: Fruit Bar is a confectionery product made by the dehydration of fruit puree into leathery sheets. In the present study, the fruit bar is prepared from amla fruit pulp in addition of amla seeds and seed coat. The motive of this study is to commercialize the value added amla fruit bar. Amla seeds and seed coat are separated from amla fruit. Amla seeds, seed coat and pulp were analysed for proximate analysis. In preparation of amla fruit bar, amla seed and seed coat is incorporated in the amla pulp. The final product is compared with control amla fruit bar for proximate analysis and so on. Amla seeds plays a vital role in curing of leucorrhea. Amla seeds contain fixed oil, essential oil and phospholipids that are very useful to relieve inflammation and infection associated with uterus and cervix. Since amla contains pectin, it is suitable in the preparation of fruit bar.

Key Words: Leathery sheets, Amla seeds, Seed coat, Proximate analysis, leucorrhea.

INTRODUCTION:

Amla is round-shaped, vertical striped fibrous fruit of the deciduous tree comes under the category of Euphorbiaceae family. It is also named as *Phyllanthus emblica* or Indian gooseberry. This species is native to India and also grows in tropical and sub-tropical regions (Charmkar and Rajesh., 2017, Sivarajasekar et al., 2019). Amla is good source of polyphenols, flavanones, tannins and other bio active compounds (Mishra et al., 2014). *Emblica* having highest amount of naturally occurring vitamin C of any ripe food used in a traditional food (Kumar, Bhowmik et al., 2012). The antioxidants present in the amla might contribute to the high health effects. It has an application of antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive, and gastroprotective (Charmkar and Rajesh., 2017). Amla seeds plays a vital role in curing of leucorrhea. It contains fixed oil, essential oil, phospholipids that are very useful to relieve inflammation and infection associated with uterus and cervix. In this study amla with seeds and seed coat is used for the preparation of leathery sheet fruit bar since amla naturally contain pectin which act as setting agents for the fruit bar processing. Proximate analysis has been analysed for seed coat, seed, pulp, amla fruit bar, amla with amla seed fruit bar and amla with amla seed coat fruit bar. Development of value added amla products was carried out to prepare the value added amla products and to assess the sensory evaluation of the products.

MATERIALS:

RAW MATERIAL PREPARATION:

Samples of amla is procured from local shops in Perundurai, Tamilnadu. Amla was thoroughly cleaned with fresh water to remove the surface dust particles and wiped with muslin cloth. From whole fruit of amla whole seed was removed manually. From the whole seed, seed coat and seed were removed manually. Seed coat and seed were dried in tray drier at 60°C. The removed amla pieces were boiled at 80°C and allowed to cooled for 10 to 15 minutes at room temperature. After that amla pieces were grinded into fine paste.

PROXIMATE ANALYSIS FOR AMLA, SEED AND SEED COAT:

MOISTURE CONTENT:

Ground amla pieces, seed and seed coat were weighed 5g and kept at hot air oven at 105°C. For every half an hour the sample was weighed, until the concordant value attained. The moisture content was analysed by the following formula,

ASH CONTENT:

About 2g of amla pieces, seed and seed coat was weighed accurately into crucible which had previously been heated to about 60°C and cooled. The crucible was then placed in a muffle furnace for about 3 hours at about 600°C, the crucible was then cooled in a desiccator and weighed. The ash was almost white or greyish in colour.

TOTAL SOLUBLE SOLIDS AND pH OF AMLA:

Total Soluble Solids (TSS) was analysed for the amla by refractometer and it was analysed as 13° Brix. pH of the fruit was analysed by potentiometric titration method which uses pH meter.

METHOD:

PREPARATION OF AMLA LEATHER FROM AMLA FRUIT PULP (ALP):

The fresh amla fruits were thoroughly washed and the whole seed is from it manually. After that, amla pieces were boiled, ground into pulp and continuously stirred until a required Brix of 75 is attained. We used water as a medium to obtain a paste form, it was necessary to ensure that the end product of TSS reaches 65-75. For 75g of pulp 50g of sugar is used to attain brix. After the preparation of paste, it is poured on a tray which is cover with butter paper is allowed to dry in tray dryer at 60°C for 8 hours. The weight of the final fruit leather is obtained as 63.46 gram. Prepared leather was analysed for TSS, Ph, moisture content wet and dry basis and total solids

AMLA LEATHER FROM AMLA FRUIT PULP AND AMLA SEEDS AL(P+S):

Seed was removed from the seed coat and it is dried in a tray drier at 60°C for 2 hours. The amla pieces are boiled, ground into pulp and total soluble solids content of the pulp was raised to 75°Brix. For 75g of pulp, 53.17g of sugar is used to attain the brix value. At the final stage dried seed is added and stirred for even distribution of seeds into the mixture. In cleaned tray, butter paper is placed and the mixture with seeds are poured onto the tray containing butter paper. The mixture is uniformly distributed on the tray and allow to dry in tray drier at 60°C for 8 hours. Weight of the final fruit leather is obtained as 62.4g. Prepared leather is analysed for TSS, pH, moisture content wet and dry basis and total solids.

AMLA LEATHER FROM AMLA FRUIT PULP AND POWDERED SEED COAT AL(P+SC)

The removed seed coat from seed is dried in a tray dried at 60°C for 2 hours. The dried amla seed coat is made into a fine powder. The amla seed coat powder along with amla fruit pulp is stirred together to obtain the brix value of 75. For 75g of pulp, 58.5g of sugar is used to attain the brix value. The cooked mass is poured onto the butter paper which is placed in the tray. The cooked mass is made into a thin leathery sheet by placing it in tray drier at 60°C for 8 hours. The weight of final fruit leather obtained is about 55.2g. Prepared leather is analysed for TSS, pH, moisture content wet and dry basis and total solids.

SENSORY EVALUATION:

All the leather sheets from ALP, AL(P+S), AL(P+SC) prepared were subjected to sensory evaluation with respect to colour, appearance, aroma, texture, taste and overall acceptability by a panel using the 9-Hedonic Rating scale.

RESULT AND DISCUSSION:

The result obtained during the analysis of percentage of moisture content for amla pulp in wet basis 89% and in dry basis 809%. pH of amla pulp analysed as 1.9. For seed moisture content analysed in wet basis 10% and in dry basis 11.11%. Moisture content analysed for seed coat in wet basis 32.5% and in dry basis 48.1%. Ash content in pulp, seed and seed coat analysed at 600°C in muffle furnace for 3 hours is 2g,1g,1g respectively.

COMPARATIVE STUDIES BETWEEN ALP, AL(P+S), AL(P+SC):

PARAMETERS	ALP	AL(P+S)	AL(P+SC)
Weight, (gram)	63.46 g (75g)	62.4g (75 g)	55.2g (75g)
TSS (°Brix)	30°	31°	27°
Moisture content (%wb)	16%	14%	19%
Moisture content (%db)	19%	16.2%	23.4%
Total Solids	84	86	81
pH	2.38	2.44	2.34

Table 1

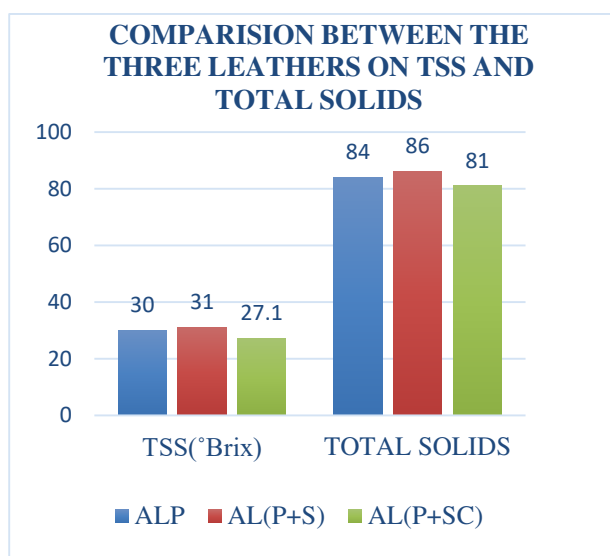


Chart 1

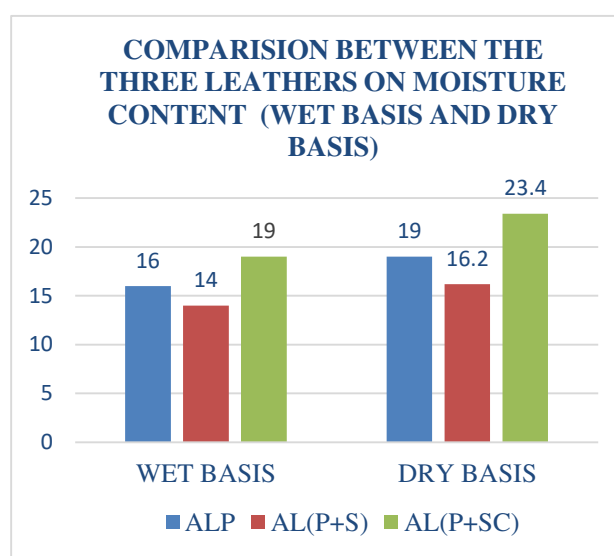


Chart 2

SENSORY ANALYSIS:

The results obtained during the investigation of sensory quality of leather sheets of ALP, AL(P+S) and AL(P+SC) presented in table 2. Sensory evaluation by panels observed colour, aroma, texture and taste. From the results the sample AL(P+S) has low bitterness than the other two ALP and AL(P+SC) samples. In addition to that raw taste of seed coat is observed in the fruit leather prepared from AL(P+SC) and also it was crumbling in nature. ALP has more appealing colour and texture. The Overall acceptability is higher for AL(P+S) than the others.

Sample	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
ALP	7.1	7	6.4	6.8	6.6	6.1
AL(P+S)	6.6	6.5	6	6	6.6	6.3
AL(P+SC)	6.8	5	6.1	5	5.5	5.5

Table 2

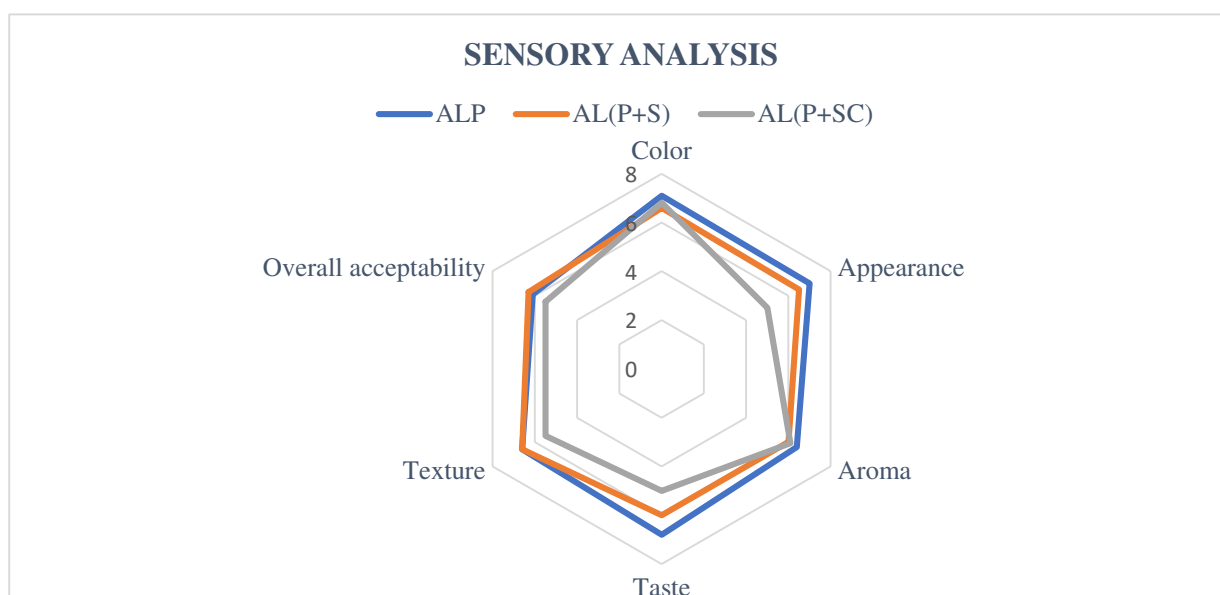


Chart 3

CONCLUSION

People may not aware of eating the amla seeds. Here, we have incorporated amla seeds and seed coat to the amla fruit bar to improve the nutritional value. On the whole the crunchiness of the seed in every bite of the fruit bar gives good mouth feel and it gained high overall acceptance compared to others.

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**Optimization of ready-to-serve pineapple-amla beverage and
Evaluation of physiochemical properties**

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Abstract: The pineapple (*Ananas comosus*) is an edible fruit, which is rich in Vitamin C and bromelain. They also contain calcium, potassium, carbohydrates, crude fibre, water and different Minerals that is good for the digestive system. Amla is very much rich in Vitamin C which helps in boosting the immune system and prevents from wide range of diseases. Fruit beverages such as RTS have become popular in comparison to synthetic drinks because of their taste, aroma, flavour and its nutritive value. The main advantage of this drink is it is easily digestible, refreshing, thirst quencher, appetizer and it is superior to other kinds of aerated drinks. The objective of this study is to optimize pineapple-Amla blend for production of Ready-To-Serve (RTS) beverage and the pH, percent acidity, ascorbic acid, total soluble solids, and colour values were evaluated. Filtered juice extract of pineapple and Amla blends were taken in different ratios; 9:1, 8:2, 7:3 and 6:4 and pineapple extract alone was used as control. Filtered and homogenized juice blends were pasteurized and hot filled in PET bottles. Results revealed that the pH of the sample decreased with increase in concentration of Amla extract. Titratable acidity of the sample was decreased to 0.5% compared to control. However there was no significant difference in the acidity was observed among the blends. Lightness (L*) value decreased with increase in concentration of Amla extract. Ascorbic acid value increased with increase in Amla concentration in the juice blend. Sensory score of pineapple-Amla blends of 8:2 ratios showed good acceptability. This study suggested that the ascorbic acid content of Pineapple RTS beverage can be increased with the addition of Amla extract.

Key Words: Amla, Pineapple, RTS beverage, Physiochemical properties.

INTRODUCTION:

India has varied agro climatic conditions and it is suitable for cultivation of different kinds of fruits in different seasons of the year. Fruits like pineapple and Amla are the most common fruits grown in subtropical area like Thailand. Pineapple has good flavor, smell, taste and has good source of ascorbic acids and is rich in vitamin B and vitamin B2. Fruit beverages such as RTS have become popular in comparison to synthetic drinks because of their taste, aroma, flavor and its nutritive value. Combining of one or two fruit juices in an appropriate proportion for the preparation of RTS beverage and it is considered as the alternative refreshing health drink and good sources for vitamins, minerals compared to artificial beverages. The need of synthetic beverages is in millions annually. The blending of one or two fruit juices or pulp in various proportions increases the palatability and increases the nutritious quality. Blending enhances appearance, flavor, aroma, consistency of the product are the main consideration for the optimization of different ratios of various blends components that meets the consumers preferences that improves the developing of new products. The RTS beverages are very popular among the consumers because it can be carried easily and consumed. Sensory, nutritional and other qualities can be improved by blending pineapple and amla juice. The main advantage of this drink is it is easily digestible, refreshing, thirst quencher, appetizer and it is superior to other kinds of aerated drinks. Pineapple is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavor and immense health benefits. Pineapple contains considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. It was carried out in different proportions, pineapple and amla were taken in different ratios; 9:1, 8:2, 7:3 and 6:4 respectively. The RTS was stored at ambient temperature conditions to study the storage behavior of the product with respect to the changes in physical, chemical and sensory qualities during storage. If the real fruit juices are could be substituted for these synthetic beverages it

could be good for fruit growers and consumers. The reason for development of the new product is there is always demand from the consumers for the development of new, nutritious product and delicately flavored product.

The present study titled “Optimization of ready-To-Serve pineapple-Amla beverage and evaluation of physiochemical properties” was done with following objectives.

- To standardize the recipe of pineapple and Amla blended RTS
- To study the storage behavior of pineapple and Amla blended RTS at ambient conditions.



Figure 1.1: Amla



Figure 1.2: Pineapple

LITERATURE REVIEW:

Spayd et al. (1984) determined that the pineapple and amla Blends stored at 250°C for 48 hours led to multiply Polymeric shade and according to cent coloration due to extended tannins, while anthocyanin awareness reduced. Deka (2000) stated a decreasing fashion in hunter L* and a* Values and growing trend in b* price and color variations during garage of amla-pineapple spiced RTS Drinks in unique containers under various storage Conditions. Rein and Heinonen (2004) suggested the L*, a*, b*, c* and h* Values of various juices of pineapple and amla in studies on balance and Enhancement of pineapple and amla juice coloration. Shashi et al. (2018) developed RTS beverage using pineapple and amla blend at four different proportions i.e. 9:1, 8:2, 7:3 and 6:4. To increase the nutritional value of the RTS beverage, Giloy was added at various proportions i.e. 0.5%, 1.0% and 2.0% to the top sensory rated RTS. Among the various proportions, the 8:2 ratio and 0.5% Giloy was accepted mostly by sensory panelists. The prepared beverages TSS were 12.01°Brix and the acidity level was around 0.3%. The addition of amla and pineapple increased the nutritional properties of the beverage. Phytochemical analysis was also done and revealed that the nutritional profile of the Giloy retained in the RTS also. They concluded that the physiochemical properties reduced with increasing storage period. Saranyah et al. (2015) developed a value added watermelon RTS beverage by blending pineapple with the RTS at different ratios. The amount of sugar, citric acid and potassium metabisulphate was kept constant for all the proportions. They checked various physiochemical properties in which the pH decreased with increase in the pineapple juice concentration whereas the other properties such as TSS, acidity, etc., increased with increasing concentration of pineapple juice. While checking the Organoleptic properties, the ratio 80:20 (watermelon: pineapple) had the better acceptability. They also concluded that the shelf life of the prepared RTS was about three months at 30±1°C without any quality changes. Ajith et al. (2019) prepared pineapple RTS by blending it with coconut milk to increase its nutritional value and the RTS is prepared by adding no preservatives and chemicals. The RTS was prepared in the different ratios and checked for physiochemical and Organoleptic properties after storing the RTS for about 14 days. They concluded that the ratio 71:29 (Pineapple: coconut milk) had the better acceptability with TSS 13% and the properties such as pH, TSS varied according to different concentration of coconut milk. Priyanka et al. (2015) conducted a study on blended RTS by preparing Jamun juice with different fruit blends such as pineapple, grapes and mango, each at 75:25, 50:50 and 25:75 (jamun: other fruits). The prepared RTS was evaluated for its Organoleptic and physiochemical properties throughout the storage period of nine months. They concluded that the 75:25 ratio jamun: grapes had better overall acceptability. The properties TSS, reducing sugars, acidity increased whereas the pH, non-reducing sugar decreased during the storage period of nine months. Hossain et al. (2017) developed RTS by blending Jackfruit and Aloe-Vera at different ratios such as 100:0, 90:10, 80:20, 70:30 and 60:40 (jackfruit: aloe vera) to increase the flavor and therapeutic value of the RTS. Sensory evaluation was done for the RTS and results showed that the 70:30 ratio (jackfruit: aloe vera) had better overall acceptability. They concluded that the RTS had a shelf life of about five months without any change in the properties at refrigeration temperature. Lokesh et al. (2017) formulated ready to serve beverage by blending aloe vera, sweet lime, amla and ginger at various proportions. These beverages were tested for physiological, microbiological and organoleptic properties. The amount of sugar and water were adjusted to get 13°Brix. They concluded that among various proportions 60:5:20:15 (aloe vera: ginger: sweet lime: amla) had the better acceptability. The study also revealed that the storage life of the RTS beverages was extended up to sixty days without any microbial load. Shashi et al. (2004) developed ready to serve fruit beverages by blending gooseberry with various fruits such as apple, grapes, pomegranate and lime. They also compared the gooseberry juice with other

fruit juice for Vitamin C content. He concluded that the Vitamin C content of gooseberry juice was higher when compared to the other juices and also showed that the Vitamin C content increased in the other fruit juices when gooseberry was added to it. Dhuru et al. (2015) prepared RTS beverage by blending bael pulp and aloe vera gel at four different compositions i.e. 100:0, 75: 25, 50:50 and 25:75 (bael pulp: aloe vera). The study showed that the composition 75:25 had the better acceptability up to three months storage at ambient air condition. This also showed that the TSS, total sugars, acidity, etc increased during the storage period whereas vitamin C and non-reducing sugar reduced during the storage period. Sasi et al. (2013) developed a therapeutic ready to serve beverage by blending aloe vera, ginger and aonla at four proportions. The RTS was checked for sensory and physiochemical properties. They concluded that the ratio 70: 15: 15 (aloe vera: aonla: ginger) had the better acceptability.

MATERIALS:

Fresh pineapples and amla were procured from local market of Coimbatore. The raw materials were free from physical damage and visible microbial and insect infestation.

METHOD:

Extraction of Juice- Pineapple and Amla:

For the extraction of juice, the ripened pineapple was washed thoroughly and later peeled and cut into small pieces. The cut fruits were made into a thick paste and then filtered using a muslin cloth. The juice was kept in refrigerator until used. For amla extraction, the fresh amla were blanched for about 13 minutes. Then the seeds are carefully removed from the blanched fruits and made into a thick paste using a mixer. Later it was filtered using a muslin cloth and kept in refrigeration condition until used.

Preparation of RTS:

For preparing the RTS, the pineapple and amla were taken in different ratios; 9:1, 8:2, 7:3 and 6:4 respectively. A control sample of pineapple RTS was also prepared. First, the sugar syrup was prepared and when it reached 60°C, the juice was added to the syrup and was pasteurized at 80°C for 1 min. Then it was checked for its TSS and the quantity of sugar and water was adjusted to obtain required TSS (12%) and the amount of citric acid was added according to the taste of the beverage. Once the TSS is obtained the RTS was hot filled in the sterilized PET bottles of 200 ml capacity.

S.NO	SAMPLE RATIO	COMPOSITION (%)	
		PINEAPPLE	AMLA
1	Control	100	0
2	9:1	90	10
3	8:2	80	20
4	7:3	70	30
5	6:4	60	40

Table 4.2: Formulation for the preparation of Ready-To-Serve beverage by blending pineapple and amla at different ratios



Figure 4.2: RTS beverage

ANALYSIS:

Physiochemical Properties:

- **Total Soluble Solids**

The TSS content in prepared RTS was determined using the refractrometer at standard temperature i.e. 20°C and the obtained values were expressed in terms of °Brix.

- **pH**

The pH of the samples was measured using pH meter. First the pH meter was calibrated using a basic and an acidic solution and then with water. Later the readings of the sample were taken.

- **Color characteristics**

The color of the RTS in terms of L*, a*, b* values are measured using hunter lab colorimeter color measuring system equipped with D65 illuminant. Three measurements were taken for each sample, and the average value was reported Rajam et al., (2014).

- **Total acidity**

The total acidity test was carried out by titrimetric method by titrating the diluted sample against 0.1 N NaOH solutions. Phenolphthalein was used as the indicator and the appearance of pale pink color indicated the end point of the experiment. The experiment was repeated two times to obtain the concordant value. By using the titre value, the % of acidity in terms of citric acid was calculated by the below given formula.

$$\% \text{acidity} = \frac{m \text{ of NaOH consumed} \times \text{acid factor} \times 100}{\text{volume of the sample}}$$

- **Ascorbic acid**

The amount of ascorbic acid present in the RTS was determined using dichlorophenol indophenol dye method. Two titrations were done, 1st to determine the dye factor where the ascorbic acid solution was titrated against the dye solution. In the 2nd titration, the diluted samples were titrated against the dye solution. Using the titre value and the dye factor the amount of ascorbic acid present in the samples were determined by using the below given formula.

$$\text{dye factor} = \frac{0.5}{1^{\text{st}} \text{ titre value}}$$

$$\text{mg of ascorbic acid per 100g} = \frac{\text{sample titre value} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{Aliquot taken for estimation} \times \text{volume of sample taken}}$$

- **Sensory values**

Composite scoring test was used for the sensory evaluation of the prepared RTS beverage with consumer panel. The average acceptability of color, appearance, flavor and overall acceptability of the RTS were evaluated.

RESULT

- **pH**

pH is the negative of the base 10 logarithm of the molar concentration of hydrogen ions in the solution. More precisely, P^H is the negative of the base 10 logarithm of the activity of the hydrogen ion.

S.NO	Sample volume	pH	Temperature(°c)
1	Control	3.81	21.3
2	9:1	3.77	21.5
3	8:2	3.72	20.9
4	7:3	3.55	20.8
5	6:4	3.46	21.1

Table 6.1: pH of the prepared RTS beverage

- **Color characteristics**

The L*, a* and b* values of the amla blended with pineapple ready to serve beverage were determined. The proportion with higher percentage of pineapple blended with minimum quantity of amla showed the highest color values.

ID	L*	a*	b*	ΔE^*
CONTROL	20.3±0.901	-3.91±0.012	9.87±0.654	22.9021±0.578
9:1	23.18±0.851	-3.95±0.128	11.64±0.378	26.2374±0.745
8:2	25.37±0.712	-4.04±0.045	12.95±0.578	28.7691±0.875
7:3	26.19±0.911	-4.12±0.279	12.27±0.479	29.2137±0.475
6:4	26.58±0.678	-4.23±0.197	10.79±0.871	28.9968±0.651

Table 6.2: Color characteristics of the prepared RTS beverage

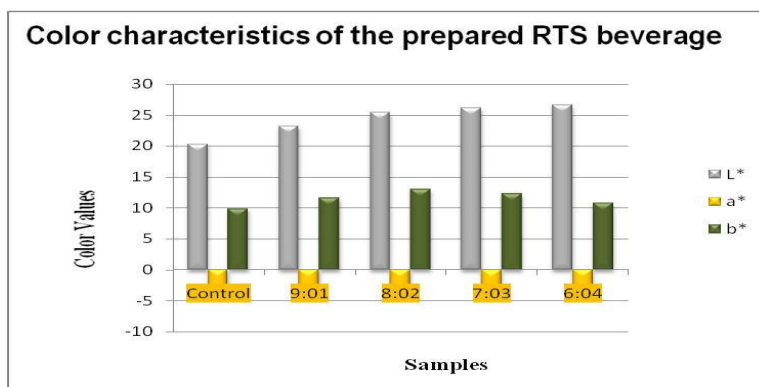


Figure 6.2: Color characteristics of the prepared RTS beverage

- Acidity Test**

Titrateable acidity is done in order to determine the acid content in the (RTS) juice. The proportion 6:4 shows the highest acidity value than any other sample.

S.NO	Sample volume	Titrateable acidity (ml)
1	Control	0.32
2	9:1	0.25
3	8:2	0.26
4	7:3	0.26
5	6:4	0.28

Table 6.3: Acidity values for the prepared RTS beverage

- Ascorbic acid test**

Ascorbic acid test is done to determine the vitamin c content in the (RTS) amla blended with pineapple juice. The proportion 6:4 shows the highest value of vitamin c content than any other sample.

S.NO	SAMPLE VOLUME	ASCORBIC VALUE (mg/100 g)
1	Control	5.712
2	9:1	5.712
3	8:2	5.712
4	7:3	8.568
5	6:4	11.424

Table 6.4: Ascorbic acid values for the prepared RTS beverage

- Sensory analysis**

The scores obtained from consumer panel for the sensory analysis are tabulated in table 6.5. It was found that color of the RTS was influenced by the addition of amla juice. Addition of amla juice at different concentrations to the pineapple juice improved the ascorbic acid value of the RTS. The addition of amla juice at different proportions showed slight difference in the sensory properties such as color, aroma, flavor and overall acceptability of RTS. These composite score test results shows that the overall acceptance of the RTS, ratio 8:2 was better.

S.NO	PARAMETERS	CONTROL	9:1	8:2	7:3	6:4
1	Color	8±0.81	7.5±0.63	8±0.26	7±0.43	7±0.18
2	Taste	8.5±0.57	8.25±0.5	9±0.31	7.5±0.56	7.25±0.56
3	Appearance	7.25±0.5	7±0.5	8.5±0.15	7.25±0.28	7±0.23
4	Flavor	7.25±0.5	7.5±0.25	8.25±0.43	7.5±0.75	7.25±0.14
5	Sweetness	8.25±0.5	8.5±0.5	8.5±0.36	8±0.25	7±0.41
6	Overall acceptability	8	7.5±0.5	8	7.25±0.36	7±0.42

Table 6.5: Sensory values for the prepared RTS beverage

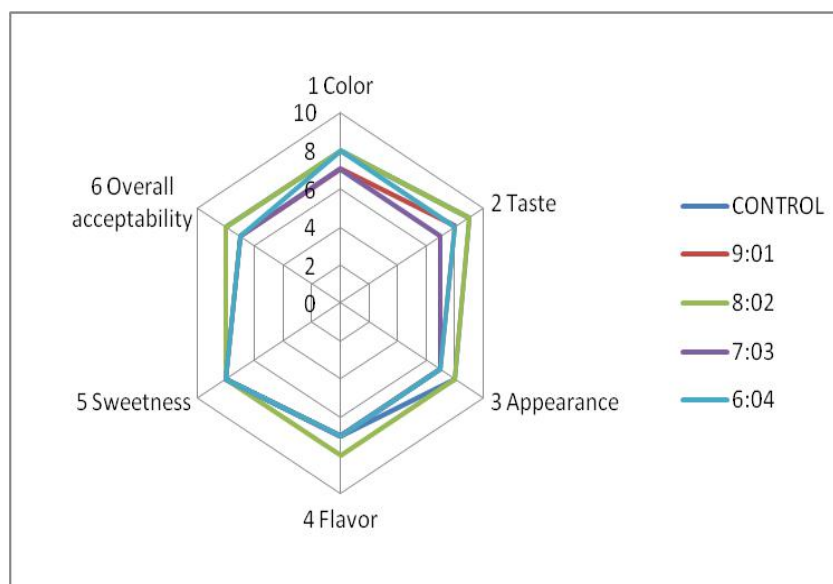


Figure 6.5: Sensory values for the prepared RTS beverage

CONCLUSION:

In the present study, the RTS beverage possesses a very good nutritional value due to blending of amla with pineapple juice. The pH value of the (RTS) amla blended with pineapple in 100ml of water varies from 3.81 to 3.46. The color value for the RTS beverage varies from dark yellow to light yellow. The titratable acidity value of the RTS varies from 0.32 to 0.28 % of citric acid. The ascorbic acid value for the RTS beverage varies from 5.712 to 11.424 mg/100 g. On the basis of the results obtained, amla blended with pineapple can be used for a variety of beneficial effects. However, further studies are carried out on microbial load and shelf-life studies using the RTS beverage.

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Effect of replacement of water with wheat milk on hydration of crackers

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Abstract: The milk extracted from wheat is a good source of protein, vitamin, dietary fibres and also good source of minerals like selenium and magnesium and other nutrients which are essential to human health. This project work presents the development of crackers by supplementing wheat flour by replacement of water with wheat milk at different levels of 0%, 30%, 60% and 100%. The selected ingredients for cracker making were kneaded, cut and baked at 180°C for 5 min. The physical attributes, proximate analysis and sensory scores were evaluated and compared with the control sample. Increase in wheat milk concentration of crackers showed a decrease in weight loss, thickness, puffiness and increase in colour, diameter, thickness, spread ratio and hardness. Proximate analysis results demonstrated that crackers with increased wheat milk concentration showed higher amounts of carbohydrates, proteins, fats but lower in moisture than the control sample. Sensory evaluation results indicated that substitution of water with wheat milk of levels up to 100% can be done without affecting the sensory acceptability of crackers, thereby increasing its nutritional quality.

Keywords: Wheat milk; crackers; physical properties; nutritional composition; sensory evaluation

INTRODUCTION:

Wheat is a cereal crop which is highly used in baking industry because of its functional properties (1). Wheat milk contains good source of protein, vitamin and dietary fiber also good source of minerals like selenium and magnesium, nutrients essential to good health. Cracker is the term used to refer salt biscuits or savory. A cracker is a flat, dry baked food typically made with flour. Flavorings or seasonings, such as salt, herbs, seeds, or cheese, may be added to the dough or sprinkled on top before baking (2). Crackers are often branded as a nutritious and convenient way to consume a staple food or cereal grain. This paper represents the development of crackers by supplementing wheat flour by replacement of water with wheat milk on different levels of 30%, 60% and 100%.

MATERIALS AND METHODS:

Good quality wheat flour was purchased from local market of Sathyamangalam, Erode district, Tamil Nadu. For the preparation of crackers the main ingredients used in this study were wheat flour, sugar and oil. Chemicals used in nutritional composition analysis were all of analytical grade.

PREPARATION OF WHEAT MILK:

Wheat milk was extracted from wheat flour. Stiff dough was made using the wheat flour and water. It was then soaked in lukewarm water for about 7 hours. Then the dough was mixed and strained well to extract the wheat milk. The wheat milk was allowed to stand for 30 minutes. The wheat milk separated into two layers with the topmost layer being water. Thick wheat milk was separated from excess water layer and used for its incorporation in crackers.

PREPARATION OF CRACKERS :

The formulation used for the preparation of crackers is shown in table 1. The concentration of wheat flour, sugar and oil was maintained constant for all the trials. The amount of water used for the preparation of crackers was varied by substituting with different concentrations of wheat milk i.e., 30%, 60% and 100%. The wheat flour and sugar was mixed thoroughly and then oil, water and wheat milk were added based on the formulation. The dough was kneaded, proofed for 10 minutes, sheeted and cut using a cookie cutter. Then the dough was baked at 180°C for 5 minutes. The crackers were cooled at room temperature and then packed in polypropylene pouches and stored at room temperature for further analysis.

S.NO	INGREDIENTS	Composition (%)			
		0% WM	30% WM	60% WM	100% WM
1	Wheat flour	61.5	61.5	61.5	61.5
2	Sugar	4.7	4.7	4.7	4.7
3	Oil	12.3	12.3	12.3	12.3
4	Water	21.5	15.05	8.6	0
5	Wheat milk	0	6.45	12.9	21.5

Table 2.1 Formulation for the production of crackers by substituting water with different concentrations of wheat milk
Note: 0% WM, crackers with no wheat milk (control); 30% WM, crackers with 30% substitution of water with wheat milk; 60% WM, crackers with 60% substitution of water with wheat milk; 100% WM, crackers with complete replacement of water with wheat milk.

PHYSICAL PROPERTIES:

To estimate the physical characteristics of crackers, parameters such as weight loss, thickness, density spread ratio and puffiness were determined. Weight loss was calculated by finding the difference between the weights of baked crackers from unbaked crackers. Digital vernier caliper of accuracy 0.001mm was used to measure the thickness of crackers. Density was determined by taking the ratio of mass to volume. Spread ratio of crackers was calculated by dividing the average value of diameter by average value of thickness. Percentage puffiness was determined using the following equation:

$$Puffiness = \frac{\text{Thickness of baked cracker} - \text{Thickness of cracker dough}}{\text{Thickness of cracker dough}} \times 100$$

TEXTURE ANALYSIS

The texture of the cracker was found using texture analyzer. The hardness of the crackers was found with the load cell of 2kg weight. Sharp cutting blade probe was used to found hardness with the pre test speed of 2mm/s, test speed of 1mm/s, post test speed of 5mm/s and distance of 3mm.

COLOR OF CRACKERS:

The color of the crackers were found in terms of L*, a*, b* values are with D65 illuminant. The measurements were taken for each sample, and the value was reported (3).

The color index of the crackers was found by using the formula

$$\Delta E^* = \sqrt{L^{*2} + a^{*2} + b^{*2}}$$

PROXIMATE ANALYSIS:

Standard methods were used for the analysis of nutritional composition of crackers. Moisture content of samples was evaluated according to International Dairy Federation Bulletin (IDF, 1993). Protein and fat were estimated using (AACC method 44–15A) and (AACC method 46–13). The total sugars in sample were estimated using Lane- Eynon method. Ash content and crude fibre content was estimated using dry ashing (AACC method 30–25) and Weende method as described by Onwunka, (2005).

SENSORY ANALYSIS:

Composite scoring test was used for the sensory evaluation of wheat milk incorporated crackers with consumer panel. The average acceptability of color, texture, hardness and overall acceptability of the crackers were evaluated.

RESULTS AND DISSCUSSIONS:

PHYSICAL PROPERTIES:

The physical properties (weight loss, thickness, density, spread ratio, and hardness) of the crackers are tabulated in the table 3.1. Weight loss of the crackers was found to decrease with increase in the concentration of wheat milk. This may be due to that, the amount of water used is less and due to increasing wheat milk concentration,

soluble solid concentration increases. Thickness of the crackers decreased as the concentration of wheat milk increases. This is one of the desirable attribute as crackers with less thickness were previously reported to have high acceptability (4).

Density is one of the important parameter for the crackers. Generally crackers with less density find high acceptability (5). But here, as the concentration of wheat milk increases, density also increases. Higher the density, lesser is the expansion ratio of crackers after baking. Higher the spread ratio, higher the quality of crackers. Increasing concentration of wheat milk was found to be positively correlated with the spread ratio, making it desirable to replace water with 100% wheat milk. Crackers with high puffiness find high acceptability (6). But here, as the concentration of wheat milk increases, puffiness decreases. But still the decrease in percentage of puffiness was not very less suggesting that 100% replacement of wheat milk can be done with not many compromises on sensory acceptability. The hardness of the prepared crackers was determined using texture profile analyzer. As the wheat milk concentration increases, the hardness of the cracker decreases. The results of the texture analysis are as shown in figure 3.2. The L*, a* and b* values of the crackers was determined and mentioned in table 3.2 The lightness value of crackers increases as the wheat milk concentration increases. The color index value of the crackers was mentioned in table 3.2.



Figure 3.1 Crackers with 0, 30, 60, 100% wheat milk

% WM	Weight loss(g)	Increase in thickness(mm)	Density(g/m ³)	Spread ratio	Puffiness (%)
0	77.09±2.1	2.53±0.038	1.2×10 ⁻³	0.86±0.007	153.2±3.074
30	71.51±1.7	2.44±0.027	1.53×10 ⁻³	1.49±0.005	144.05±2.496
60	70.83±1.4	2.16±0.021	1.41×10 ⁻³	1.72±0.009	115.81±4.723
100	65.58±2.4	2.03±0.017	2.01×10 ⁻³	2.21±0.017	103.41±3.165

Table 3.1 Physical properties of crackers Note: %WM: Wheat Milk

%WM	L*	a*	b*	ΔE*
0	67.96±0.954	8.62±0.041	33.58±0.654	76.29±0.399
30	64.18±1.004	9.45±0.057	34.79±0.214	73.61±0.745
60	57.22±0.843	14.35±0.062	35.54±0.347	68.87±0.632
100	54.57±0.994	14.49±0.876	33.86±0.871	65.84±0.419

Table 3.2 color attributes of cookies Note: %WM: Wheat Milk

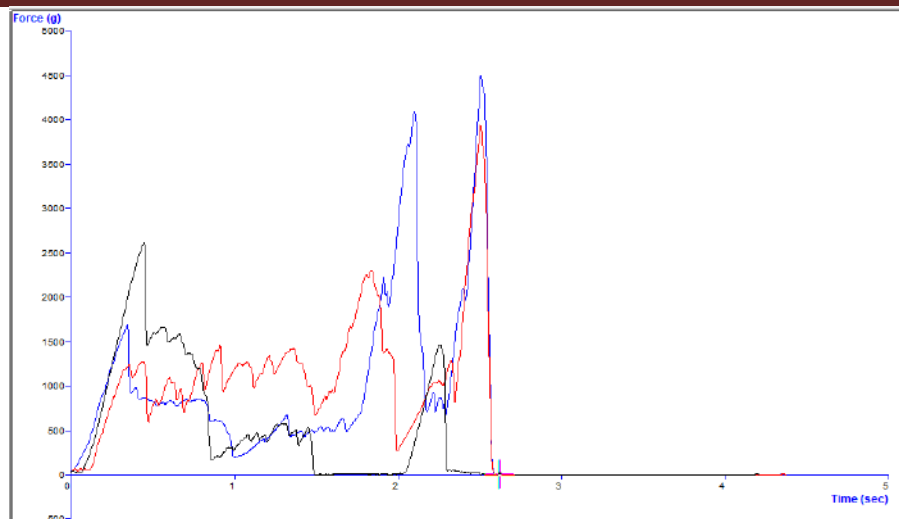


Figure 3.2 Texture analysis

PROXIMATE ANALYSIS:

The physical properties (Moisture content, ash, crude fiber, total sugars, protein and fat) of the crackers are tabulated in the table 3.3. The moisture content is one of the important parameter to determine the characteristics of the crackers. The moisture content of the crackers decreases with increase in wheat milk concentration. This may be due to the increase in soluble solid concentration in wheat milk. Ash content measures the inorganic residue remaining after the ignition of organic matter in the food. When the wheat milk concentration increases, the ash content increases which indicates the presence of inorganic residues is higher in replacement of wheat milk. Crude fiber gives the quantity of indigestible matters such as cellulose, lignin, pentosans, etc. Increasing concentration of wheat milk was found not to affect the crude fiber levels. The content of total sugars in the crackers increases when the wheat milk concentration increases. Protein is one of the important parameter for the crackers. The protein and fat content was found to gradually increase as the wheat milk concentration increases.

%WM	Moisture content	Ash content	%Crude fiber	%Total sugars	Protein	Fat
0	8.04±0.15	0.0091	22.32±0.134	35.51±1.065	6.45±0.052	13.23±0.254
30	4.17±0.032	0.0099	23.02±0.288	39.68±1.882	6.74±0.014	13.52±0.361
60	3.19±0.057	0.0100	21.21±0.143	44.33±0.958	6.81±0.078	13.59±0.214
100	2.67±0.033	0.0113	21.85±0.357	48.64±0.842	6.88±0.063	13.66±0.417

Table 3.3 Nutritional composition of crackers Note: %WM: Wheat Milk

SENSORY ANALYSIS:

The scores obtained from consumer panel for the sensory analysis are tabulated in table 3.4. It was found that color of the crackers was not influenced by the substitution of wheat milk. Addition of wheat milk to crackers improved the texture of the crackers. The hardness of the crackers was found to increase with increasing wheat milk concentration. But the The substitution of wheat milk does not affect the sensory properties such as color, aroma, flavor and overall acceptability of crackers. These composite score test results shows that the overall acceptance increases as the concentration of wheat milk increases. Hence the substitution of wheat milk in the crackers does not affect the texture and aroma of the crackers.

Parameters	0% WM	30% WM	60% WM	100% WM
Colour	14.8±0.207	13.3±0.301	14.8±0.369	14.8±0.224
Texture	22.5±0.292	29.5±0.352	32.1±0.210	32.1±0.277

Hardness	20.9±0.334	31.3±0.451	31.6±0.251	32.0±0.366
Total score	58.2±0.774	74.1±0.146	78.6±0.139	79.0±0.217

Table 3.4 Sensory analysis Note: %WM: Wheat Milk

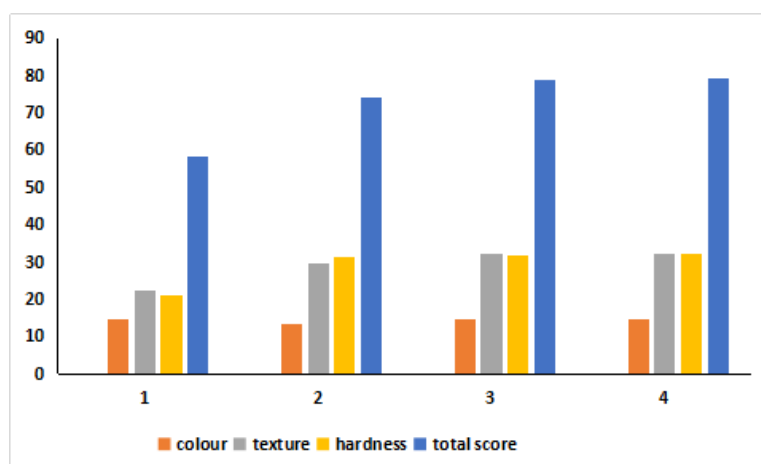


Figure 3.3 Sensory analysis

Note: 1, crackers with no wheat milk (control); 2: crackers with 30% substitution of water with wheat milk; 3: crackers with 60% substitution of water with wheat milk; 4: crackers with complete replacement of water with wheat milk.

CONCLUSIONS:

The crackers prepared by substituting wheat milk showed higher protein, fat, ash and total sugars compared to the control sample. The substitution of wheat milk instead of water at levels of 100% showed good overall acceptability with a decrease in physical properties such as puffiness and colour lightness value. However, all wheat milk substituted crackers showed high acceptability compared to the control crackers. Therefore, wheat milk has a potential to be supplemented in crackers. In this regard, wheat milk can improve the nutritional profile and sensory qualities without compromises on physical properties.

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Edible antimicrobial film incorporated with Carica papaya puree, defatted soy fibre and *Eugenia caryophyllata* oil

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Abstract: Food industries recognize edible packaging as a useful alternative or addition to conventional packaging to reduce waste and to create novel applications for improving product shelf life, stability, quality for consumer convenience. Diverse usage of biopolymers with certain food components provides antimicrobial properties. This idea is implemented to produce degradable food packages with antimicrobial properties. Edible cellulosic films made are with hydroxypropylmethylcellulose (HPMC). But it has inadequate moisture barriers. This can be improved by the addition of Papaya puree and defatted soy fibre. Nisin is a peptide effective against gram-positive bacteria. Nisin incorporated films shows increased antibacterial properties. The resultant films, with the addition of papaya puree will show significant increase in colour properties, tensile strength and seal strength. The addition of defatted soy protein to papaya puree, shows significant increase in elongation, water permeability and decrease in water solubility. Clove oil (*Eugenia caryophyllata*) is rich in plant-derived phenylpropanoids, with the main compound eugenol in edible film increases antifungal properties. The other bioactive compounds like thymol, carvacrol, and cinnamaldehyde add several functional properties. This innovative film is a boon and will play a vital role in food packaging sector and this shows a vibrant change in food processing industries.

Key Words: Biodegradable, Antimicrobial, cellulose film, papaya puree, defatted soy fibre, Clove oil.

INTRODUCTION:

Packaging material plays a vital role in Food industries in processing sectors. Usually plastics and glass are used for food packaging. Though plastic plays an inevitable role in packaging sector as a nondegradable material it pollutes the environment. In order to reduce its usage degradable films are formulated. With this antimicrobial agents are incorporated to increase the shelflife of packed food. Edible films provides several functional properties such as modifying the respiration effect of fruits and vegetables, improving structural integrity, reducing the usage of additives, retaining volatile flavor and they can also reduce packaging waste related with synthetic polymers [Silvia fores et al (2006)]. HPMC(Starch) forms a continuous matrix upon heating. This process is involved in making biodegradable film by gelatinization (swelling of starch molecules) and retrogradation (realignment of starch molecule). Plasticizing agents are added to remove the bitterness of starch by intermolecular forces. Flexibility and extensibility of the films can be improved upon the addition of glycerol. Glycerol is the most widely used plasticizer for improving the mechanical properties and transparency of the edible films [Maran et al (2012)].

LITERATURE REVIEW:

HPMC:

Hydroxypropylmethylcellulose(HPMC) commonly known as hypromellose, an inert ingredient usually used in ophthalmic preparations. This has high viscosity hence it requires no addition of solidifying agents in film preparation. HPMC has increased water vapour barrier with the addition of plasticizers.(Fernando A Osorio,Procedia food science).

CLOVE BUD OIL:

Clove essential oil (*syzygium aromaticum*, l) is known for its antimicrobial activity against several pathogenic bacteria. It showed in vitro inhibitory and bactericidal affect against *s.aureus*, *e.coli*, *l.monocytogenes* & *s.typhimurium* (food chemistry, volume 276, marjana randunzk).Application on meat products showed that clove oil microcapsule have good antiseptic effect on meat products at an effective fungicidal concentration of above 0.070%.As a novel alternative, clove oil microcapsule had practical application as preservative in meat products, especially in foods requiring heat processing.(LWT,volume 89,yu-feng Wang).

NISIN:

Nisin is a polypeptide together with acids and peroxides. It is a biological inhibitor that is produced by *Lactis streptococci*. The function of nisin is that it has a regulatory action in the growth of producer organisms. Biosynthesis of nisin takes place after 50% mass of producer organisms is formed. Nisin has a main advantage is that it acts as a food preservative

DEFATTED SOY FIBER:

Defatted soy flour is incorporated in HPMC based films to impart resistance to extension. Though specific volume increases it provides ample amount of extension resistance (D Indrani, Journal of Food Science and Technology). Defatted soy fiber contains 50% protein, 40% carbohydrate and micronutrients in smaller quantities.

PAPAYA PUREE:

Papaya puree is used in manufacturing of edible anti microbial packaging films. It has uniform moisture content hence it toughens the packaging film. Papaya puree increases the tensile strength and seal strength of the film. It contains antioxidant beta-carotene hence it reduces the cancer risks. It consists of enzyme called papain and this enzyme improves digestion. Here toughness of papaya puree is utilized by its addition with HPMC base.

GLYCEROL:

Natcharee et al (2016) analyzed the effect of different types of plasticizer (glycerol, xylitol and sorbitol) on physical and mechanical properties of potato starch films [Riku et al (2006)]. Their results showed that, water vapor permeability increase with increasing concentration of plasticizer. They concluded that, effects of properties of the films are larger for glycerol than the sorbitol. Hence for providing water vapour barrier glycerol is added.

MATERIALS:

Hydroxy propyl methyl cellulose (hpmc), clove bud oil, defatted soy fibre, nisin are purchased from reputed companies in India.

METHOD:

Preparation of papaya puree:

(Stafford et al 2) describe a procedure for preparing a puree from peeled papayas of the Solo variety grown in Hawaii. The papayas were harvested when mature-green, and then ripened at room temperature spontaneously. The ripe papayas were sorted, washed with brushes, trimmed, cut into halves and the seeds were removed by hand. Unpeeled fruit sections were further cut into smaller pieces and mashed in a comminuting mill using a No.4 screen. The mashed fruit was then fed by gravity into a Srew pulper fitted with a 0.060-inch screen. The pulp was transferred to a paddle finisher equipped with a 0.033-inch screen. The puree is obtained by this method.

Preparation of film:

Casting method was used to produce HPMC based edible film. The homogeneous film forming solution was prepared by dissolving the required amount of HPMC, DSF, CBO, PP, Nisin in the 100 ml of distilled water and solution is heated up to 70°C with stirring in a water bath. After the gelatinization of HPMC, it is kept for 30 min at a temperature of 70°C. After cooking the film forming solutions are poured into the 9 cm internal diameter petridishes and drying the solution with plates at room temperature for 48 hrs. The dried films were carefully peeled off from the petridishes and equilibrated at 25°C, 58% relative humidity for 72 h prior to further analysis.

Tensile stress and elongation at break:

Tensile strength, elongation at break and Young's modulus were studied a universal tensile strength tester. Each film was cut into (100 × 10 mm). The sample was mounted between the grips and tightened. The top grip was hung on the load cell hook. Then ON button is pressed to start the machine. The strips were stretched by the grips until broken. Then the reading was taken. The tensile strength and elongation of film were calculated according to Eq. (1.1) and (1.2), respectively [Sivarrooban et al (2008)].

$$TS \text{ (MPa)} = \text{Peak load (N)} / \text{Initial cross-section area (m}^2\text{)} \quad (1.1)$$

$$E \text{ (\%)} = \text{Increase in length at breaking point (mm)} / \text{Original length (mm)} \times 100\% \quad (1.2)$$

Water vapour permeability:

Water vapour permeability (WVP) was determined using the cup test. The films were cut into square pieces (90 × 90 mm). The thickness of the film cut was measured at 3 selected locations (top, center, and bottom). The mean of the film thickness was used to calculate WVP. The test cup was filled with 50-mL distilled water; the cut circular SPSF film was mounted on the top of the test cups. An initial weight was measured before placing the individual test cup in desiccators at 23 °C and 50% RH. The weight of cups was recorded after 2 h for steady state to be reached, and 6 weights were measured at 1-h intervals to calculate the weight changes of the test cups. The WVP was calculated using Eq. (1.3)

$$WVP = \frac{24 \times M}{A \times t} \text{ (g/m}^2\text{/day)} \quad (1.3)$$

Where,

M – Loss in mass (g)

T – Time between weighing
A – Internal area of dish (m²)

$$A = \frac{\pi d^2 \times 10^{-6}}{4} \quad (1.4)$$

Where,

d – Internal diameter of dish (mm)

Moisture content (AOAC, 1999):

Weigh the sample to the dish. Place the dish with the sample in a hot air oven at 105°C for 3 hrs. After drying reweighs the sample and dish separately. The moisture content was calculated using the following equation.

$$M.C(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Where,

W₁ – weight of sample before drying

W₂ – weight of sample after drying

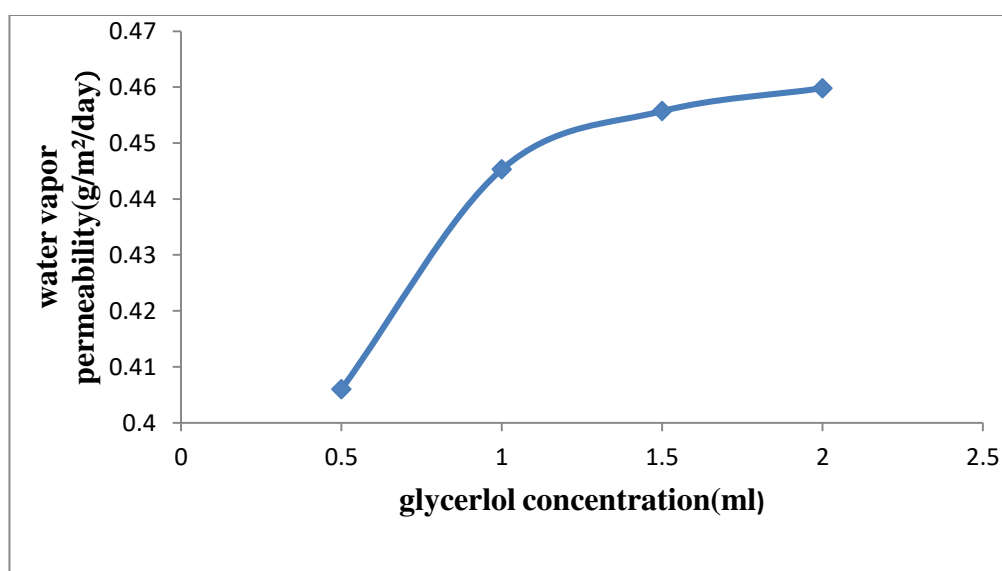
DISCUSSION:

TENSILE STRESS AND ELONGATION AT BREAK:

A good quality packaging material requires high stress with deformation [Alves et al (2007)]. It must provide structural integrity or reinforce food structure. The glycerol concentration significantly affected mechanical properties of modified HMPC films. The role of glycerol in film is, modified the structure in cellulose network, so the less dense film and also improving the film flexibility. The plasticizing effect of glycerol in edible films of have been extensively reported [Alves et al (2006), Riku et al (2006), Muhammed et al (2015), Prakashmaran et al (2012) and Andrea and Cecilia et al (2014)]. The tensile stress of HMPC based PP and DSF films fabricated with glycerol from 0.5% to 2% significantly ($P \leq 0.05$) decreased from 9.56 – 7.25 MPa caused by it reduces the interaction between the polymer chains, thereby reducing film strength and increasing film flexibility [Sorthornvit (2016)].

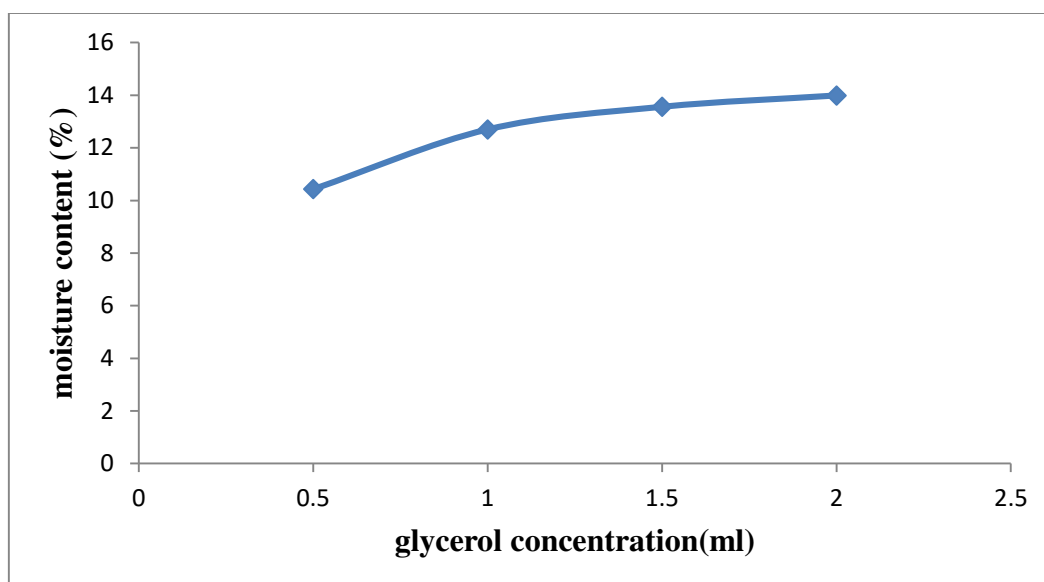
WATER VAPOR PERMEABILITY:

Food packaging film must have a strong retention to water permeability and its maintained at the lowest value. WVP decreased significantly (Tukey test, $p \leq 0.05$) with lower glycerol concentration. The water vapor permeability of film was significantly affected by the concentration of glycerol as presented in Fig 4.3. The water vapor permeability of the films significantly increased with the increasing glycerol concentration from 0.5% to 2% increased WVP values from 0.406 g/m²/day, 0.4453 g/m²/day, 0.4557 g/m²/day and 0.4598 g/m²/day. The hydrophilic character of starch is responsible for the high WVP of HMPC based films compared to synthetic plastic films [Sanyang et al (2015)].



MOISTURE CONTENT:

Moisture content is an important parameter of a packaging film. The glycerol concentration in SPS film was significantly affected the moisture content ($p \leq 0.05$). The moisture content of the film was increasing with the increasing concentration of glycerol [Maran et al (2013)] reported that high concentration of glycerol favour the adsorption of water molecules due to its hydrophilic nature, which retains water in the film matrix and form hydrogen bonds. High water resistance of a film is one of the most important properties for a food packaging applications.



ANALYSIS:

At different Glycerol concentration of HPMC based payapa and defatted soy fiber film tensile stress, Elongation at break, Moisture content, Water vapour permeability for the samples are noted on the average basis of three samples.

Run	Glycerol concentration (%)	Tensile stress (MPa)	Elongation at break (%)	Moisture content (%)	Water vapor permeability (g/m ² /day)
1	0.5%	(9.53±0.25) ^a	(48.4±0.2) ^b	(12.5±0.1) ^d	(0.41±0.01) ^a
2	1%	(8.50±0.20) ^b	(48.53±0.35) ^b	(13.6±0.1) ^c	(0.46±0.02) ^a
3	1.5%	(7.63±0.3) ^c	(49.36±0.20) ^a	(15.5±0.2) ^b	(0.52±0.02) ^a
4	2%	(7.43±0.2) ^c	(49.5±0.20) ^a	(16.6±0.3) ^a	(0.46±0.15) ^a

FINDINGS:

From this experiment edible antitmicrobial films were developed, an alternative for plastic films. The obtained film found to have reasonable value of Tensile stress, water vapour permeability. Upon different glycerol concentration several factors varies. The resultant film shows increased antimicrobial properties on comparison with base HPMC base films.

RESULT:

Edible antimicrobial films are developed with HPMC base and the effect of the glycerol concentration on properties were studied. The prepared films were transparent and homogenous. The textural properties and the water vapour permeability increases along with glycerol concentration. . But the tensile strength of the film was decreased with increasing the concentration of glycerol. It is clear that, properties are controlled mainly by plasticizer concentrations.

CONCLUSION:

As a alternative for plastic films, cellulose based edible films can be used. This innovative film is a boon and will play a vital role in food packaging sector and this shows a vibrant change in food processing industries. Further studies on improving microbial properties in under process.

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Sensory Studies on Development of Probiotic Guava *Shrikhand*

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Abstract: *Shrikhand* was prepared using different levels of Guava pulp to increase nutritional quality and overall acceptability. The Development of Probiotic Guava *Shrikhand* using full cream milk *Chakka* with constant level of sugar (42% by weight of *Chakka*), *Lactobacillus rhamnosus* GG and Allahabad Safeda variety of Guava pulp at different concentrations i.e. T₀ (0%), T₁ (2%), T₂ (4%), T₃ (6%), T₄ (8%) and T₅ (10%). All the treatments were evaluated for physico-chemical, microbiological and storage stability. Guava fruit pulp was added to *Chakka* to enhance its colour and appearance, body and texture, sweetness, flavour and overall acceptability of T₄ was liked very much up to 12 days of storage. The viable Probiotic count of Guava *Shrikhand* was recorded as 10x10⁷cfu/g in fresh product. Although the Probiotic count was decreased to log₁₀9 cfu/g from the initial count of log₁₀10 cfu/g upto 12 days of storage. An effort was made to produce a functional fermented Probiotic product. It was concluded that the organoleptic quality of Probiotic *Shrikhand* was enhanced by using of Guava pulp at the level of 8% against the control and storage stability up to 12 days at refrigeration temperature.

Key Words: Guava, *Shrikhand*, Probiotic, Sensory Evaluation.

INTRODUCTION:

Fermented milk products are generally sour milk products prepared by fermenting milk by means of specific dairy starter cultures. Fermented milk has become increasingly popular in recent times on account of being important sources of Probiotics in our diet. Fermented milk products like *Shrikhand* have some advantage over fluid milk because of more keeping quality, digestibility and palatability. *Shrikhand* is one of the fermented milk delicacies and is derived from the Sanskrit word “*Shrikharini*”, meaning a curd preparation with added sugar, flavouring material, fruits and nuts (Desai and Gupta, 1986). Probiotics are defined as live microbial feed supplement that beneficially affects the host by improving its intestinal balance (Gorbach, 2000). The term “Probiotics” meaning “for life” was first coined by Lilly and Stillwell in 1960s (Lilly and Stillwell, 1965). In the early 20th century, Metchnikoff (1907) first proposed the concept of Probiotics. The market of Probiotic dairy foods is increasing annually. An increased demand for dairy Probiotic products comes from health promotion effects of Probiotic bacteria which are originally initiated from milk products, bioactive compounds of fermented dairy products and prevention of lactose intolerance (Bastani *et al.*, 2016). Probiotics are the most natural and safe means of maintaining this balance (Anuradha and Rajeshwari, 2005). Because of the change in the economic status and food habit of consumer, the other varieties of *Shrikhand* such as fruit *Shrikhand* are also in great demand (Nadaf *et al.*, 2012). Guava is an important fruit crop in tropical and subtropical regions of the country and often marketed as “super-fruits” which has a considerable nutritional importance in terms of vitamins A and C with seeds that are rich in omega-3, omega-6 polyunsaturated fatty acids and especially dietary fiber, riboflavin, as well as in proteins and minerals salts (Singh, 2008). Guava has excellent digestive and nutritive value, pleasant flavour, high palatability and availability in abundance at moderate price (Kadam *et al.*, 2012). *Shrikhand* may be prepared by addition of Guava pulp to enhance its colour, appearance and flavour. Keeping in view of above facts, the present study is planned for development of *Shrikhand* with addition of Guava pulp and Probiotic bacteria.

LITERATURE REVIEW:

According to FSSAI (2011), *Shrikhand* means a product obtained from *Chakka* or Skimmed milk *Chakka* to which milk fat is added. It may contain fruits, nuts, sugar, cardamom, saffron and other spices. It shall not contain any added colouring and artificial flavouring substances. It is very popular in the state of Gujarat, Maharashtra and part of Karnataka (Sonawane *et al.* 2007). It shall conform to the following specification given in Table 2.1

Table 2.1 FSSR (2011) Standards for *Shrikhand*

S.N.	Particulars	<i>Shrikhand</i>	Full Cream <i>Shrikhand</i>	Fruit <i>Shrikhand</i>
1.	Total solids, minimum, %, (m/m)	58.0	58.0	58.0
2.	Milk fat, minimum, %, (m/m), on dry basis	8.5	10.0	7.0
3.	Milk protein*, minimum, %, (m/m), on dry basis)	9.0	7.0	6.0
4.	Titrateable acidity, maximum, %, (as lactic acid)	1.4	1.4	1.4
5.	Sugar (sucrose), maximum, %, m/m, (on dry basis)	72.5	72.5	72.5
6.	Total Ash, maximum, %, m/m, (on dry basis)	0.9	0.9	0.9

* Protein content is 6.38 multiplied by the total nitrogen determined

The curd mass known as “*Chakka*” is the base material for *Shrikhand*. It is obtained by the removal of whey from *Dahi*. The quality of *Shrikhand* is largely influenced by physical and chemical properties of *Chakka*. According to FSSA (2006), *Chakka*-means a white to pale yellow semi-solid product of good texture and uniform consistency obtained by draining off the whey from the Yoghurt/*Dahi* obtained by the lactic fermentation of cow's milk, buffalo's milk, skimmed milk and recombined or standardised milk which has been subjected to minimum heat treatment equivalent to that of pasteurization. It shall have pleasant Yoghurt/*Dahi* like flavour. It shall be free from mouldness and free from signs of fat or water seepage or both. It shall be smooth and it shall not appear dry. It shall not contain extraneous colour and flavours. The requirement for *Chakka* according to FSSR is tabulated in Table 2.2.

Table 2.2 FSSR (2011) Standards for *Chakka*

S.N.	Parameters	<i>Chakka</i>	Skimmed Milk <i>Chakka</i>	Full Cream <i>Chakka</i>
1.	Total solids, minimum, %, (m/m)	30.0	20.0	28.0
2.	Milk fat, %, (m/m), on dry basis	33.0 (minimum)	5.0 (maximum)	38.0 (minimum)
3.	Milk protein*, minimum, %, (m/m), on dry basis	30.0	60.0	30.0
4.	Titrateable acidity, maximum, %, (as lactic acid)	2.5	2.5	2.5
5.	Total Ash, maximum, %, (m/m), on dry basis	3.5	5.0	3.5

* Protein content is 6.38 multiplied by the total nitrogen determined

Devshete *et al.* (2012) prepared *Shrikhand* using yoghurt culture and reported that the *Shrikhand* prepared by using yoghurt culture was comparable in composition, physical and sensory attributes with *Shrikhand* prepared using *Dahi* culture. The main advantage of this product was that, it contains live cell of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which provide therapeutic benefits to consumers. Singh and Paswan (2015) was carried out for the development of *Jamun* enriched *Shrikhand*. The process was optimized with 12.78 per cent *Jamun* pulp powder and 30.66% concentration of sugar shows the higher impact on the colour, flavour, sweetness, body and texture and overall acceptability where as the sugar concentration greatly affect the sweetness and body and texture. The optimized JES retains higher amounts of nutritional and functional compounds with excellent sensory score. Gupta *et al.* (2018) carried out the enhancement of organoleptic qualities of *Shrikhand* by using fruit pulp conducted to evaluate the effect of fruit pulp on organoleptic and sensory quality of *Shrikhand* and also enhancing keeping quality, flavour, lowering cost of production. There were three types of fruit Mango, Banana and Papaya with four different levels of fruit pulp as 0, 20, 40 and 60 per cent and stored at five various storage periods viz. 0, 7, 14, 21 and 28 days at 5°C. The organoleptic quality of *Shrikhand* was enhanced by using of Banana pulp at the level of 20 per cent. This combination was accepted up to 21 days of storage period at 5°C of refrigeration temperature. Dadarwal *et al.* (2005) added the Banana, Guava and Sapota pulps in milk as well as in *Chakka*. Addition of 5 per cent fruit pulps in milk before pasteurization and lactic fermentation enhanced drainage of whey. The *Shrikhand* prepared by addition of 5 to 10 per cent pulp in milk and 10 to 20 per cent pulp in *Chakka* were acceptable in organoleptic qualities. However, the *Shrikhand* with 5 per cent pulp addition in milk and 10 per cent pulp addition in *Chakka* were equivalent or superior to *Shrikhand* made from milk alone in sensory attributes.

MATERIALS:

- Information about Place of Work

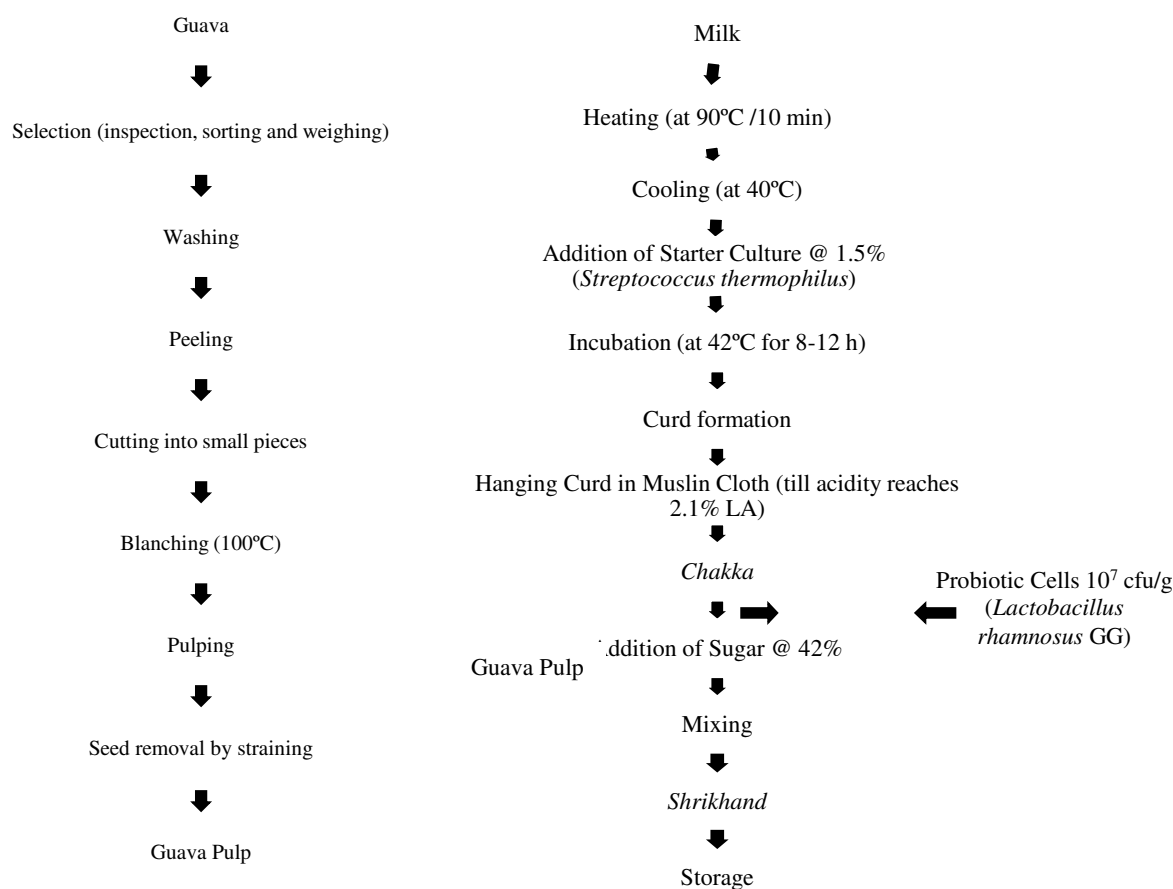
The present study on “Development of Probiotic Guava *Shrikhand*” was carried out at the Department of

Dairy Microbiology, College of Dairy Science and Food Technology, C.G.K.V., Raipur (C.G.).

- Collection of Milk
The fresh full fat cream milk was procured from local market.
- Starter Culture
The freeze dried *Streptococcus thermophilus* (NCDC-325) and *Lactobacillus rhamnosus* GG (NCDC-347) was procured from NDRI, Karnal.
- Guava
The fresh Guava (Allahabad Safeda) fruit was procured from Department of Horticulture, College of Agriculture, I.G.K.V., Raipur (C. G.).
- Sugar
Clean crystalline cane sugar was procured from local market and used as per requirement.
- Mixer/Grinder
Electric mixer was use for making Guava pulp and grinding sugar.
- Muslin cloth
Muslin cloth was procured from local market for draining of whey from *Dahi* during *Chakka* making.
- Plastic Cup
Plastic cup was procured from local market for storing and serving of *Shrikhand*.
- Skim Milk Powder
Skim milk powder (*Manthan*) was used for standardization of milk and for the propagation of starter cultures.

METHOD:

Fresh good quality full fat cream milk was converted to *Shrikhand* as follows. The milk was heated and then inoculated by lactic starter culture @ of 1.5% and incubated, at 42°C for 8 to 12 h until a firm coagulum was formed. The *Dahi*, so formed was broken and transferred to muslin cloth and hanged, for drainage of whey. The coagulum so obtained is called *Chakka*, was mixed with 42% sugar, Guava fruit pulp for different treatments and probiotic culture to obtain Probiotic Guava *Shrikhand*. Flow Chart for Preparation of Guava Pulp and Probiotic Guava *Shrikhand*



Treatments

Treatments	Chakka (g)	Guava Pulp (g)	Sugar (g)	<i>Lactobacillus rhamnosus</i> GG
T ₀	70	0	30	10 ⁷ cfu/g
T ₁	68	2	30	10 ⁷ cfu/g
T ₂	66	4	30	10 ⁷ cfu/g
T ₃	64	6	30	10 ⁷ cfu/g
T ₄	62	8	30	10 ⁷ cfu/g
T ₅	60	10	30	10 ⁷ cfu/g

DISCUSSION:

The effect of different levels of Guava pulp on colour and appearance, body and texture, sweetness, flavour and overall acceptability of Probiotic Guava *Shrikhand* was evaluated. It is confirmed that the different combinations of Guava pulp had not been significantly effect on the colour & appearance and sweetness and statistically there was significant difference on body and texture, flavour and overall acceptability on the control and test Probiotic *Shrikhand*.

Table 5.1 Sensory Evaluation of Different Treatments of Guava Pulp in Probiotic *Shrikhand*

Treatments	Colour and Appearance	Body and Texture	Sweetness	Flavour	Overall Acceptability
T ₀	7.00 ^e	7.74 ^d	7.00 ^d	7.72 ^c	7.74 ^e
T ₁	7.40 ^d	7.80 ^c	7.10 ^{cd}	7.80 ^c	8.00 ^d
T ₂	7.68 ^c	8.28 ^{ab}	7.50 ^c	7.88 ^c	8.29 ^c
T ₃	8.22 ^{bc}	8.50 ^a	7.28 ^c	8.10 ^{ab}	8.50 ^{ab}
T ₄	8.86 ^a	8.91 ^a	8.80 ^a	8.80 ^a	8.90 ^a
T ₅	8.43 ^b	8.65 ^a	8.50 ^b	8.65 ^a	8.75 ^a

ANALYSIS:

• Propagation of Cultures

The culture was propagated and maintained in reconstituted skim milk and MRS agar (*Lactobacillus rhamnosus* GG) and M17 agar (*Streptococcus thermophilus*) and broth medium during the investigation.

• Physico-chemical Properties

The entire test Guava enriched Probiotic *Shrikhand* along with control was subjected to physico-chemical analysis viz. titratable acidity, fat, moisture, protein, ash and total solids.

Table.6.2 Physico-chemical Properties of Probiotic Guava *Shrikhand*

Parameters	Treatments	
	Without Guava Pulp based <i>Shrikhand</i> (Control)	Guava Pulp (8 per cent) based <i>Shrikhand</i> (T ₄)
Titratable Acidity (per cent)	1.10	1.18
Moisture (per cent)	37.95	38.84
Total Solids (per cent)	59.30	59.34
Fat (per cent)	8.73	8.74
Protein (per cent)	6.33	6.37
Ash (per cent)	0.69	0.71

RESULT:

✓ Sensory Analysis

• Colour and Appearance

The highest scores secured for colour and appearance was 8.63 (T₄). It is confirmed that the different combinations of Guava pulp had not been significantly effect on the colour and appearance of the control and test Probiotic *Shrikhand*.

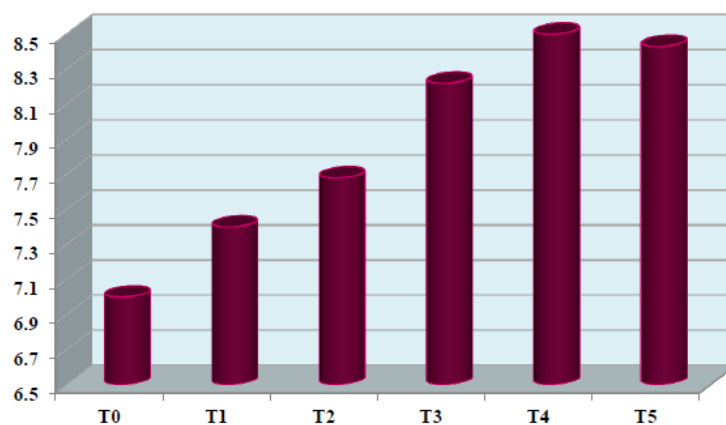


Figure 1. Colour and Appearance Score of Probiotic Guava *Shrikhand*

- Body and Texture**

The highest sensory score of 8.91 (T₄) was secured with respect to body and texture. The statistical analysis revealed that the different combination of Guava pulp had been significant effect on body and texture of control and test Probiotic *Shrikhand*.

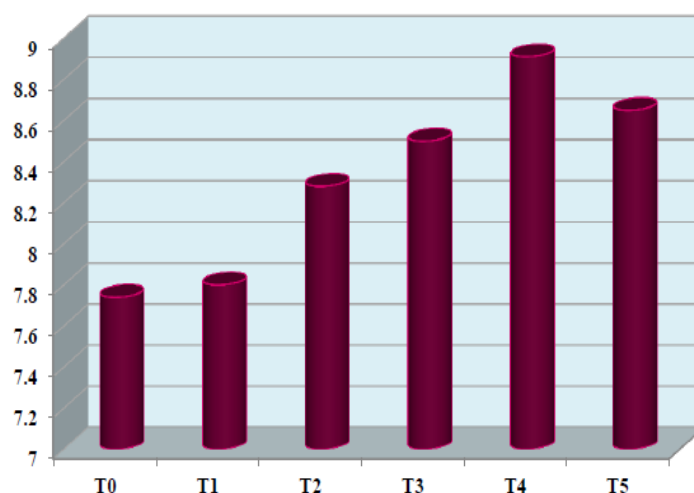


Figure 2. Body and Texture score of Probiotic Guava *Shrikhand*

- Sweetness**

The highest sensory score of 8.80 (T₄) was secured with respect to sweetness at 8 per cent level of Guava pulp. The statistical analysis revealed that the different combination of Guava pulp had not been significant effect on sweetness of control and test Probiotic *Shrikhand*.

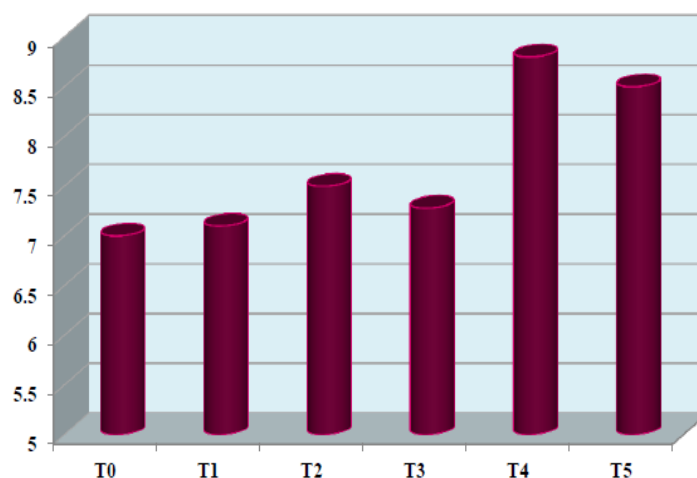


Figure 3. Sweetness score of Probiotic Guava *Shrikhand*

- Flavour**

The highest sensory scores of 8.80 (T₄) was secured with respect to flavour. Statistically there was significant difference on flavour between control and test Probiotic *Shrikhand* samples.

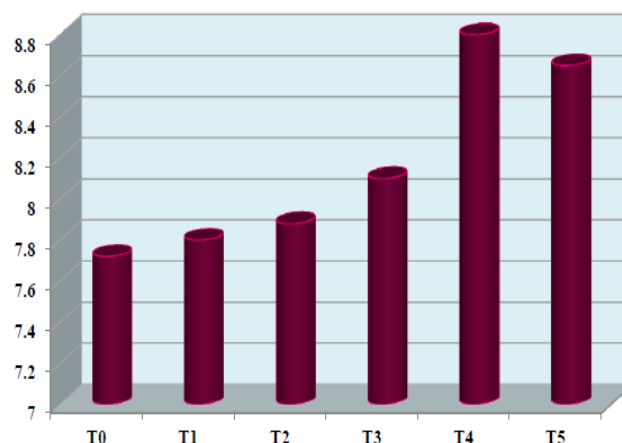


Figure 4. Flavour score of Probiotic Guava *Shrikhand*

• Overall Acceptability

The highest sensory score of 8.90 (T₄) was secured with respect to overall acceptability at 8 per cent level of Guava pulp. The statistical analysis revealed that the different combination of Guava pulp had been significant effect on overall acceptability of control and test Probiotic *Shrikhand*.

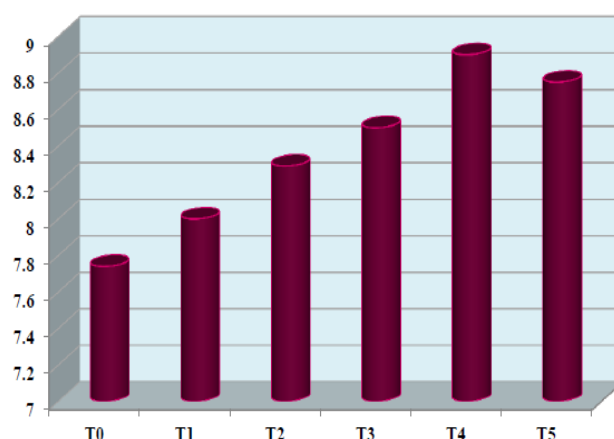


Figure 5. Overall Acceptability score of Probiotic Guava *Shrikhand*

✓ Microbiological Analysis

The Probiotic count was decreased to log₁₀9 cfu/g from the initial count of log₁₀10 cfu/g upto 15 days of storage. The coliform as well as yeast and mold counts were absence during the storage period.

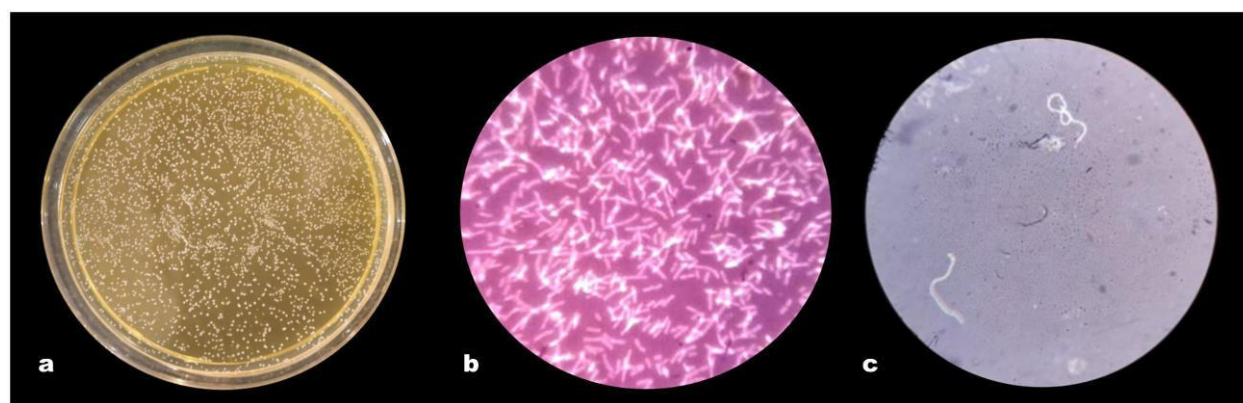


Figure 7.2 (a) Colonies of *Lactobacillus rhamnosus* GG on MRS agar (b) Photograph of *Lactobacillus rhamnosus* GG and (c) *Streptococcus thermophilus*

CONCLUSION:

It may be concluded that Probiotic Guava *Shrikhand* can be prepared successfully from full fat cream milk. The highly acceptable sensory characteristics i.e. colour and appearance, body and texture, sweetness, flavour and overall acceptability of T₄ was selected on the basis of sensory attributes without any significant deterioration in

microbiological and sensory qualities. The storage stability of T₄ having added health and nutritional advantages of Guava and Probiotics was found suitable for consumption up to 12 days of refrigeration temperature.

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Development of whey beverage incorporated with sugarcane juice

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Abstract: The aim of this study was to develop a low cost nutritious beverage using whey and sugarcane juice. Here by considering the waste utilisation as a factor, the whey from the dairy industry which being dumped as sewage and resulting into pollution is being reperculated. The level of sugarcane juice addition was optimized based on sensory quality evaluation. From the sensory evaluation 7:3 was accepted by most of the panel members and got the maximum overall acceptability. For the developed product, physio-chemical properties viz., pH, acidity, colour, viscosity and mineral content were calculated. For the time period of 15 days, pH value got decreased from 5.41 to 3.85. Acidity got increased from 0.081 % to 0.726. Viscosity values varied for all the compositions. In ash content there is a significance difference between the samples and control. Lightness (L*) value increased with increase in concentration of sugarcane juice. Control got the low ash content as 3.2 % where the ash content of 7:3 was found to be 5 %.

Key Words: Whey, sugarcane juice, beverage.

INTRODUCTION:

In recent few years many studies have been made to transform the whey obtained from dairy industry to be a value-added food product. The whey obtained by curdling or straining of milk is a good source of vitamins and minerals and contains high quality protein among them sulphur amino acids have high anticancer property. This whey can be applied in the formulation of RTS beverages or soft drinks and some protein beverages to be nutrition for various therapeutic purposes. Utilization of whey can be done with various fruit beverages at different compositions. Whey is watery part of milk that remains after separation of coagulated product that results due the acid mediated coagulation of milk. It is the major by-product of dairy industry manufacturing paneer, channa, chakka, cheese, casein etc. Whey contains about half of the total solids of milk and is a source for many nutrients such as whey proteins, lactose and minerals. Nutritional and health benefits are Whey contains proteins, albumins and globulins which have outstanding nutritional qualities. The glutathione in whey protein is an antioxidant which reduce the risk of cancer in animals suggesting an avenue for future medical research. They have antimicrobial, antiviral and antioxidant properties, which be protection against cancer and heart diseases and assist at the enhancement of immune defence. The freshly obtained juice extracted from pressed sugarcane was mixed with whey liquid at different composition and optimisation was done. The health benefits of the sugarcane are it has a great preventive and healing source. Sugarcane strengthens the stomach, kidneys, heart, eyes, brain, and sex organs. It refreshes and energizes the body instantly as it is rich in carbohydrates.

LITERATURE REVIEW:

Silviya R Macwan an et-al proposed that whey the by –product obtained from dairy industry which were being considered as an insignificant and was either can be used as animal feed or it can be disposed as waste. By considering that a study based on therapeutic study was made on transformation of whey as a utilisation in animal feed. Alak Kumar singh and et-al said that whey obtained from dairy industry is generally disposed into sewage which creates a major problem of pollution beside which many losses of valuable nutrients. So, they aimed at the developed a low-cost nutritious whey beverage by hydrolysing lactose with immobilized beta-galactosidase enzyme. Dhananjay statue and et-al carried out study on incorporation of Mentha arvensis extract to prepare a herbal beverage with four different combination .The acceptability of whey beverage was measured in terms of sensory attributes such as colour, flavour, consistency, and taste using 9 point hedonic scale by a panel of five semi expert judges. By developing such herbal beverage based on whey, beetroot and methanol to exhaust nutritional therapeutic as well as medicinal properties of beetroot and menthol were done. Pareek N and et-al (2014) conducted a study on blended RTS of mixing orange juice with whey at different ratios like 70:30,60:40, and 50:50. They carbonated the RTS manually in which 70:30 was the most acceptable. In the nutrient analysis 70:30 ratio shows the drastic increase in nutrients.

MATERIALS:

Fresh milk and sugarcane were procured from local market of sathyamangalam. The procured raw materials were free from adulteration and microbial contamination.

METHOD:

- **Extraction of juice- Sugarcane**

For the extraction of juice, sugarcane stems were washed thoroughly and inserted into the mill where the fresh sugarcane juice were collected and kept in refrigeration condition until it is used.

- **Preparation of whey**

For the preparation of whey, the raw milk was procured from dairy farm. Then it was boiled to 72°C for 5min and cooled slowly till it reaches 62°C. After obtaining the temperature the coagulant was added (citric acid 2g for 1L) and stirred continuously till the whey separates and settles. Then the whey was separated and clarified using centrifugal separator (5000-6000rpm at 40c) and it was filtered using double layer muslin cloth. Then it was refrigerated until it is used.

- **Preparation of beverage**

For preparing the beverage the sugarcane juice and whey was taken in different ratio 4:6, 5:5, 6:4 and 7:3 respectively. Optimisation was purely based on the sensory analysis and 7:3 ratio had the high acceptance. The sugarcane juice was mixed with whey at different ratios and was filled in pre sterilized bottles with head space of 2.5m. Then in bottle sterilization was carried out and the bottles were labelled and stored at temperature of 5°C.

ANALYSIS:

- ✓ **Physiochemical Properties**

- **Total Soluble Solids**

The TSS content in prepared RTS was determined using the refractrometer at standard temperature i.e. 20°C and the obtained values were expressed in terms of °Brix.

- **pH**

The pH of the samples was measured using pH meter. pH meter was calibrated using a buffer solution and with water.

- **Color characteristics**

The color of the sugarcane beverage in terms of L*, a*, b* values are measured using hunter lab colorimeter color measuring system equipped with D65 illuminant.

- **Total acidity**

The total acidity test was carried out by titrimetric method by titrating the diluted sample against 0.1 N NaOH solutions. Phenolphthalein was used as the indicator and the appearance of pale pink color indicated the end point of the experiment. The experiment was repeated two times to obtain the concordant value. By using the titre value, the % of acidity in terms of citric acid was calculated by the below given formula.

$$\% \text{acidity} = \frac{\text{ml of NaOH consumed} \times \text{acid factor} \times 100}{\text{volume of the sample}}$$

- **Viscosity**

The viscosity of the sample was measured using rotational viscometer. Appropriate spindle was chosen and at different rpm centipoise was checked at maximum accuracy. Rotational viscosity determines the property of a fluid at which angular momentum differences are equilibrated.

- **Ash**

Ash content determination was carried out using muffle furnace. The temperature of the furnace was maintained at 550°C. It determines the amount of mineral present in sample.

- **Sensory values**

Composite Scoring test was used for the sensory evaluation of prepared beverage with consumer panel. The average acceptability of colour, appearance, flavour and overall acceptability of the beverage was evaluated.

RESULT:

- ✓ **pH**

pH is the negative of the base 10 logarithm of the molar concentration of hydrogen ions in the solution. More precisely, P^H is the negative of the base 10 logarithm of the activity of the hydrogen ion.

S.NO	DAYS	7:3	CONTROL	TEMPERATURE (°c)
1	0 th Day	5.41	5.54	22
2	5 th Day	4.94	4.77	24
3	10 th Day	4.20	4.21	21
4	15 th Day	3.85	3.50	23

Table 6.1: pH

✓ Color characteristics

The L*, a* and b* values of the sugarcane blended with whey ready to serve beverage were determined. The proportion with higher percentage of sugarcane blended with minimum quantity of whey showed the highest color values.

S NO	SAMPLE	L*	A*	B*	ΔE^*
1.	7: 3	21.50	4.75	10.63	24.45

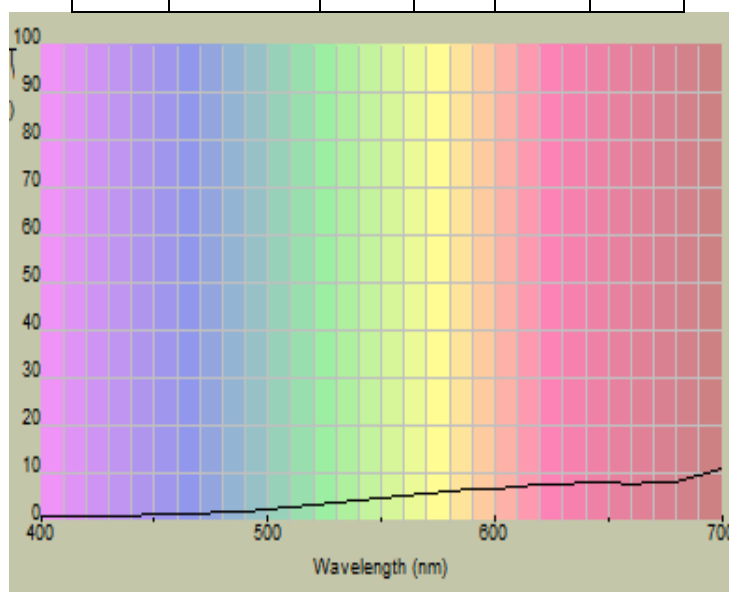


Figure 1. Colour Characteristics

✓ Acidity Test

Titrateable acidity is done in order to determine the acid content in the (RTS) juice. As per the optimization the 7 :3 ratio beverage had an acidity

S.NO	DAYS	7:3 (ml)	CONTROL
1	0 th Day	0.081	0.027
2	5 th Day	0.111	0.156
3	10 th Day	0.345	0.478
4	15 th Day	0.726	0.887

Table 6.3: Acidity test

✓ Viscosity

In 7:3 the viscosity was found to be less when compared with other ratio due to the high amount of sugarcane juice.

S.NO	RATIO	CENTIPOISE	RPM	ACCURACY
1	4:6	14	30	98%
2	5:5	17	30	95%
3	6:4	18	50	96%
4	7:3	12	50	90%
5	Control	20	30	93 %

Table 6.4: Viscosity

✓ **Ash**

The mineral content was found to be less in 7:3 ratio due to the less amount of whey content.

S.NO	SAMPLE	MINERAL (%)
1	4:6	5.6
2	5:5	5.4
3	6:4	5.3
4	7:3	5.0
5	Control	3.2

Table 6.5: Ash

✓ **Sensory analysis**

The scores obtained from consumer panel for the sensory analysis are tabulated in table 6.5. It was found that color of the RTS was influenced by the addition of sugarcane juice. The addition of sugarcane juice at different proportions showed slight difference in the sensory properties such as color, aroma, flavor and overall acceptability of RTS. These composite score test results show that the overall acceptance of the RTS, ratio 7:3 was better.

S.NO	PARAMETERS	4:6	5:5	6:4	7:3	CONTROL
1	Color	7.25±0.19	7±0.43	7.5±0.63	8±0.26	4.25±0.19
2	Taste	7±0.56	7.5±0.56	8.25±0.5	9±0.31	4±0.57
3	Appearance	7±0.23	7.25±0.28	7±0.5	8.5±0.15	4±0.22
4	Flavor	7±0.14	7.5±0.75	7.5±0.25	8.25±0.43	4.25±0.15
5	Sweetness	7.25±0.40	8±0.25	8.5±0.5	8.5±0.36	4±0.39
6	Overall acceptability	7±0.42	7.25±0.36	7.5±0.5	8	4±0.42

Table 6.6: Sensory values for the prepared RTS beverage

CONCLUSION:

The whey beverages was optimized using composite scoring test. For the trial four different variations of sugarcane juice and whey were taken viz 4 :6, 5 :5, 6 :4, 7 :3. Among the ratio 7 :3 was chosen best and obtained higher overall acceptability among the other samples. The viscosity values were found to be high in 4 :6 and to be low in 7 :3 due to the composition of whey. The obtained colour was influenced by the sugarcane juice. The pH values tend to decrease and the acidity values tend to increase as the day increases. Though the sugarcane juice with many health benefits when mixed with whey gives enhanced benefit to the beverage. Hence here waste utilisation takes place and it can good considered as a low cost nutritious beverage.

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Promising Role of Polymers in Agriculture

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Abstract: The application of polymers in agriculture dates back to early 1950s. But in recent times, it has gained much recognition because of its ability to increase the agricultural productivity without any threat to the environment. Raising population paved the way for extensive use of agrochemicals to maximize food production which in turn had a downbeat on nontarget organisms and the natural resources. This urged the researchers to opt for polymer technology which is in no way harmful to the environment owing to its biodegradability and nontoxicity nature. One of its kind, superabsorbent polymers or crosslinked hydrogel have demonstrated to be soil conditioners by enhancing the water holding capacity, permeability and minimizing soil erosion or compaction which in turn leads to reduction in the irrigation frequency, enhanced plant growth and fertility of the soil. The potential of the polymer to be easily tailored and incorporation of nanotechnology has led to the development of polymer nanocarrier in releasing agrochemicals in a sustained manner thus increasing their efficacy of action and preventing environmental pollution. This article elucidates the role of polymer in agricultural field highlighting its current trend and future aspect.

Key Words: Polymer; Agriculture; Superabsorbent polymer; Hydrogel; Soil conditioner.

INTRODUCTION:

Our planet has provided us with ecosystem and natural resources which we depend upon to harbor life. Right from the rise of human civilization, development in agriculture has been commendable with own set of traditional agricultural practices across the world. About 37 percent of the world's population depended on agriculture for their livelihood which started to deteriorate since 2011. Currently world's population has reached over 7.6 billion feeding which has become a challenge since the agricultural population is shrinking and the non-agricultural population is soaring. Thus expansion of urbanization to accommodate the rising population and growth of industrialization for their livelihood has destroyed agricultural lands and other natural resources. Unlike traditional agricultural practices such as polyculture farming or shifting cultivation, monoculture farming which involves cultivation of a single type of crop in a given area throughout the year came into practice to meet the global demand for food. This resulted in the depletion of nutrients essential to that crop and buildup of pests in the soil. To prevail over such situation pesticides and fertilizers were used. Though the usage of agrochemicals had given a remarkable yield, their excessive use has left the land and natural water resources with contaminants rendering the ecosystem unfit to live [1]. Furthermore, considering the present day distress caused by climatic change at the global level that results either in flood or drought conditions, it is a mandate to look for a promising technology which aids in meeting the global demand for food without devastating the ecosystem as well. Recent developments in the polymer technology have demonstrated betterment of soil condition and other agricultural aspects though the use of polymers in agriculture has began in 1950s. This review article emphasizes the use of both natural and synthetic polymers in soil conditioning, water conservation and controlled delivery.

CROP PROTECTION:

Crop protection deals with the practice of defending the crops from weeds, insects, temperature, humidity, rainfall and wind which hinders the plant growth and development. It is very important to guard the crop so as to enhance its yield and quality. Conventional methods of crop protection includes use of pesticide, pest control by biological means such as use of trap crops or other living organisms, constructing a barrier between pest and plant like net or mesh and use of bird scarers. For protection against the abiotic stress, greenhouses or tunnels, the structures in which walls and roofs are covered of usually glass supported by metal, steel or wooden frame, were used for protection against extreme climatic conditions and from birds and insects as well [2]. Greenhouse covered with glass is much preferred in colder countries owing to its transmittance of light waves, reflectance of long wave heat radiations in the inside and resistance towards wind and snow. But their brittleness oils up the safety concern [3].

Thus polymeric flexible greenhouse films came into play which was economically feasible to construct greenhouse with simple hollow metal frame support.

GREENHOUSE FILMS:

Polymeric films are the most potential materials that exhibits enhanced light transmittance essential for photosynthesis and plant development, reflectance of heat radiations thus acting as thermal insulators and are highly long-lasting and light weighted [4]. Rigid plastic materials such as polyvinyl chloride (PVC), polycarbonate (PC), polymethylmethacrylate (PMMA) and glass reinforced polyester (GRP) in the form of sheets were used as greenhouse covering. The performance of these rigid polymers alters based on their extruded structural difference namely, double sheet or single sheet. Double sheet greenhouse covering demonstrates a reduction in light transmittance owing to its thickness which in turn escalates its production cost. Despite these limitations double sheets exhibit higher impact resistance in case of hail and lesser risk in case of fire [3]. And it is vital to look after the choice of framework support material and the number of contact points between the sheet and the frame to minimize creation of local hotspots [5]. Apart from rigid polymeric sheets flexible polymers like low density polyethylene (PE) films, which came in to play early in 1960s, were much exploited for agricultural purposes due to its relatively lower cost, better mechanical properties and improved light transmittance. Flexible PVC transparent films despite its high stability and fungus resistance, attracts dust particles from air which requires frequent washing [5]. PVC films reinforced with fibers demonstrate enhanced mechanical strength and stability compared to PE films. But Asian countries prefer PVC sheets over PE or PVC films which tend to be stronger in case of blustery weather despite its disposal issues [6].

Moreover integration of additive materials to the polymeric film alters their properties. For instance, the light wave entering the earth includes UV, Visible and infrared regions (both near and far). The visible part of the electromagnetic radiation is important for the photosynthesis process in plants and UV region contributes to the plant development such as leaf and pigments [7]. But the higher energy content of UV region may end up in degradation of the polymeric film. Thus incorporation of UV blockers as additives in the films is essential for better durability. Furthermore, the temperature difference between the inside and outside of the greenhouse may cause water vapor to condense and form water droplets on the inside of the film which in turn may reduce the transmittance of light waves and the water dripping from the film may damage the crop as well. This can be mitigated by inclusion of anti-drop additives to the film resulting in lower surface tension which makes condensation process to form water film instead of droplets followed by the water flowing along the sides [3]. Also, the presence of additives like ethylene tetra fluoroethylene (ETFE) despite enhancing the direct light transmittance and longer lifetime also blocks the transmittance of long wave heat radiation emitted by plants and soil at night, outside the greenhouse which protects the crops from cold weather by acting as thermal insulator. In contrast to this, Hemming et al., (2006) [8] has demonstrated a greenhouse design for tropical countries with a special polyethylene film covering that blocks the near infrared waves from entering the greenhouse to protect the crops from heat. Another remarkable design was the polycarbonate polymeric sheets with the zigzag cross-section which was destined to increase the light transmittance. This zigzag cross-section makes the reflected part of the incident light to again hit the polymer film surface unlike in flat surface thus again transmitting the light [9]. Photo-selective films such as colored films which blocks particular wavelength or film incorporated with fluorescent molecules which absorbs and re-emits photosynthetic wavelengths have demonstrated enhanced growth, yield and shorter maturation time [10 & 11]. Apart from regulating the abiotic conditions inside the greenhouse, polymeric meshes are used for protection of crops from pests. The climatic conditions provided by the greenhouse film covering not only favors plant growth but also insect pests. But the closed environment provides a better platform to carry out pest control treatments. Fine meshes made of polymers like polyolefin, polyethylene can prevent insects from attacking the crop plants [12 & 13].

DIRECT COVERS:

Polymeric films can also be made to cover the crops directly without any framework supporting them [14]. These films will be free lying over the crops with only their edges grounded. The crops grown under these direct covering films tend to grow faster and the cover spread out as the plants grow taller. These direct covering films exhibits good light transmittance, heat management, and protection from blustery weather, downpour and pests. But air ventilation is one of the significant factors for plant growth. Thus if the covering film is a very fine mesh or fleece or non woven fabric, it provides better protection for the underlying crop. Perforated PE films are widely used as direct covers owing to its light weight, channel for irrigation and better ventilation. Studies have evaluated the performance of different blends of perforated, non- perforated PE and spun fabrics on the production of muskmelons, pest control and soil conditions [15].

SHADE COVERING:

Shading is an important aspect for plants grown in tropical regions or very hot countries. This can be provided by painting or use of meshes or nettings in greenhouses. These artificial shading polymer meshes assures the same

level of benefit as natural shade and in addition these covering can be made momentary based on the season. Polyethylene shading fabrics with diverse color are commercially used to prevent decaying and pest control.

FUNCTIONALIZED POLYMERS FOR AGROCHEMICAL DELIVERY:

Agrochemicals namely, pesticides and fertilizers are being used for crop protection and production respectively. Their persistence in the soil is determined by their physico-chemical properties. Agrochemicals that are leached away in a shorter period, call for frequent application which results in economic and safety concern. Highly persistent agrochemicals have higher chances of getting into the human food chain, water resources and reduce the soil fertility by killing the beneficial microorganisms. Recent advancement in polymer technology has helped in mitigating these tribulations. Natural or synthetic polymers functionalized with active groups alter their properties rendering them highly active material which can be exploited in controlled release of agrochemical or they themselves can be utilized as a pesticide or plant growth promoter [16]. Polymer held pesticide or fertilizer, triumph over the environmental issues since the slower degradation of polymer results in controlled release of these agro compounds over a longer period of time from the polymer matrix and thus the optimum concentration is upheld and best utilized for the plant development unaffected the environment and nontarget organisms. The agro compounds are apprehended by the polymer by either physical (encapsulation or heterogeneous distribution) or chemical means (through chemical reaction) and the efficiency of the system is based on these physicochemical interactions between them and the biological and chemical properties of the agro compound [16]. A broad variety of polymer materials have been employed as the carrier for the agro compounds such as polyethylene, acrylic acid and polysaccharides among which acrylamide based gels being the most frequently used [17]. Recently environmental friendly materials such as organic polymers, hybrids or composites of organic and inorganic compounds are used as carrier. Agrochemicals that have been widely investigated with a polymer carrier includes, herbicides such as 2,4-dichlorophenoxy acetic acid (2,4-D), pentachlorophenol (PCP) and 4- chloro-2-methylphenoxyacetic acid (CMPA) [18], which are usually introduced as pendant groups, fertilizers such as NPK (nitrogen, phosphorous and potassium), urea, calcium carbonate, and other micro and macro nutrients (P, K, Zn, Mn, Cu, Mo, etc.) [17] and pesticides namely, atrazine, tebuconazole, chlorpyrifos, etc. [19]. Nanotechnology, the most proficient technologies in recent times, has been introduced in this aspect for the betterment of release mechanism. Various acrylates such as diethylene glycol methacrylate, Octaethylene glycol methacrylate, acrylamide, etc. have been investigated for the release of CMPA and 2,4-D. Derivatives of 2,4-D namely, tartarate and glutarate, which acts as both pesticide and fertilizer have been made to bind with hydrophilic polymers such as polyamides, polyureas and polyesters and their release rate was studied. A work by Kenawy et al., (1998) [20] has discussed the release of 2,4-D from acrylamide based crosslinked gel derived from various diammine. The release rate of the active compound from the polymer matrix depends upon the hydrophilicity nature of the polymer and the pH and temperature of the surrounding medium which affects the hydrolysis of the hydrophilic polymers. Microencapsulation technique was utilized to load 2,4-D within the biodegradable ethyl cellulose based polymer capsules, which protects the loaded herbicide from light and renders it less harmful to the environment [21]. In this study, process control parameters such as stirring speed, polymer concentration, polymer-herbicide ratio and pH of the medium were analyzed for better particle size, entrapment efficiency and herbicide release mechanism. Likewise, polymers like natural rubber, PE, copolymers of VC-acrylic acid esters, etc. were studied for giving a water insoluble coating over the fertilizer granules that render stability to the fertilizer and enhances its delivery. Some of the polymers used as carriers of agrochemicals is tabulated in Table 1.

Table 1. List of polymeric materials used as carriers of agrochemicals

S.No	Polymer Carrier	Agrochemical	Technology used	Reference
1	Chitosan microspheres	Urea	Emulsification and cross linking	22
2	Polyhydroxybutyrate and ethyl cellulose	Urea	Coating	23
3	Chitosan and Gellan-gum	Monopotassium phosphate	Hydrogelation	24
4	Cellulose acetate	NPK	Hydrogelation	25
5	Alginate and chitosan	Paraquat	Encapsulation	26
6	Calcium alginate and starch	Chlorpyrifos	Encapsulation	27
7	Montmorillonite	Hexazinone	Adsorption	28

Thus, the polymeric carriers holds advantage of less frequency of application of agrochemicals, avoids quicker leaching, controlled and continuous release to the plants, reduction of environmental pollution. But the prediction of release profile of the agrochemical at the field and the fate of the compound released or the left out debris is still difficult. Also, the chance of initial burst release of agrochemical may damage the crop. Thus, several mathematical models have been developed to predict the mechanism of release. But despite these disadvantages polymer based carrier systems are found to be most promising for agricultural production.

SOIL CONDITIONING:

Soil is one of the most significant factors in agricultural production since it acts as a medium to hold plants and as a reservoir for water and plant nutrients essential for plant growth. Water retention capacity of the soil depends upon the type of the soil, its thickness and its compactness i.e., more compact the soil lesser the water retained. The fertility of the soil is determined by the mixture of macro and micro nutrients (95%) and the organic material (5%) which may be animal, plant, fungus or bacteria that are beneficial for plant growth and development. In addition to these organic and inorganic nutrients, the pore space between the soil particles is filled by the air. The top layer of the soil containing the maximum amount of nutrients and organic matter enters the layer beneath it through leaching. Thus a soil with perfect shape and conditions is essential for enhancing agricultural production. Tropical soils which are exposed to frequent rainfall suffer from lesser aggregate stability and greater compaction which results in reduced water retention capacity and greater soil erosion. Sandy soils have a higher infiltration rate and thus very poor water holding capacity. Clayey soils which are capable of forming crusts remain a threat to seed emergence. Thus, it is crucial to condition the soil for amplifying the agricultural production. Polymers are added to the soil to perk up soil grain structure and composition suitable for holding plant and its growth, improving water holding capacity, increasing soil infiltration rate and permeability, reducing soil erosion, compaction and irrigation frequency [29].

SUPERABSORBENT POLYMERS:

Superabsorbent polymers (SAPs) are polymeric materials that are capable of absorbing and swelling several times their original weight and size owing to their minimal crosslinking between their polymeric chains [30]. Some of the commercially available SAPs include partially hydrolyzed products of starch-acrylonitrile copolymers, starch-acrylic acid copolymer and crosslinked polyacrylic acid. These materials degrade into ammonia, carbon dioxide and water, which in general may take about 5 to 7 years. Thus, the degradation of these materials has become an important concern taking into account the environmental pollution [31]. SAPs used in sandy soils acts as a mini water reservoirs from which water is utilized by the plant via roots through osmotic pressure difference. These hydrogels also take up the nutrients present in the surrounding medium and prevent their faster leaching thus making it readily available for the plants [32, 33]. In addition, SAPs also aid in seed germination and development when used as seed additives, seed coating, root dips or fertilizer and pesticide immobilized to it [34]. Abedi-Koupai et al., (2006) has investigated the performance of an ornamental plant, *Cupressus arizonica*, under the effect of SAP and with reduced irrigation regime [35]. A composite of polymer and clay has received great attention recently owing to their lower production cost and higher water absorbency. Example includes acrylic acid and acrylamide composite on attapulgitic micropowder which acts as a good substrate for the composite with OH reactive group on its surface. Acrylamide has a good salt tolerant property that adds up to its higher water absorbency nature [36]. Thus, the soil stabilizing and flocculating properties of SAPs make them a very promising material for enhancing agricultural production and the plant quality.

BIODEGRADABLE POLYMERIC FILMS:

Conditioning the soil by covering with polymeric films has been introduced back in 1970s, the approach being called as solarization. These polymeric films were mechanically stable and allow visible light to pass through simultaneously resisting infrared radiation from passing through. These films shows greenhouse effect which warms the soil during the day as it is permeable to visible light and during the night when the soil emits infrared radiation, the film traps it as it is impermeable to it thus retaining the warmth. Usually polyethylene films are used with fillers such as phosphates which impart higher opacity to IR radiation. Films made of synthetic polymers face a serious drawback of the need for removal and disposal, which adds up the cost or sometimes, these films are burnt which results in environmental pollution. Thus, films made of biodegradable polymers such as alginate, polyvinyl alcohol (PVA) and glycerol are found to be advantageous which eliminates the need for removal from the soil after use [37, 38].

CONCLUSION:

Thus considering the current state of agriculture, which is achieved through the exploitation of natural resources with simultaneous use of agrochemicals at large scale, polymer technology has found to be a potential technology owing to its ease of tailoring for various agricultural applications. The successful application of polymers in agriculture shows the increasing trend of using polymers across various industries in place of habitual materials considering the cost, easy processability and high performance. Polymers now encompass all aspects of agriculture

with variety of products. Thus it is vital that the use of polymers is economically, technically and environmentally feasible and efficient.

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CLEAN MEAT: A FUTURE HOPE OR HYPE (A REVIEW ON IN-VIVO MEAT)

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Abstract: “One cannot think well, love well, sleep well, if one has not dined well.” said by Virginia Woolf portrays the importance of food. India, being the second most populous country in the world, we are in a situation where there is an urgent need to immediately meet the demands of people. There are lot of sectors which needs to be spotted and developed for the welfare of the country, one such area is food. Focusing on the issues of food especially meat, which predominantly involves brutal killing of animals just for food. Conventional meat production has its own advantage and disadvantage. In-vivo meat or clean meat or hydroponic meat means the meat produced from lab instead of killing the animals. By applying the basics of cell culture, the animal cells are isolated and cultured in lab with suitable nutrients where they develop into clean meat. They are pollution free, animal friendly, avoids the existing problems of conventional meat and can be fortified or maybe in future can used for therapeutic purposes. This review focuses on in-vivo meat, problems with conventional meat production, in-vivo meat production techniques and its benefits on a briefer scale.

Key Words: in-vivo meat, animal friendly, cell culture, food, clean meat.

INTRODUCTION:

The escalating world population appeals to extensive food demands. Adding to which over 70 % of the total population are non-vegetarian. It is helpless, since people choose non veg over veg for its taste and high protein content. To meet this enormous demand and prevent abundant slaughtering of meat animals for their flesh, it is important to switch to an alternate yet efficient food source. The concept of ‘cultured meat’ or ‘in vivo meat’ involves bio-fabrication of living cells by bioengineering techniques, which can possibly avoid the problems of conventional meat and can produce animal free meat or vegan meat. The cultured meat is developed in *in vitro* condition, for which the stem cells are derived from its meat animal. Then they are further transferred to a suitable medium which contains essential nutrients, growth factors, energy sources and components that are required for growth and differentiation of stem cells that later matures into muscle cells in a bioreactor. Further cell culturing is performed to help small amount of muscle cells proliferate into large cell mass, better known as meat. The first culturing of stem cells from animals was done in 1990s and small quantities of tissue were produced. Researches on muscle culture from turkey were conducted by NASA [1,2]. Stem cells with collagen matrix, aids in the proliferation for muscle strip generation [3]. Many techniques have been evolved to generate bone, skeletal muscles, fat, fibrous tissue and cartilage [4]. Using the meat cultured from bovine stem cells, the world’s first burger was made in 2013 [5]. It is possible to develop meat by culturing loose myosatellite cells in a suitable substrate and further harvesting mature muscle cells after they have differentiated, processing them into various meat products [6]. There are still lot of technical difficulties to be resolved for the cultured meat products to go acceptable by the consumers. All the principal steps involved in the production of cultured meat should work well in the aspects of both biological as well as commercial points of view, in order to bring the vision of ‘vegan meat’ to reality.

PROBLEMS ASSOCIATED WITH CURRENT MEAT PRODUCTION:

Day by day, the demands of people keep on sprouting. Meat requirement is expected to be increasing among people in both developed and developing countries [7,8]. The meat consumption has grown from 234 pounds per person in 1980 to 273 pounds in 2007 in the USA [9] and to quench the demands of this mushrooming growth 10 billion terrestrial food animals were slaughtered in 2007 in the USA and almost 25 percent of the world’s total of non-aquatic animals killed just for food [10]. Traditional meat production involves rearing of animals and slaughtering

them for meat purposes, though animal suffering is an evil, increased addiction to meat has lead to merciless killing of animals for food [11]. Some of the problems due to conventional meat are addressed below.

ANIMAL BRUTALITY:

Foremost the animals are highly stressed and killed for meat production. A huge of world's 17 billion hens and meat chickens are forced to live in a space less than a size of a paper [12]. Due to all these stress, they develop abnormal diseases and they are not healthy for consumption too [13].

ENVIRONMENTAL ISSUES:

Conventional method involves killing of animals and it generates lot of waste by products which increases the greenhouse gas emissions. It is expected that annual meat production will increase from 228 in 2000 to 465 at present, as a result of which annual greenhouse gas emission is expected to rise. A total of 30 percent of land surface is used for livestock production globally and 33 percent of land is for livestock feed crops and 26 percent is used for grazing of these farm animals [14]. About 1kg of beef leads for to GHG emissions of 36.4 kg, CO₂-equivalents that is almost equivalent to 14 liters of diesel or 12 liters of fuel [15].

DISEASE AND HEALTH ISSUES:

Conventional meat has devastating effects on human health due to development of the meat under stress and increased use of antibiotics [16]. Incase of epidemics like chicken flu they can cause serious illness to the people [17]. Also increased use of antibiotics and use of hormones has caused so many health risks to the consumers especially for women. Also they affect non meat consumers by spread of pathogens and infections by discarded meat [18].

INEFFICIENT CONVERSION:

Present meat production techniques are inefficient, about 1 kg poultry, pork and beef requires 2 kg, 4 kg and 7 kg of grain, respectively. About 85 percent of total soy production is used for livestock production [19], for a country like India with a detonating population, it is inefficient to waste the food grains. If this situation prevails then the country may face a serious starvation.

EXTINCTION OF RARE SPECIES:

Many species have become extinct due to extensive rearing of animals for meat and conversion of forest areas as cattling area is one of the major cause for extinction of many rare species of animals [20].

SOCIAL TABOOS:

Conventional meat involves killing of animals for food, this is ethically and socially not accepted by many religions. But clean meat can solve this issue. They do not have such an issue and they can be ethically accepted by many people [21].

TECHNIQUES FOR PRODUCING CULTURED MEAT:

Muscle tissue is formed during embryogenic development as mono nucleated myoblast with limited proliferation capacity [22] and fuse with each other to form multi nucleated myofibres. Multinucleated myofibres upon maturation form non proliferative myofibres [23]. These mono nucleated myofibres are always in quiescent and dormant state [24] until they are activated in vivo by injury, stress and they divide into self-renewing myoblast and committed myofibres [22,25]. Different culture techniques and methods are available for developing meat from lab animals, some of them are conventional cell culture and tissue culture, engrossing organ printing, bio photonics and nanotechnology [16].

SCAFFOLD BASED TECHNIQUES:

This technique involves culturing of embryonic myoblasts isolated from farm animals by biopsy and culturing them, allowing them to proliferate. After which they are added to scaffold i.e.: micro carriers made of collagen and they are added to bioreactor which are either stirred or stationary filled with nutrient medium. Taking all these cues the myoblast develops to myotubes, as a result of which large number myoblast cells can be harvested from the culture and they can be used as boneless meats which are soft and don't have form of high structured meats. Two protocols are available for culturing of meat by scaffold based meat preparation one of them is Vladimir Mironov for the NASA [26] whereas the other one is a patent in the name of Willem Van Eelen [27]. Both of these involve culturing of meat in collagen; the former involves culturing it in collagen fibres whereas the later involves culturing of it in collagen mesh work. After which due to various factors the myoblast combine to form myotubes and they can be renewed later.

SELF ORGANIZING TECHNIQUES:

Another technique for producing in vivo meat is self-organizing technique or tissue culture techniques. Extensive work on Gold fish (*Carassius auratus*) muscle explants in vitro was done by Benjaminson. The explants of gold fish were grown in nutrient medium for 7 days. When different components are used for with medium showed different efficiency of growth as tabulated below,

Media component	Growth percentage [%]
Fetal bovine serum	13.8
Maitake mushroom extract	15.6
Fishmeal extract	7.1
Shiitake extract	4.8
Carassius skeletal muscle cells	79

Table 3.1 Reference [26]

The meat was marinated with olive oil and garlic deep fried and kept for panel observation. Though it was not eaten by the panel they showed positive results by sensory evaluation. [22, 29, 30]. Thereby this can pave a remarkable method for production of lab based meat. But the meat become necrotic when separated from nutrient for longer periods. Addressing these issue edible porous polymers within which nutrients can profuse was given by Vladimir Mironov.

ORGAN PRINTING:

Though meat can be prepared by cell culture and organ culture, they cannot be vascularized, beat the consistency of conventional meat and its taste. Organ printing involves use of printers where single or balls of cells are used which are sprayed on a gel that acts as a printing paper that can be made into desirable shape and structure. They not only produce cell structure which mimics the natural one, they also produce vascularized one providing blood supply to the entire organ [31, 32, and 33].

BIOPHOTONICS:

This is an emerging field where the cells are organized by the action of lasers. Here, the defining point is that the action of lasers can move the particles which can organize the cellular structures to produce meat. Blood cell arrays and hamster ovaries are created so far with these methods [34, 35], thereby leading to a doable way to produce in vivo meat and eliminating the need for scaffoldings [36, 37].

NANOTECHNOLOGY:

The very basic concept is that everything is made of atoms virtually constructed in different ways to produce different components, thereby using this concept, nanotechnology can be applied to simply produce desired compound from scratch. Thus all these technologies provide a possible solution for producing meat by culturing the animals without killing them. Though at present it is not feasible for bio-fabrication of meat, or it may take years for its development, yet it can be a promising way to avoid killing of animals for meat and make the vision of vegan meat factual.

ATROPHY AND EXERCISE:

Hydroponic meat will undergo atrophy or wasting when there is reduction in cell size or wasting of cell due to lack of use, thereby various electrical and mechanical stimulus required to be provided from outside addressing this issue. Growth of myoblast, especially its proliferation and differentiation are found to be affected by mechanical, electromagnetic, fluid flow and gravitational fields. Repeated stress and relaxation of about 10% of length and 6 times an hour increases the myoblast growth [36].

BENEFITS OF CULTURED MEAT:

The conventional meat production processes raise issues of serious consequences like nutritional related diseases, antibiotic resistant pathogenic strains, food borne illnesses and environmental backlash of livestock that include massive emissions of methane which is a major cause for global warming [22]. With communities developing increased concern over the threat being imposed, the in vitro meat production could revolutionize the food industry by reducing the risks of environmental pollution and livestock required for the conventional meat production.

DESIGNER MEAT:

In vitro meat can be cultured to be a healthier and more functional just by manipulating the composition of the nutrients in the medium. Healthy fats such as omega-3 can be incorporated replacing the harmful saturated fats. Thus, meat can be produced much healthier than conventional ones having a beneficial effect on health [27].

ANIMAL VIRTUE:

Opting out for cultured meat could completely eradicate the act of slaughtering of animals since the technique only requires removal of cells from the donor. The recovered stem cells are further cultured in mushroom extract avoiding animal blood serum [9, 33, 35]. We could yield around 50000 metric ton of meat from continuously differentiating ten stem cell lines for two months. By theory, one cell line would be satisfactory to feed the entire world [35].

REDUCTION IN DISEASES:

Since the in situ conditions practice Good Manufacturing Practices, the probability of food borne diseases spreading will be the lowest as there would be no existence of potential harmful organisms. The risk of liability towards hazards such as pesticides, heavy metals and dioxins are also significantly reduced.

FASTER PRODUCTION PROCESS:

Unlike the long production time of conventional meat, the in vitro meat production requires significantly less time. The current meat production systems having long conversion time with months for chicken production and years for beef and pork can be countered by fast in vitro production processes.

REDUCED LIVESTOCK:

The conventional meat production processes require large land for animal grazing and comparatively huge water need. The amount of energy consumed for the whole meat conversion processes is also significantly higher. Whereas, the bioreactors used for production of cultured meat occupies only 1% of the total space needed for animal domestication. These bioreactors can also be built vertically, thus can be conveniently placed in or near cities without affecting the location [37, 38]. Some researches states that greenhouse gas emissions could be grossly reduced around 90% by opting out for cultured meat [39].

SOCIAL AND ENVIRONMENTAL BENEFITS:

Several scientific, environmental and animal rights communities greatly support the welcoming production technique. The extensive reduction in the usage of land results in the returning of habitat to the wilderness which may restore the lives of endangered species [40]. The major vegan population can opt out for the best alternate for meat if they keep their ethics aside. It can prove as a best source of food in space stations and settlements.

CONCLUSION AND FUTURE PERSPECTIVE

Hydroponic meat can be an envisaging boon of future to avoid all the detriments of conventional meat ie: pollution, animal brutality, waste generation and ethical issues. It requires sophisticated technology and zealous research at present, but it may stand a chance in future for production of clean meat [38]. But the problem arises not only in the cost and the technology but also consumer acceptance of these lab grown meat. These kind of areas are still in infant stage and they require lot of efforts to bring these test tube meat to menu hoping for a better future.

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Traditional Rearing Practices of Indigenous Pigs

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Abstract: Indigenous pigs were domesticated and well adapted to our ecosystem. A study was conducted on pig rearing system among the people who rear pigs as their main source of income for their livelihood. The study revealed that the indigenous pigs were reared under traditional system by the particular community people and were evenly distributed among all parts of Karnataka and is the main source of income. The phenotypic characters showed that these pigs were indigenous to this state and their existence was noted since many centuries. People use various indigenously made materials/utensils for housing and feeding. Pigs were fed mainly with locally available feed resources, through scavenging and rooting. Commercial sale of animal and meat was also practiced. Certain tribe/community practices century old established indigenous technical knowledge in pig rearing and they are passed through many generations. Indigenous traditional knowledge would significantly contribute to the generation and pave the way for exploitation of technology to benefit tribal/rural populations

Key Words: Indigeneous pigs ; rearing ; feeding

INTRODUCTION:

The geological pattern makes this state as unique and very suitable for floral and faunal biodiversity. Among the indigenous livestock germplasm, pig contributes a major source of income for certain community in Karnataka and also it is a part of traditions and culture. It is believed that pig production and pork consumption is strongly associated with specific communities and piggery is invariably a small scale backyard enterprise. Traditional management continues to dominate production system with exception that indigenous pigs were more preferred than the exotics or crossbreds.

OBJECTIVE

- Study on Pig Rearers and Their Livelihood.
- Study on Traditional Pig Rearing Practices.

SURVEY AND METHOD:

A field survey was conducted to study the traditional pig farming systems in Bellary. Survey was conducted in 44 villages of Bellary. The data was primary data collected from 144 farmers using Urban and rural areas of Sc Castes only in Koracha and Korava communities. pre-framed questionnaire and recorded from randomly selected people both through personal interviews and observations with suitable photographs.

RESULT AND DISCUSSION

PIG REARERS AND THEIR LIVELIHOOD:

Women also involved in rearing pigs. All individuals irrespective of age were involved in rearing of pigs and 40% of the farmers were between 30-40 years of age. 45 % of the farmers completed their primary education. Individual's average herd size is 25. The indigenous pigs were reared by 85 % of poor and landless farmers belonging to specific communities. Mostly the pigs were reared under scavenging system. Few farmers also rear cattle, sheep and goat along with pig. The rearing of pig is profitable when compared to other livestock enterprise. Other than local indigenous pigs, Large White Yorkshire, cross bred pigs was also reared by the people. Farmers prefer the indigenous pigs because of their good adaptation to the local climatic conditions.

TRADITIONAL PIG REARING PRACTICES:

HOUSING:

Pig serves as food and nutritional security for certain communities of people. Most of them are reared under free range / scavenging system. Scavenging systems allow pigs to roam freely day and night. The pigs roam freely and

may or may not return to the shelters and usually shelter underneath the trees. Few people have separate enclosure/shelters for piglet and rarely for adult. The type of housing varied based on the local climatic adaptation and locally available resources. Materials such as mud bricks, cement bricks, bamboo, concrete, wood and iron sheets were commonly used. The roofing material is usually thatched leaves and some people use asbestos / iron sheets.

FEEDING:

Indigenous pigs are omnivorous. Usually pigs were fed with the kitchen waste from hotels, hostels, etc. Most of the people feed the pigs once daily either in the morning or in the evening. No specific sounds are made to call the pigs. The feed is dumped in a place; all pigs make way to that place for feeding at a particular point of time. In some circumstances, they also provide commercially available feed which includes grains, cereals, meat, fish meal, etc. No separate feed for piglets, weaner and for adults. They are fed in groups irrespective of their age or size or sex. The water trough is usually made up of tyre with the half cut in it for easy feeding. Usually they feed banana leaves, rice, and food waste from hotels, canteen, etc. They don't feed concentrate feed.

PIG REARING PRACTICE:

Pigs were reared in free range system and few at backyard. The pig farmers practice low input production system. Breeding is mainly by the natural service and no selection was practiced. The breeding boar is very difficult to find or catch. No extra care was given to the pigs. Pigs are reared mainly for pork consumption. Farmers are not aware of the common diseases of pig and their incidence due to lack of attention / knowledge. Further, the people approach/seek veterinary assistance very rarely and in case of any unnatural death they rarely report to the Veterinarian Department of Animal Husbandry for conducting post mortem and for further investigation.

HEALTH:

Monitoring the health of pigs periodically is not possible in scavenging system. But, in case of any sudden outbreak of diseases, they isolate the diseased pigs based on their experience and treat on their own. Mostly the sick animals will be slaughtered or sold immediately. The animals are mostly under scavenging system, hence, no deworming/vaccination has been carried out.

MARKETING:

The animals are purchased at the farmers' place or through the middle men. There is no organized marketing channel for indigenous pigs. The slaughter of the animals is by jatkhah method. The pigs were slaughtered twice in a week preferably on Sunday. Each individual market their own meat and is usually on the road side shops. No special products are made from meat of indigenous pigs. In some areas, the meat is cooked and sold for the people depending upon their needs. The pigs were sold based on the live weight (Rs.50-60/kg). Demand for younger pigs is higher than the adult pigs, both in terms of frequency and numbers. Usually few adult pigs are reared in sheds. It is considered as a social act for safeguarding traditional needs. During religious functions of certain communities, the demands for indigenous pigs are so high and hence, pay more for live weight during this period. Mostly adults over one and half years were preferred for religious functions.

CONCLUSION:

The study revealed that the marketing systems were controlled by informal marketing channels and hence no defined market place or traders for farmers to sell their pigs. The weight of the pig is lesser compared to their age, indicating poor growth performance and poor returns to pig farmers. To improve smallholder pig production, marketing systems and clean pork production, there is a need for strategic development of pig value chain. Native pig rearing plays a vital role in improving the socio-economic status of a downstream people. It ensures food and nutritional security. This unique indigenous pig germplasm is considered as prosperity of Bellary. The native pigs are reared by farmers of certain community of this state ecosystem over the centuries and they believe that animals perform well under this local agro climatic conditions. District should chart out strategies for improving the farming system management, genetic improvement and conservation.

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Department of Biotechnology, Kumaraguru College of Technology, Tamil Nadu, India

Bioethanol Production from Ionic Liquids by *Saccharomyces Cerevisiae*

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Abstract: The productions of bioethanol from Lignocellulosic materials are abundant feedstock. In this work, the complex structure of the lignocelluloses is the main obstacle in the conversion of lignocellulosic biomass into valuable products. A promising new pretreatment method for lignocellulosic materials is the use of Ionic liquids (ILs). Ionic liquids provide the opportunities for their efficient pretreatment for biomass. Fermentation of banana waste to bioethanol by *saccharomyces cerevisiae* was investigated at different temperature, different pH, and different incubation period and at different agitation speed.

Key Words: Optimization; Bioethanol, *Saccharomyces cerevisiae*, Ionic liquids, Fermentation.

INTRODUCTION:

Technologies that are being developed for commercial pre-treatment and value addition of Low Cost Waste face technical and economical impediments. Therefore, cost effective technical innovations that allow cost-effective conversion of biomass into fuels and chemicals are mandatory. These technologies include low-cost thermo chemical pre-treatment, highly effective enzymes and efficient and robust fermentative microorganisms. The high cost of enzymes presents a significant barrier to commercialization of bio-based products. In the simplest terms, the cost is a function of the large amount of enzyme protein required to break down polymeric sugars in cellulose and hemicelluloses to fermentable monomers. In recent years, significant effort has been expended to reduce the cost by focusing on improving the efficiency of known enzymes, identification of new, more active enzymes, creating enzyme mixes optimized for selected pre-treated substrates, and minimization of enzyme production costs [1].

Ethanol's future role as a fuel hinges on several factors including feedstock availability, processing costs and supportive political framework, improvements in pre-treatment and advance in biotechnology, especially through process combinations can bring the ethanol production overall process efficiency to 68%. Also, a combined effect of higher hydrolysis-fermentation efficiency, lower specific capital investments and increase of scale, cheaper biomass feedstock costs and using genetically engineered microorganisms that can convert xylose and/or pentose to ethanol can greatly improve ethanol production [2, 3]. Processes that produce only ethanol from lignocellulosics display poor economics. The large market for ethanol makes it possible to achieve economies of scale that makes it possible to achieve economies of scale that reduce sugar cost, and co-producing chemicals promises greater profit margins or lower production costs for a given return on investment. For the large processing plants, the production costs are significantly reduced compared to small plants, the production costs are significantly reduced compared to small plants [4]. Yield improvements in all major steps of LCW processing would enable lower capital requirements, thus improving the economics and lowering. The actual work deals with the process bioconversion of cellulose from Banana Pseudo stem waste, obtained from an agricultural waste, into bio ethanol by using the methods of acid pre-treatment, hydrolysis and fermentation by *Saccharomyces cerevisiae*.

MATERIALS AND METHODS:

- **Sample preparation:** The gathered banana stem were chopped into small pieces approximately 2-4 cm in length using knife. The pieces were then sun dried under mild sunlight for two days and then dried at 60°C in an oven for one day. The cut pieces were then crushed in the grinder. *Saccharomyces cerevisiae* (wild strain) and *Saccharomyces cerevisiae* NCIM 3495 are used in this study. [6, 7].
- **Sample pre-treatment:** The separate samples autoclaved at 15psi pressure for 30 min. After autoclaving the sample allowed to cooled then filtered.

- **Pre-treatment of biomass with ionic liquid:** 1ml of IL and 250mg of biomass were mixed and heated at 120° C for 6h under continuous stirring. Diluted sulphuric acid was added to the sample from pre-treatment steps. The sample was hydrolyzed in the reactor between 100 °C for 30 min. After hydrolysis, pH adjustment was carried out with 1M NaOH until the pH reached a pH of 7. Insoluble particles were separated from the hydrolysis by filtration [8].
- **Ethanol Fermentation:** The 5g of yeast culture was added into the flasks. The samples were placed in shaker incubator at 200 rpm at 30°C, for 3 days. Distillation is the most dominant and recognized industrial purification technique of ethanol. The basic principle that by heating a mixture, low boiling point components are concentrated in the vapour phase. By condensing this vapour, more concentrated less volatile compounds is obtained in liquid phase. Water is obtained from the bottom of the tower and ethanol is obtained from the top of the tower.
- **Analytical methods:** Ethanol concentration was determined by the Standard potassium Dichromate method [9, 10].

RESULTS AND DISCUSSION:

- ✓ **Growth Curve for *Sacchromyces cerevisiae* NCIM 3495:** The samples were collected at the interval of 2hours for 4days and the concentration of cells was calculated by absorbance at 600nm.

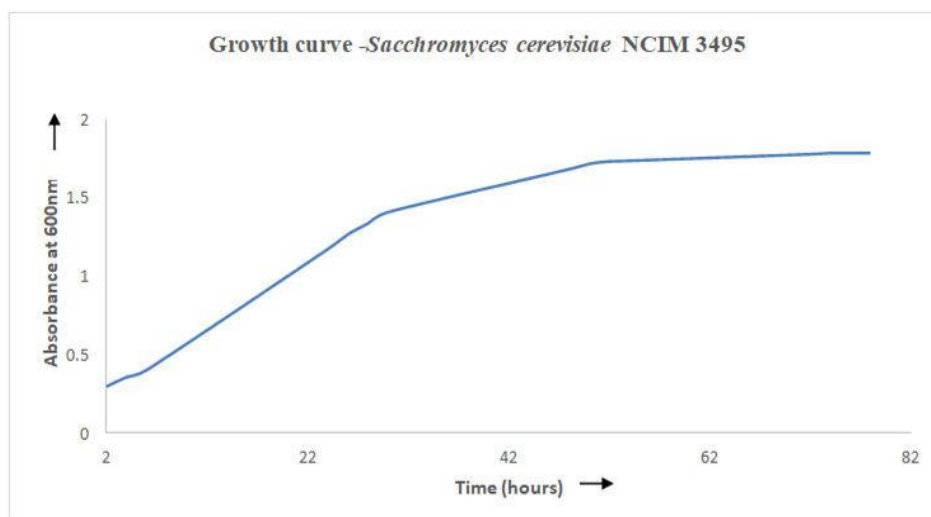


Figure 1. Growth curve for *Sacchromyces cerevisiae* NCIM 3495

- ✓ **Estimation of ethanol by ionic liquids:**

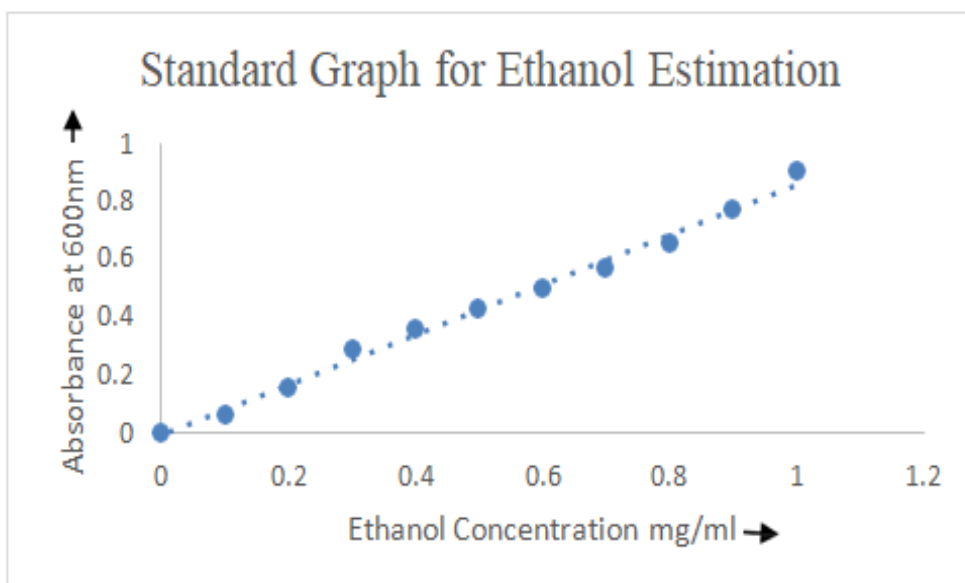


Figure 2. Estimation of ethanol by Ionic liquids

✓ **Optimization of fermentation parameters:**

- **Effect of temperature:** The results in Fig 5 indicate that maximum ethanol yield of 0.762mg/ml was produced at temperature of 30°C. The ethanol yield increased with the increase of temperature from 20-30°C up to 48 h of incubation after which is declined. Thus, optimum temperature of fermentation of banana stem was found to be 30°C with the maximum ethanol yield 0.762mg/ml at 48hr incubation. Therefore the future experiments were conducted at incubation temperature of 30°C.

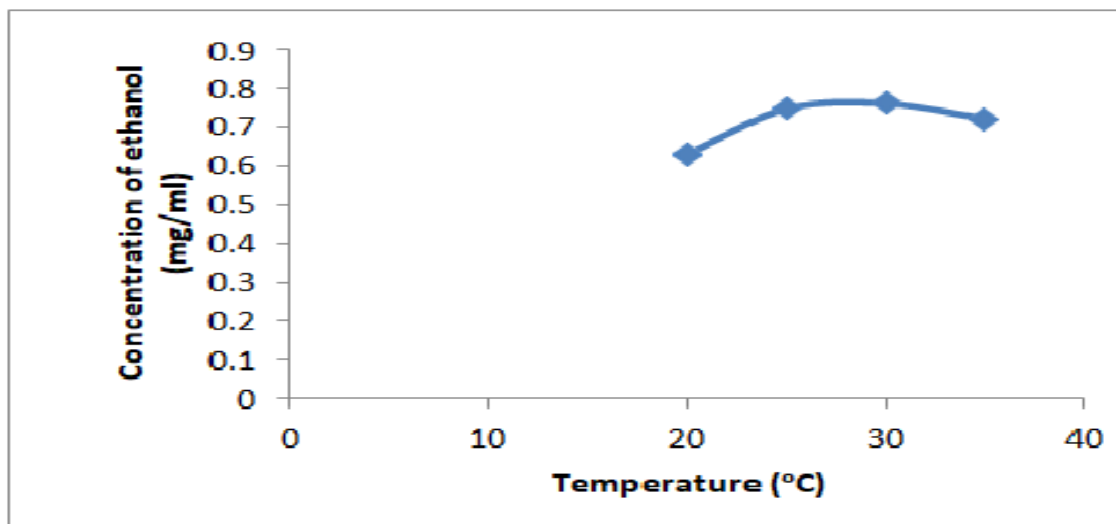


Figure 3. Effect of Temperature

- **Effect of pH:** Fig 6. Shows that effect of pH on ethanol production. The ethanol yield increased with the increase of temperature from 4-6 up to 48 h of incubation after which is declined. Thus, optimum pH of fermentation of banana stem was found to be 6 with the maximum ethanol yield 0.802 mg/ml at 48hr incubation [11, 12].

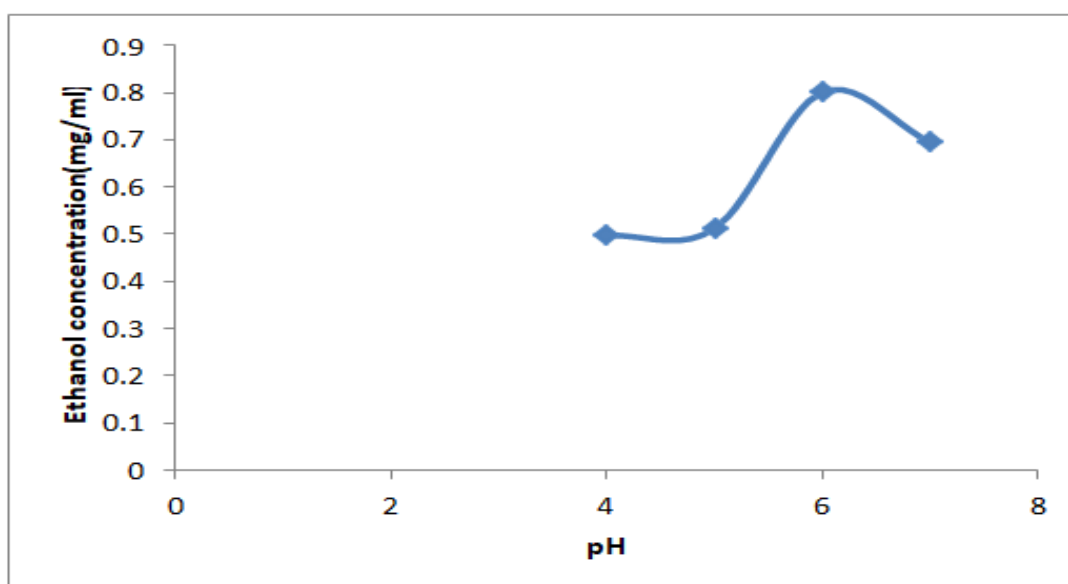


Figure 4. Effect of pH

- **Effect of incubation period:** In this study carried out on effect of incubation period on ethanol productivity. *Sacchromyces cerevisiae* exhibited maximum ethanol yield (0.638mg/ml) at 5 days of incubation [13].

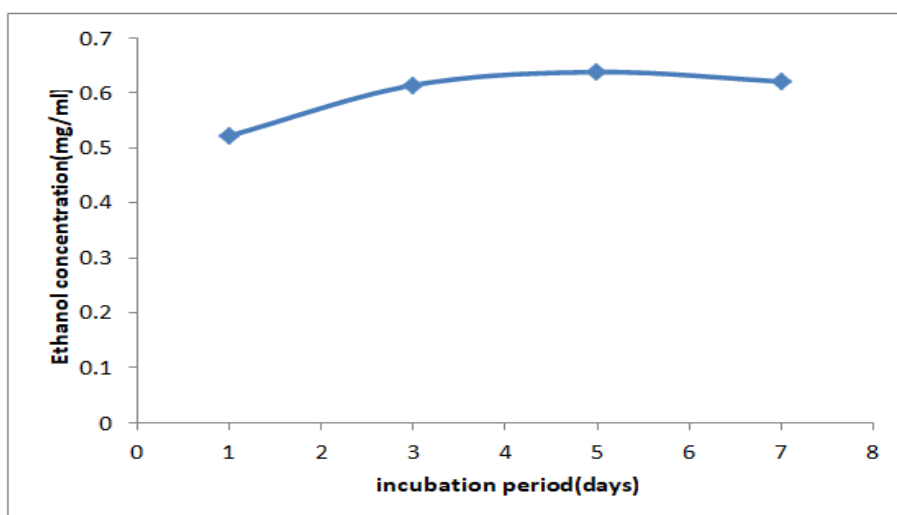


Figure 5. Effect of Incubation period

- **Effect of agitation speed:** Fig 7 shows that effect of agitation speed on ethanol production. It is clear from the fig that lower agitation speed was found to be suitable for ethanol production. The highest ethanol yield of 0.74mg/ml observed with 200rpm of agitation which is primarily due to initial oxygen requirements of yeast cells. Excess oxygen in the fermentation medium could lead to increased cell growth at the cost of ethanol productivity [14].

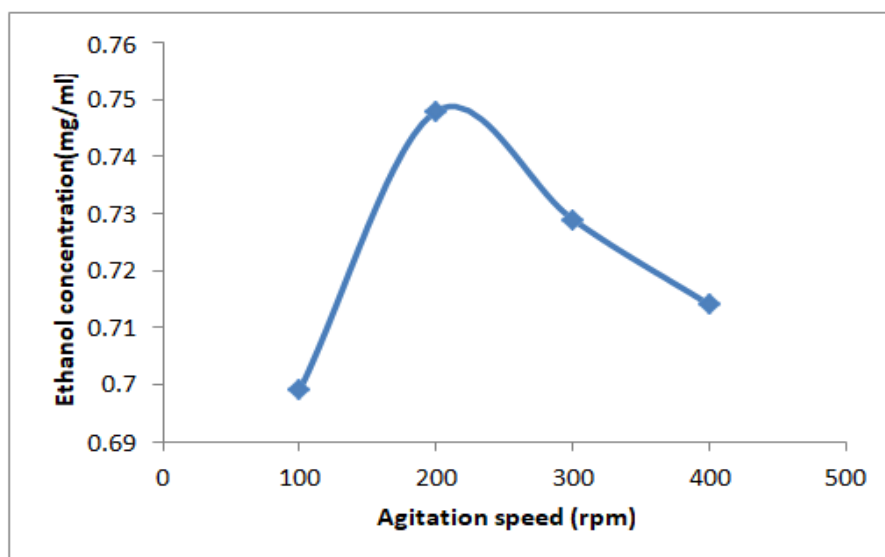


Figure 6. Effect of Agitation speed

CONCLUSION:

This study could establish that banana stem which have not been exploited commercially for any industrial application and are poorly disposed could effectively be used for ethanol production through the process of ionic liquids pre-treatment and fermentation. Ionic liquids treatment can significantly reduce the volume of the waste material. The process with optimized fermentation parameters described in the project could be used for scaling up of the process to a pilot scale or commercial fermented level thereby making the process more cost effective.

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Process optimization for the extraction of beta carotene from the edible seaweed – *Ascophyllum nodosum* and incorporation in food products

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Abstract: Nutraceuticals are food extracts that have been demonstrated to produce a physiological benefit or provide some protection against chronic disease. Nutraceuticals are the intersection of nutrition and pharmaceutical and is an umbrella term that can also include functional foods and dietary supplements (Eg. Omega-3 fatty acids that are both) Biocompatible and Bioavailable. In this work the aim was to produce nutraceuticals from extracted β -Carotene. β -Carotene used in this study were extracted from *Ascophyllum nodosum*. The Functional role of β -Carotene were assessed by HPLC and antioxidant assay was studied.

Key Word: Nutracuticeals, *Ascohyllum nodosum*, β -Carotene, antioxidant assay.

INTRODUCTION:

β -Carotene is a C40 carotenoid an organic compound - a terpenoid, a red-orange pigment abundant in plants and fruits. As a carotene with beta-rings at both ends, found in all green plants where it is localized in the chloroplast. the most common form of carotene. It is a precursor (inactive form) of vitamin A. Being highly conjugated, it is deeply colored, and as a hydrocarbon lacking functional groups, it is very lipophilic. Its main commercial use is in the food industry where it is used as the colouring agent in margarine, baked goods and other food products. Beta carotene has also been shown to be a good scavenger of free radicals and other reactive species and appears to have a role as a protective agent against cancer. In recent years, much clinical evidence has accumulated on this potential cancer protective activity of Beta carotene. The structure was deduced by Karrer et al. In nature, β -carotene is a precursor to vitamin A via the action of β -Carotene 15,15'-monooxygenase. β -Carotene is also the substance in carrots that colors them orange. β -Carotene is biosynthesized from geranylgeranyl pyrophosphate. Isolation of β -Carotene from carotenoids abundant fruits is commonly done, using column chromatography. The separation of β -Carotene from the mixture of carotenoids is based on the polarity of a compound. β -Carotene is a non-polar compound, so it is separated with a non-polar solvent such as hexane

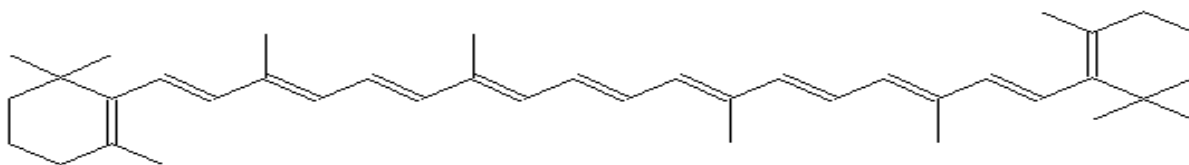


Figure 1. Structure of β -Carotene

Beta-Carotene is made up of eight isoprene units, which are cyclised at each end.

MATERIALS AND METHODS:

The method used for the extraction of β -Carotene from the seaweed *Ascophyllum nodosum* was Soxhlet extraction the Solid – Liquid extraction.

SOLVENT SELECTION:

Though β -Carotene a fat compound the desired extraction efficiency will result only when polar solvents are used and also polar solvents along with the combination of water gives more efficiency. Since water helps in the rupture of cell wall and also in release of cell components, it is used in solvent composition for extraction.

SOXHLET EXTRACTION:

Sample of 20g was measured. The sample was placed into the cellulose thimble and plugged with cotton. It was then placed into the extractor fitted with a pre-weighed RB flask. The solvent of 200 ml was added from the top of the apparatus using the funnel as the solvent passes through the sample packed into the thimble for efficient extraction of the extractor and material. The condenser was fixed and a current of cold-water was allowed to flow; the temperature was adjusted according to the boiling point of the solvent used for the extraction. The extraction was continued till the total content is extracted. Then extractor was removed and the thimble was taken out and solvent was allowed to evaporate from it. The extraction process was held for about 4 hrs.

S.NO	SOLVENT COMPOSITION	TIME (HR)	PRETREATMENT	COMPOUND EXTRACTED
1	Isopropanol 99%	4	-	No extraction
2	IP 80% + Water 20%	4	-	No extraction
3	IP 80% + Water 20%	4	Overnight soaking	Compound extracted
4	Hexane 99%	4	-	No extraction
5	Hexane 80% + IP 20%	4	-	No extraction
6	IP 80% + Hexane 20%	4	-	No extraction
7	IP 80% + Acetone 20%	4	Overnight soaking	No extraction
8	IP 50% + Acetone 30% + water 20%	4.5	-	Compound extracted
9	IP 60% + water 40%	4	-	Compound extracted
10	IP 70% + Water 30%	4	-	Compound extracted
11	Acetone 70% + Water 30%	4	Overnight soaking	Compound extracted
12	IP 70% + Water 30%	6	Overnight soaking	Intense extraction

Table 1: Trials

BULK EXTRACTION:

Pre-raised the 1Kg of seaweed with 2% Formalin wash and raise with the traces of the water to remove the traces of formalin solution. It was then dried to 20% moisture Content. Extraction was done using the solvent (70% Isopropanol + 30% H₂O + 1g of NaOH). 1Kg of seaweed was taken and extracted with 4 lit of solvent. The collection of the extract and concentrated by using the Rotary Vacuum evaporator. For 1 kg of seaweed 2 lit of miscella was obtained, this miscella contains 4% of active compound. The extract was then concentrated up to 10% solid content (i.e) 1000 ml of extract concentrated to 100ml

COLUMN CHROMATOGRAPHY:

A clean, dry column is aligned vertically; a very small plug of glass wool is placed at the bottom of the column. Silica gel was used as a packaging material to fill column about 2/3 full is added and gently tapped to level it. The eluting solvent 90% hexane-10% acetone solution (by volume) was added gently so that no cracks in the column formed it was then allowed to drain. 2-3 cm of solvent was allowed to stand at the top of the column the stopper was closed. The top of the column was covered with parafilm to avoid evaporation of solvent. The concentrated extract from bulk extraction (i.e) 100 ml concentrate was filtered by Whatmann no 1 filter paper. The filtered extract of 25 ml was poured into prepared column. The eluting solvent was poured along. Three bands were formed and each band was collected separately in a beaker and analyzed by HPLC technique for presence of Beta carotene.

HPLC ASSAY:

TRAIL-1	TRAIL-2
Mobile phase was assayed: ACN/Methanol/water (60:30:10 v/v)	Mobile phase was assayed: Hexane/ Acetone (82:18 v/v).
The HPLC grade solvents are used as the mobile phase.	The HPLC grade solvents are used as the mobile phase.
The mobile phase was then degassed ultrasonically for 15 min prior to use.	The mobile phase was then degassed ultrasonically for 30 min prior to use.
The mobile phase flow rate was 1 ml/min.	The mobile phase flow rate was 1.2 ml/min.
The column temperature was 30 °C and the absorbance was read at 450 nm.	The column temperature was 30 °C and the absorbance was read at 470 nm.

Table 1.1 : Chromatographic Conditions Of Trail-1&2 in HPLC

ANTIOXIDANT ASSAY:

Extracts were mixed with DPPH (0.1mM) in methanol solution. 0.1mM of DPPH was prepared by dissolving 1.2 mg of DPPH in 20 ml of methanol. 10µl volume of extract and standard of concentrations like 10µg/ml, 25, 50, 75, 100 µg/ml were taken in the ELISA plate. To the 10 µl of extract and different concentrations of standard 190 µl of DPPH (0.1mM) was added. It was allowed for incubation. After 30 min incubation at room temperature dark atmosphere, the absorbance was read at 517 nm. The inhibitory percentage of DPPH was calculated according to the following equation:

$$\% \text{inhibition} = \frac{\text{absorbance}(\text{cont}) - \text{absorbance}(\text{sam})}{\text{absorbance}(\text{cont})} \times 100\%$$

INCORPORATION IN FOOD – MILK CHOCOLATE:

The cover of the cardamom was removed and the seeds are ground. 1 litre of milk was boiled till it was reduced to its $\frac{1}{2}$ the quantity. Sugar, cocoa powder and flour were sieved together. The sieved mixture was then added into the milk slowly and stirred continuously till the mixture becomes thick. Ghee was added and stirred well. Cardamom powder was added and the extracted Beta carotene was incorporated and immediately transferred the content vessel and kept for setting.

RESULTS AND DISCUSSION: MOISTURE CONTENT

$$\text{MC}\% = 100 \times \frac{(W_1 - W_2)}{(W_1 - W_3)}$$

(W = 48.32, W₁ = 53.42, W₃ = 49.27)

$$= 100 \times \frac{(53.42 - 48.27)}{53.42 - 48.24}$$

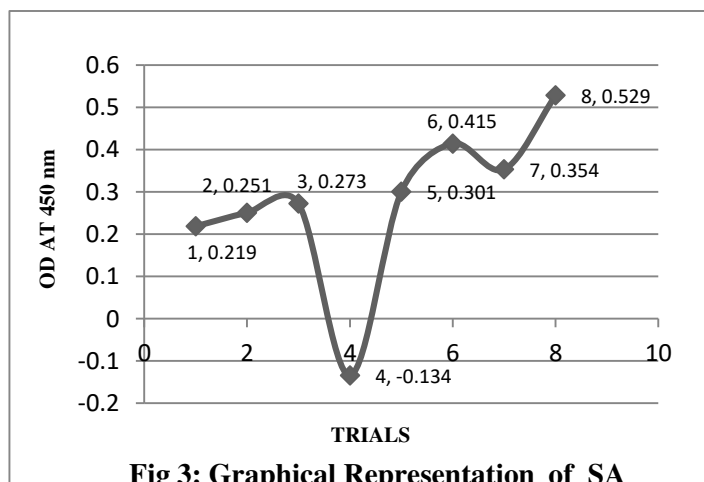
$$= 100 \times \frac{4.15}{5.1}$$

$$= 81.31\%$$

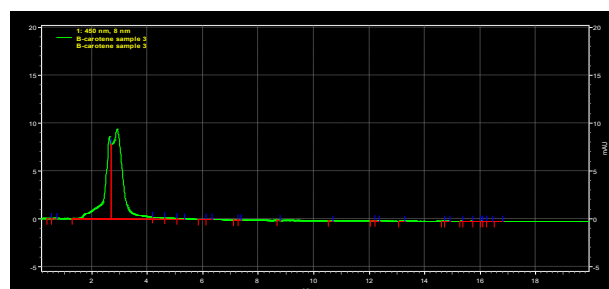
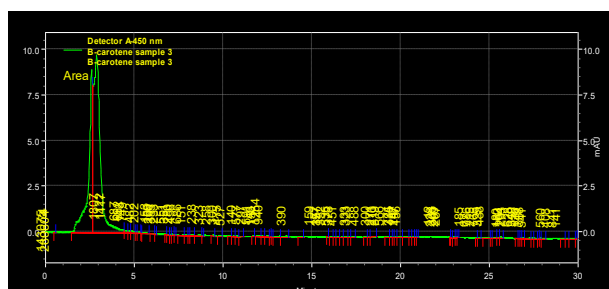
SPECTROPHOTOMETRIC ANALYSIS

Trial	OD (450 nm)
1	0.219
2	0.251
3	0.273
4	-0.134
5	0.301
6	0.415
7	0.354
8	0.529

Table 3: Spectrophotometric Analysis of Trials



HPLC ANALYSIS:



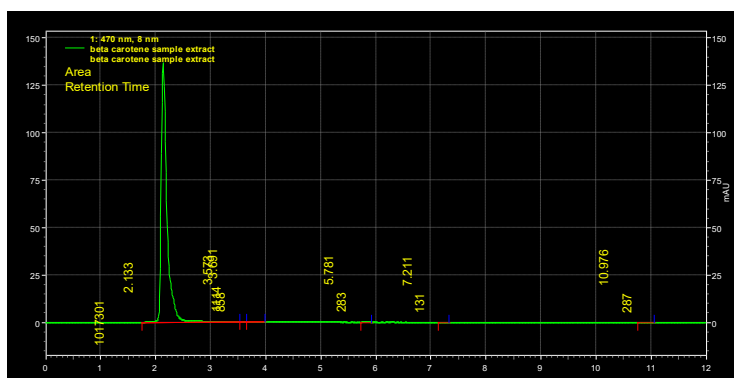


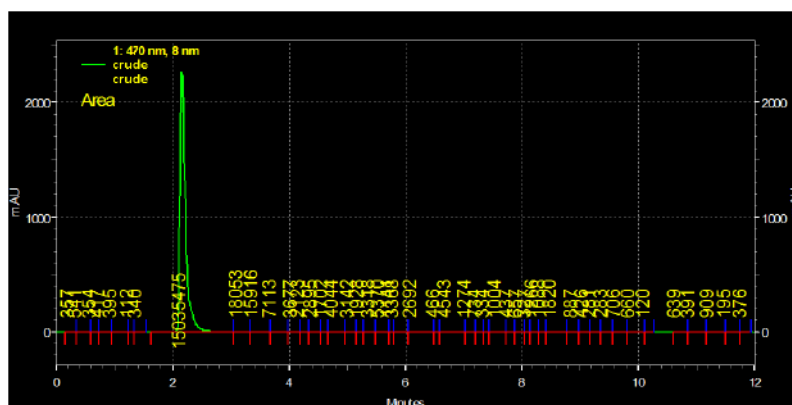
Fig 3.3: Chromatogram For The Trial 12 Isopropanol 70% + Water 30% (Overnight Soaking)

BULK EXTRACTION:

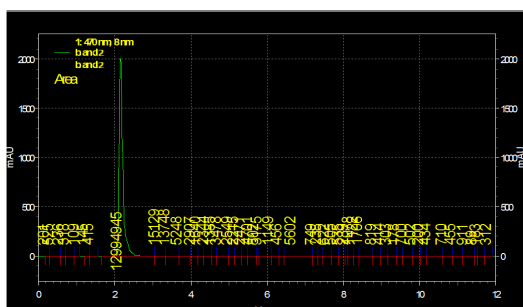
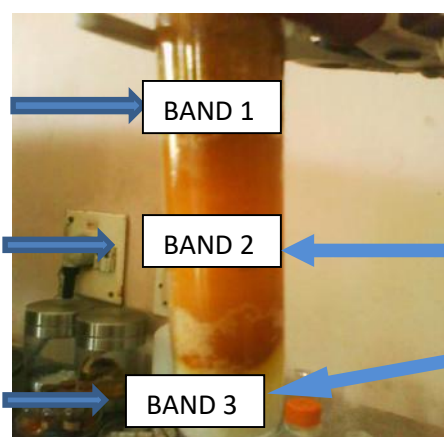
Fig 3.4: Bulk Extract



Fig 3.5: Chromatogram For Crude Extract From Bulk Extraction



Column Chromatography



INCORPORATION IN FOOD:

The purified extract - Beta carotene using column chromatography and analyzed by HPLC was concentrated and weighed according to the ADI recommendations, (i.e) 1 mg from seaweed *Ascophyllum nodosum* was incorporated into food milk chocolate and it was subjected to sensory evaluation.

HEDONIC RANKING ANALYSIS FOR CHOCOLATE:

Like extremely	=	No
Like very much	=	Yes
Like moderately	=	No
Like fairly	=	No
Like nor dislike	=	No
Dislike fairly	=	No
Dislike moderately	=	No
Dislike very much	=	No

CONCLUSION:

β -Carotene is an organic compound - a terpenoid, a red-orange pigment abundant in plants and fruits. It is a precursor (inactive form) of vitamin A. A powerful antioxidant with radical quenching properties. *Ascophyllum nodosum* a brown algae rich in β -Carotene being the only species in the genus *Ascophyllum*. It is seaweed of the northern Atlantic Ocean. The extraction of β -Carotene from *Ascophyllum nodosum* was done by solid – liquid extraction method by Soxhlet apparatus. The extraction efficiency was high in the twelfth trial with solvent composition 70% Isopropanol + 30% water, 6 hrs and 80°C. The pretreatment overnight soaking in the solvent gives better efficiency. The extract was concentrated using rotary vacuum evaporator, and the HPLC analysis was done using the parameters like mobile phase of hexane: acetone (82:18v/v), with the flow rate of 1.2 ml/min and the absorbance was read at 470 nm. The bulk extraction was done, for 1 kg of seaweed 4 lit of solvent (70% Isopropanol + 30% water) was used. The extract was concentrated to 10 folds. The concentrated extract was analyzed using HPLC and purified by column chromatography. The extracted beta carotene was incorporated into chocolate it was given to 25 members for sensory analysis.

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Polygalacturonase production by mutant *Aspergillus carbonarius*

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Abstract: Pectinase enzymes plays a significant role in different industries such as textile industry, fruits and vegetables processing, Polygalacturonase is one type of pectinase which plays a vital role in fruit juice industry. Pectinase can be produced naturally by all *Aspergillus* species. To increase the yield of the enzyme, strain improvement (mutant strain 42 KDa) process was carried out to get higher yield in Submerged fermentation. *Aspergillus carbonarius* subjected to ultra violet radiation to produce mutant strain. The yield of polygalacturonase (U/mL) obtained in Sub merged Fermentation was 150.63 kg/batch from 50kg/batch of corn flour. The results shows that submerged fermentation and mutant *Aspergillus carbonarius* both contributed to the high yield of enzyme production.

Key Words: Pectinase, Polygalacturonase, *Aspergillus carbonarius*, Ultraviolet radiation.

INTRODUCTION:

Polygalacturonase, the major constituent of pectinase for mulations is produced from the fungus, *Aspergillus carbonarius*. Enzymes produced from the fungi *Aspergillus*, are gener-ally regarded as safe [7] Polygalacturonase is a depolymerizing pectinase, Which catalyzes the hydrolysis of -1,4 glycosidic linkages in homopolygalacturonan. About 90enzymes are produced by SmF using genetically manipulated microorganisms owing to several process advantages over SSF [2]. The natural isolates of *Aspergillus carbonarius* produce polygalacturonases, strain improvement to increase enzyme production. *Aspergillus carbonarius* mutants that formed aggregates during submerged fermentation were described to produce higher quantities of enzyme and production was reasoned to the better fermentor behavior such as less viscosity, better growth, higher aeration and efficient mixing of medium [3] Production of polygalacturonase by filamentous fungi is repressed by readily available carbon source. Synthesis of the enzyme was induced by galacturonic acid or polygalacturonic acid at transcription level. A number of reports showed that repression of polygalacturonase synthesis in *Aspergillus carbonarius* during submerged growth by simple sugars was very effective and stronger than in SSF [1]. This study revealed that enzyme production by the fungus in shake flask cultures at differential regulation. As the yields of polygalacturonase during shake flask growth was postulated and to evidence the hypothesis, Mutants of *Aspergillus carbonarius* were isolated to examine the phenomenon governing polygalacturonase secretion by *Aspergillus carbonarius*. In this study, a mutant producing the enzyme during fermentation process. The isolation of polygalacturonase overproducing mutant of *Aspergillus carbonarius* suggested its application for industrial enzyme production by submerged fermentation. A mutant strain *Aspergillus carbonarius* secreted different forms of polygalacturonases when grown in SmF culture [8]. Porous membranes have been employed generally for the concentration of enzymes [4].

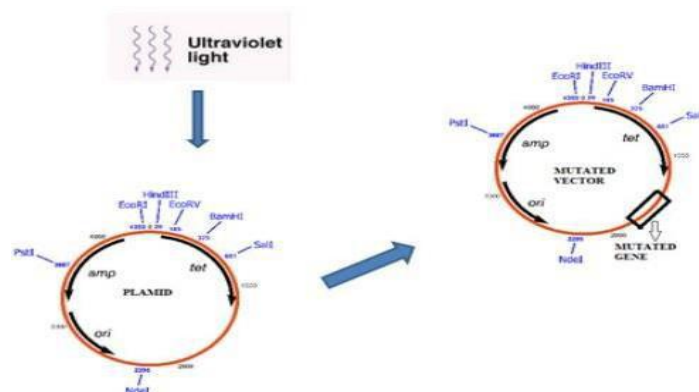


Figure 1. Vector mutation by UV radiation.

MATERIALS AND METHODS:

Process description:

Aspergillus carbonarius was used for enzyme production in Submerged fermentation. Process description for Smf, the fungus was grown in a medium made of 7.5 percentage (w/v) corn flour, and 2.6 percentage ammonium dihydrogen phosphate [8]. Smf was carried out in a fermentor of 75L using an inoculum in above medium in shake flask (200rpm) and incubated at 30°C for 48 h with spores from one week slants. The inoculum developed in shake flask was used to inoculate in Bioengineering fermentor. Fermentation conditions were maintained as above. The culture broth was harvested in fermentor for enzyme production. These process conditions were maintained in the fermentor with following Table.1.

TABLE 1. THE PROCESS CONDITIONS OF THE FERMENTOR

PARAMETERS	PROCESS CONDITIONS
volume	1000L
Temperature	30° c
Domestic waste	3.0
pH	5.5
Air flowrate	300 - 375
Fermentation period	24h

RESULTS AND DISCUSSION:

Polygalacturonase production in *Aspergillus carbonarius* has been shown to be induced by pectin and products released due to degradation of pectin [9]. The regulation of polygalacturonase expression during submerged cultivation of *Aspergillus* has been reported [1]. The production of polygalacturonases using *Aspergillus carbonarius* was performed by Submerged fermentation. The isolation of mutant strain of *Aspergillus carbonarius* which constitutively produced polygalacturonase. The mutant strain of *Aspergillus carbonarius* when grown in shake flask does not produce polygalacturonase.

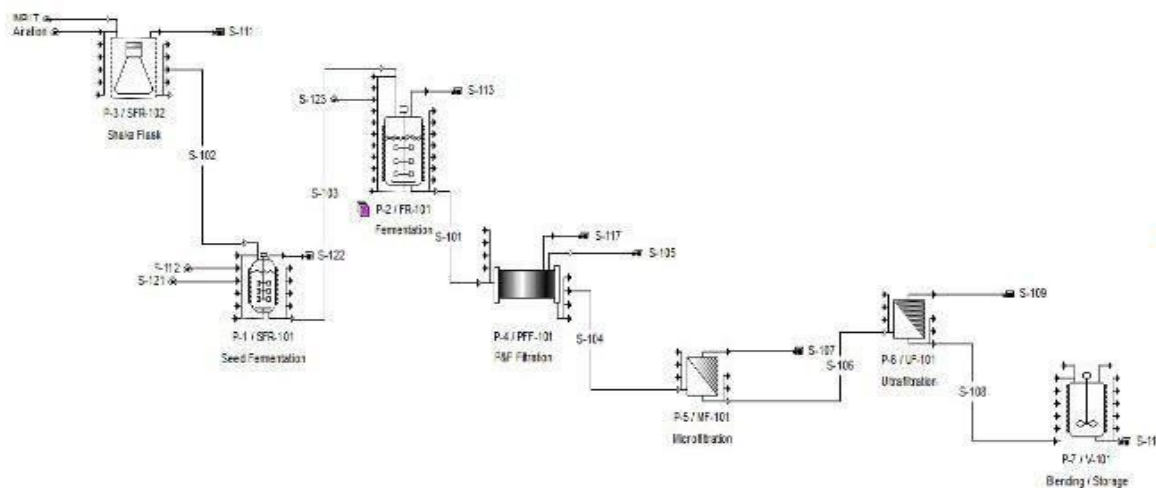


Figure 2. Process flow diagram of *Aspergillus carbonarius* of Polygalacturonase production in submerged fermentation.

To induce the enzyme production the corn flour and glucose are added. The media contain magnesium sulphate and ammonium dihydro- gen phosphate as a nitrogen source, Yeast as a mineral required for growth. After 72h growth, Polygalacturonase in culture was filtrated using microfiltration and ultrafiltration. The fermentation period of SmF is about 72 h which provides a system with larger enzyme yields and longer fermentation periods [6]. In the present study, the yield of polygalacturonase (U/mL) obtained in SmF was 150.63kg/ batch from 50kg/batch of corn flour. The process performance of SmF for *A. carbonarius* polygalacturonase production and purification is listed in Table 1. The polygalacturonase productivity obtained by SmF used mutant of *Aspergillus carbonarius* was more effective in strain improvement strategy [5].

TABLE 1. MASS COMPOSITION OF THE COMPONENTS USED IN FERMENTOR

COMPONENTS	MASS COMPOSITION (IN PERCENTAGE)
Corn flour	10.9682
Ammonium di-hydrogen phosphate	2.5974
Yeast	4.9172
Water	24.5802
Aspergillus carbonarius	7.0040
NaCl	3.0439
media	17.4900
Magnesium sulphate	4.6330
Calcium chloride	5.1948

CONCLUSION:

The current work on enhancement of polygalacturonase production from *Aspergillus carbonarius* has been achieved through strain improvement by using mutant of *Aspergillus carbonarius*. This work confirmed that mutant *Aspergillus carbonarius* provides higher enzyme yield. This report emphasizes that mutant *Aspergillus carbonarius* results in higher polygalacturonase production using submerged fermentation under optimal conditions. This enzyme is being used extensively in agricultural productivity, fruit ripening process, Food industry etc.

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Department of Biotechnology, Kumaraguru College of Technology, Tamil Nadu, India

**COMPARATIVE STUDY OF ENZYME HYDROLYSIS AND
CHEMICAL HYDROLYSIS FOR BIOETHANOL PRODUCTION
FROM BAGASSE**

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Abstract: Bagasse a major by-product from sugarcane industry serves as an important raw material for the production of ethanol and power generation. Ethanol is mainly used as a substitute for petrol. Ethanol is fermented from sugarcane molasses enzymatically and by chemical hydrolysis method. In this present study, a comparison of enzymatic and chemical hydrolysis method has been carried out. In addition, various parameters were taken into consideration to compare the efficiency of the plant in terms of production. Results obtained from the simulation confirms that the chemical hydrolysis method produced ethanol of 34.5 kg/batch compared to that of the enzymatic hydrolysis of 34.25 kg/batch for 100 kg of sugarcane bagasse.

Key Words: Superpro , enzymatic hydrolysis, chemical hydrolysis.

INTRODUCTION:

During the last decade, concerns regarding global warming, fossil fuel depletion, and energy security resulted in a wide interest in renewable and environmentally friendly fuels [4]. Alternative for gasoline by liquid fuels produced from various renewable sources is a high-priority goal in many countries. The main aim of using renewable sources is for a secure and sustainable energy supply and a desire to lower greenhouse gas effect. Bioethanol is seems to be one of the most dominating renewable biofuels in the transportation sector within the coming 20 years, and has already been introduced on a large scale in Brazil, the USA and some European countries .The advantages of bioethanol are that it can be produced from a variety of raw materials and it is non-toxic to the environment

.Bagasse is a major by-product of the sugar industry, which is utilized in the same industry as an energy source for sugar production [7]. Sugarcane bagasse a major lignocellulosic materials that can be employed for bioethanol production and act as fuel for providing heat and power generation for the entire production plant [2]. Certain bioproducts can also be extracted during ethanol production. Surplus bagasse and trash that burnt during harvesting can also be collected and used for energy consumption of plant [3]. Pentose and lignin, byproducts obtained in bioethanol production, may be used as fuels [5] increase the amount of surplus bagasse, in an optimized process condition . The main aim of the process is to compare the enzymatic and chemical hydrolysis for the high yield of bioethanol production. The two conversion steps can be considered key processes: hydrolysis of the hemicelluloses and cellulose to sugars, [6] and fermentation of all the sugars. The enzymatic process is regarded as the most attractive way to degrade cellulose to glucose [8]. However, enzyme-catalyzed conversion of cellulose to glucose is very slow unless the biomass has been subjected to some form of pretreatment, as native cellulose is well protected by a matrix of hemicelluloses and lignin. The effective pretreatment should result in high recovery of all carbohydrates and high digestibility of the cellulose in the following hydrolysis process. Liming is the pretreatment that has been used in this process. Cellulose which has regular and cross-linked chains forms a very efficient barrier against penetration of the enzymes into the fibre [1]. Swelling of the pores can be achieved by pretreatment through soaking of the material in an liming solution. After pretreatment the resultant mixture is subjected to hydrolysis by both enzymatic and chemical hydrolysis methods. The enzymes used in this process are cellulase and glycosidase. These enzymes are produced using *Trichoderma reesei* and *Aspergillus niger* fungus and in chemical method sulphuric acid is used. The bottleneck process of this process is hydrolysis since hydrolysis of cellulose to glucose is the mandatory step to produce

bioethanol.

Unit procedure	v	o	l	u	m	e	Height/Depth	Pressure (bar)
steam explosion (R-101)	5	0	0	0	L		2 . 5	1 . 5 2 0
Delignification (R-102)	2	0	0	0	L		2 . 5	1 . 5 2 0
cellulose hydrolysis(R-103)	1	0	0	0	L		2 . 5	1 . 5 2 0
Fermentor (FR-101)	1	0	0	0	L		2 . 5	1 . 5 2 0

TABLE 1
REACTORS USED IN DESIGN FLOW SHEET

MATERIALS AND METHOD:

The design and simulation for the ethanol production plant was performed by using SuperPro designer. The following process operations were considered in anhydrous ethanol production in an autonomous distillery: juice extraction from sugarcane, juice treatment and concentration, fermentation, distillation, filtration and dehydration of ethanol, cogeneration (combined heat and power generation)(Dias et al., 2010). The dimensions of the equipment used in treatment plant are :

<u>Filter</u>	<u>Filter area</u>
Pentose filter(RVF-101)	50m
Black liquid filter(RVF-102)	10m
Glucose filter(RVF-103)	10m

TABLE II
FILTER USED IN DESIGN FLOW

<u>Parameters</u>	<u>Process conditions</u>
Temperature	37°C
Cycle time	24h
Batch time	35.33h

TABLE III
THE PROCESS CONDITIONS OF THE FERMENTER

Bagasses is produced when sugarcane juice is separated from the fiber and is used to produce steam and electricity. Sugarcane bagasse may be used as feedstock for ethanol production. Since it is composed of cellulose, hemicellulose and lignin glucose, it may be converted into fermentable sugars through pretreatment and hydrolysis processes. These process run with reactors like fermentor and filters to obtain ethanol in successive manner, and the details of reactors and filters are listed in the Table 1. and Table 2. In this process, steam explosion is considered as a pretreatment method, and hemicellulose is converted into pentose and cellulose. cellulose bagasse is burnt in cogeneration systems to produce steam and electricity for the plant [2]. Celluligin obtained from pentose in the pretreatment process produces ethanol by anaerobic digestion. At second level, alkaline delignification improves hydrolysis , which removes lignin and produce cellulose to enzymatic attack. At third level, the recovered pentose are recycled to steam explosion.

The glucose liquor obtained after chemical and enzymatic hydrolysis is fermented to produce ethanol. The proposed scenarios were simulated using SuperPro designer to generate process data to simulate in cogeneration system .By this study, compared that production of ethanol using enzyme hydrolysis and chemical hydrolysis. which is represented in the following figures below Fig 1. and Fig 2.

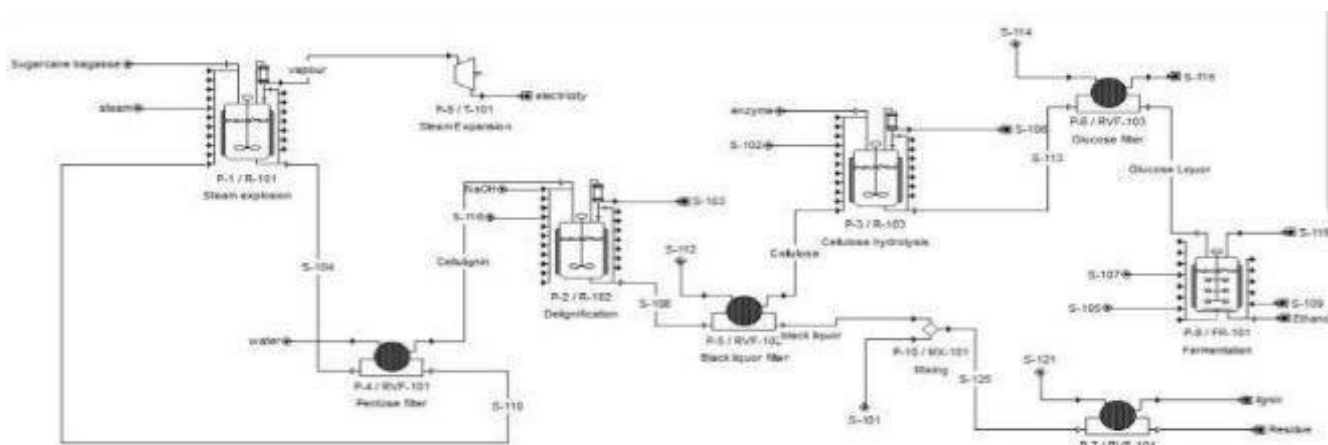


Fig. 1. Flowsheet representing the simulation of ethanol production using enzyme hydrolysis .

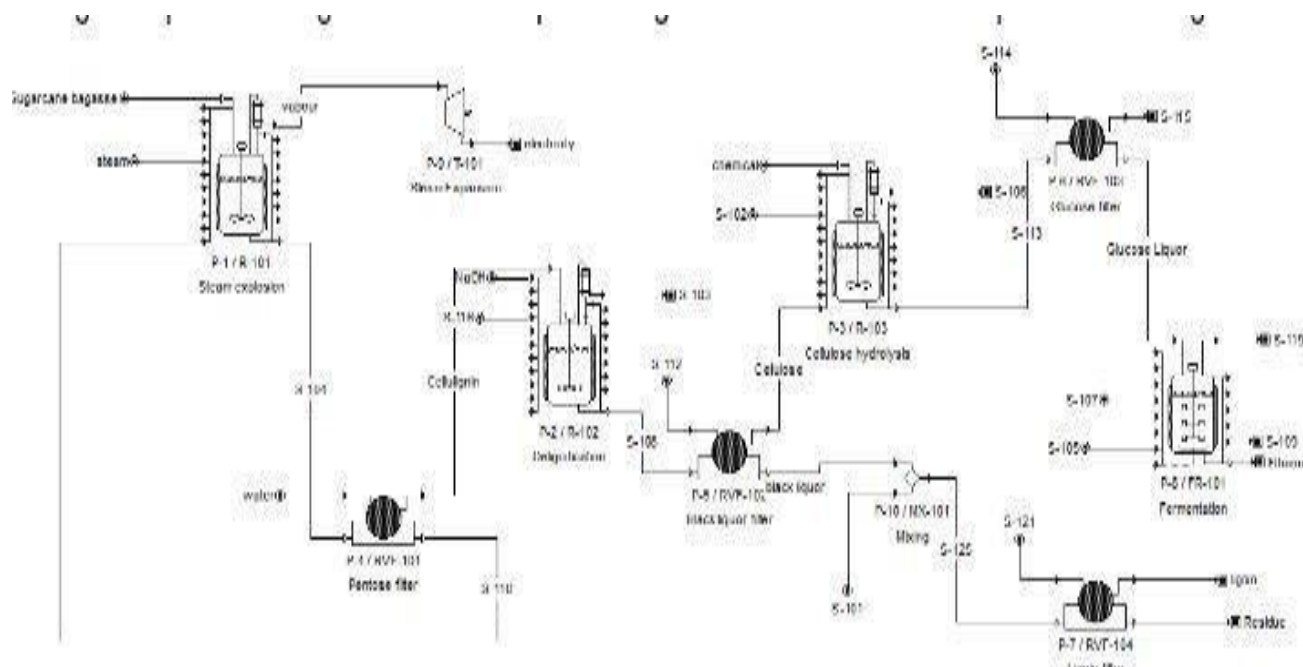


Fig. 2. Flowsheet representing the simulation of ethanol production using chemical hydrolysis .

The total amount of ethanol produced (kg/batch) in chemical and enzyme hydrolysis were 34.59 kg/batch and 34.25kg/batch for 100kg/batch of sugarcane bagasse. The high yield was observed in chemical method. As the ethanol is produced from the glucose obtained from the bagasse (sugarcane trash) and hydrolysis technologies. The current hydrolysis technology is used to produce ethanol in a decreased production cost.

RESULTS AND DISCUSSION:

Enzyme hydrolysis (Cellulase and glycosidase) which cleaves bond in molecule with addition of elements in water and acid hydrolysis (Sulphuric acid and Hydrochloric acid) was performed to breakdown bagasse into simple sugars, which cleaves bond in molecule with addition of elements in water and consumed as substrate for yeast. Which results production of ethanol in varying amount. As a result, Ethanol production was simulated, by which electricity was produced using cogeneration production. In Ethanol production, the total amount of ethanol produced (kg/batch) in chemical and enzyme hydrolysis were 34.59 kg/batch and 34.25kg/batch for 100kg/batch of sugarcane bagasse. The high yield was observed in chemical method. In delignification process, lignin was removed which is from 50 to 35.31 kg /batch for both chemical and enzyme hydrolysis. Glucose produced during hydrolysis 57.64 (kg/batch) in chemical hydrolysis and in enzyme hydrolysis glucose produced was 57.07 (kg/batch). scenarios. These hydrolysis

technologies relatively produced low yield (34.59), as the glucose obtained from the lignocellulose is only converted into ethanol while the pentose obtained from the pretreatment process is anaerobically digested to produce biogas hence it is employed in the cogeneration system. But this hydrolysis technology reduces the ethanol production cost and produces biogas and electricity which turned this process profitable.

CONCLUSION:

The production of ethanol using sugarcane trash (bagasse) decreased the overall production cost of ethanol when compared to conventional distillery, because in this process bagasse is used as a raw material while integrated process use sugar-cane as raw material and 50 percentage of trash is used for steam and electricity production. These hydrolysis technologies relatively produced low yield as the glucose obtained from the lignocellulose is only converted into ethanol while the pentose obtained from the pretreatment process is anaerobically digested to produce biogas hence it is employed in the cogeneration system. But this hydrolysis technology reduces the ethanol production cost and produces biogas.

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Design and Simulation of a Municipal Waste Water treatment by Aerobic Digestion

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Abstract: Recycling of waste is creating major impact in the environment. Especially waste water treatment and recycle the waste water is becoming more important. Removing different types of contaminants from waste water needs many operations. Waste water from different source is subjected for different waste water treatment. In this study, we have designed a municipal waste water treatment bioprocess model using SuperPro Designer. In which, we used operation units such as Mix, Aerobic Bio-Oxidation, Clarification, Flow Splitting, Belt Filter and Sludge Dryer including a recycling process. Using SuperPro Designer simulation and optimization were done. Before looking for an industrial level large scale process, this bioprocess model output data would help to design a waste water treatment plant. And then, the model design for large scale waste water treatment plant. In this report, efficient removal of domestic waste and ammonia was achieved using biological method. Using aerobic bio oxidation is costly but, it gives good quality of the effluent water. Efficient removal of sludge can be achieved using incinerator. This process is mainly helps to remove contaminants from municipal waste water and convert it into an effluent that can be returned to the water cycle with minimum impact on the environment.

Key Words SuperPro Designer, Aerobic Bio-Oxidation.

INTRODUCTION:

Increased environmental awareness together with very stringent regulation standards has make different industries to project them in adopting waste water treatment technologies [1]. The main sources of contaminants are the domestic waste and industrial waste which includes suspended solids, biodegradable organics (proteins, carbohydrates and fats), total dissolved solids (TDS) etc. [2]. These compounds create problems in sewage treatment plants and if not treated properly, it will contaminate the water resources. To overcome this, researchers had developed various technologies to achieve high efficiency with minimum cost. The technique includes membrane filtration, ion-exchange, electrolysis, adsorption, etc. [3]. Biological treatment methods have been reported to be a possible solution in removing these compounds without generating toxic end products [4]. Previously conventional anaerobic-aerobic treatment plants were used mostly for the treatment of wastewaters, in recent time, bioreactors (anaerobic-aerobic) with high rate efficiency have been in use to increase the wastewaters treatment with high chemical oxygen demand (COD) [5]. his process needs primary and secondary treatment which includes mixing, aerobic digestion, clarification and filtration. Aerobic biological waste water treatment plants are designed to convert complex organic molecules to carbon dioxide, nitrate, biomass and water. Municipal Waste Water Treatment plants are classified into three categories with specific characteristics such as sewage forms with mechanical purification, biological waste water treatment plant consisting of large tanks with several thousand cubic meters of volume, in which the flow conditions are not predicted in very poor oxygen transfer rate, WWT plants which can be used even in densely populated cities and are relatively inexpensive [6], [7].

SuperPro Designer is one of the best bioprocess simulators to obtain a process model. This simulator is used to get maximum expected result data by changing the input feed value and other operation parameters. In this study, SuperPro Designer used to design a process model for Municipal Waste Water Treatment (MWWT).

REVIEW ON RELATED PAPERS:

Authors demonstrated that by using an integrated bioreactor with multiple specification in treating high strength wastewaters is advantageous because it needs minimal space requirements, low capital cost investment and excellent COD removal efficiencies [5]. Coagulation and flocculation both process were considered as most used and important process in treatment of waste water because it will remove the pollutants from the waste water effectively and give a good quality of effluent [1].

OBJECTIVES:

Objective of this work is to design a model for Municipal Wastewater treatment using aerobic bio-oxidation. Though, the use of these aerobic tanks are extremely costly, they exhibit high efficiency, high operation flexibility, no scaling-up problems and possibility of process control [7].

METHODS AND MATERIALS:

The design and simulation for the waste water plant was performed by using SuperPro Designer software (Fig1).

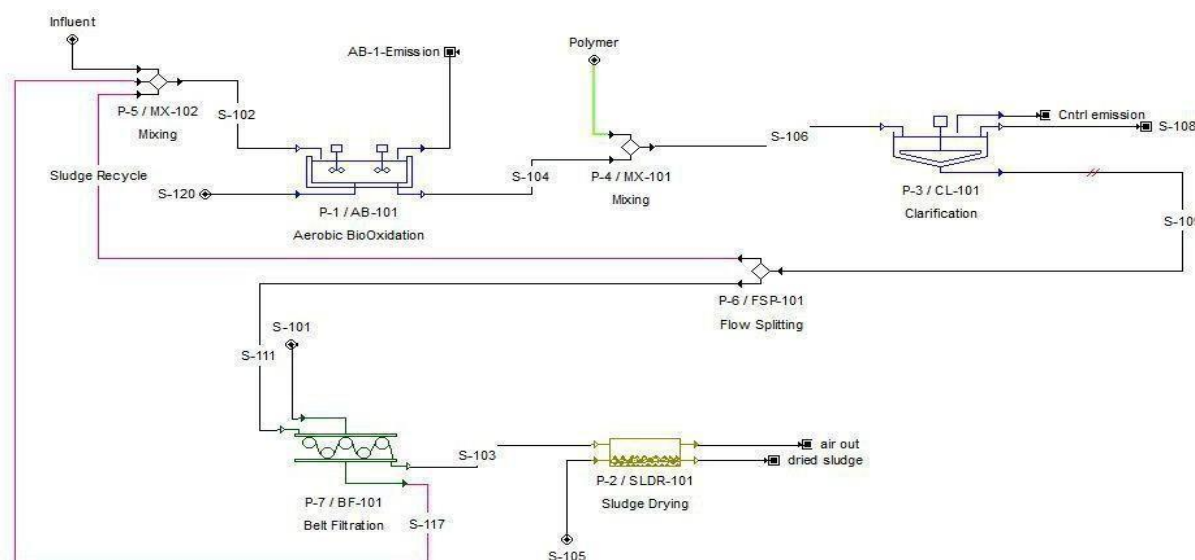


Figure 1: Flow Diagram of Municipal Waste Water Treatment Plant

Overview of the Process:

Influent is municipal wastewater and it send to the aerobic bio-oxidation unit (AB101) for the processing of wastewater through biological treatment. The treated water shipped to the mixer (MX -101) by adding polymer to the treated wastewater which makes it to undergo coagulation and flocculation. After this the polymer blended product sent to clarifier (CL-101) to get the clarified liquid out by liquid effluent (S-108) and remaining were send to the flow splitting (FSP -101) unit where the liquid and solids were separated and liquid dispatched to mix (MX-102) for recycling and solid sent to belt filter unit (BF-101). Belt filter used to filter the solid from the liquid for that water (S-101) is added to dilute the solid particles to get slurry and liquid to be separated. Filtered liquid dispatched to recycling (S-117) and the slurry ship to sludge dryer (SLDR-101) for dried sludge removal. Dried sludge collected in the effluent.

Equipment Specification:

The main equipments of this treatment plant are:

- Aeration Basin – a tank having vessel volume of 150000.00 L with a depth of 2 m (AB101).
- Clarifier – a tank having a volume of 60000.00 L with a depth of 3 m (CL-101).
- Belt Filter – a belt filter press with belt width 0.003 m (BF-101).
- Sludge Dryer – one unit with a evaporative capacity of 22.00 kg/h (SLDR-101).

Pure Components Registered:

Table 1: The pure components were added to the feed stream as described below

COMPONENT	FLOWRATE (kg/batch)	CONCENTRATION (g/L)
Ammonia	6.0	0.15802
Carbon dioxide	1.45	0.03832
Domestic waste	3.0	0.079091
FSS	1.72	0.04543
Nitrate	0.75	0.01975
TDS	8.7	0.22913
water	28372.5	747.24889
X-Vss-h	1.5	0.03951
X-Vss-i	1.45	0.03832
X-Vss-n	0.09	0.00237

RESULT AND DISCUSSION:

The concentration of the TDS (Total Dissolved Solids) in the influent was 0.229 g/L (Table 1). In aerobic bio-oxidation, domestic waste and ammonia are efficiently digested and degraded by micro-organisms in the presence of oxygen and reduced to nitrate and carbon dioxide. Domestic waste was degraded insignificant amount. After aerobic bio-oxidation and clarification, CO₂ and ammonia was efficiently removed. Biomass concentration was increased when compared to the influent. In clarifier chamber the polymers react with waste water where the solid particle coagulates and settle at the bottom of the tank and the liquid effluent is removed (Table II). After clarification 95% of the slurry is sent for sludge recycling and remaining 5% slurry is sent for further sludge processing. Impurities from the influent were removed as much as possible, remaining was sent for recycling to remove the impurities completely.

Table 2: Input and Output data of Municipal Waste Water Treatment

COMPONENTS NAME	CONCENTRATION(g/L) (IN)	CONCENTRATION(g/L) (OUT)
Ammonia	0.15802	0.029705
Carb. Dioxide	0.03832	0.001467
Dom waste	0.07901	0.000362
FSS	0.04543	0.221065
Nitrate	0.01975	0.226185
TDS	0.22913	6.505368
Water	747.24899	943.621464
X-Vss-h	0.03951	0.498333
X-Vss-i	0.03832	0.188107
X-Vss-n	0.00237	0.029796

In waste water treatment, sludge processing is an important part as there is an increasing demand to minimize the final sludge disposal which greatly impacts the environment. 5% of concentrated slurry is then subjected to belt filtration for dewatering purpose and about 15% of cake obtained from belt filtration is further dried using sludge dryer. The remaining 85% of water is recycled back to MX101 (Figure 1). In sludge dryer, 35% of solid concentration is obtained as dried sludge.

CONCLUSION:

This work mainly involves the design and simulation of municipal waste water treatment plant by biological method. Domestic waste and ammonia feed is efficiently reduced by aerobic digestion. Polymer utilization improves the waste water treatment and good quality liquid effluent in the output.

RECOMMENDATIONS:

The nitrogen and nitrate removal can be improved by the use of anoxic bioreactors and the sludge removal can be further improved by the use of incinerator. It also helps to generate and a self-sustainable plant.

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Design and analysis of nitrogen removal from municipal wastewater using Modified Ludzack-Ettinger process

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Abstract: In recent years, usage of water has been increased, so this study has been reported for the alternative ways of the waste water treatment. Waste water treatment plays a major role in re- cycling process so the process utilizes both anoxic and aerobic reaction to do it effectively. The design was to optimize the possibility to increase the recycled water content and to remove the nitrogen content from wastewater. The entire analysis was performed by intelligen superpro designer using modified Ludzack-Ettinger process. The carbon:nitrogen (C:N) ratio of the influent fed into the aerobic reactor is considered as the most important factor that affects the nitrification rate. Due to nitrification and denitrification in anoxic basin, nitrogen has been removed. Therefore, liquid effluent efficiency and solid effluent efficiency were estimated as 94.38% and 0.10% respectively.

Key Words: Anoxic and aerobic reaction, modified Ludzack-Ettinger process, nitrification, denitrification.

INTRODUCTION:

Treatment of waste water usually consists of chemical physical pretreatment followed by the biological process which effectively removes nitrogen and remaining organics. Chemical-physical process create substantial quantities of sludge to be dewatered and eliminated [1]. The modified Ludzack-Ettinger process is patterned to use nitrate produced by the aerobic bio-oxidation as an oxygen source for facultative bacteria in the breakdown of raw wastewater in the anoxic basin. Initial process in the treatment flowsheet, a pre-anoxic basin where influent wastewater form the feed, return activated sludge from the clarifier, and nitrate rich mixed liquor pumped from the effluent end of the aeration the carbon source for the microorganism, the anoxic recycle pumps furnish nitrate as an oxygen source and release nitrogen [2].

Nitrates are introduced to the anoxic tank through the recycled activated sludge. By increasing the influent carbon:nitrogen (C:N) entering the aerobic reactor, nitrification rate decreased asymptotically [3]. Nitrate removal efficiency in an anoxic reactor is controlled by inlet nitrate level entering in the system. Nitrification and denitrification are the key biological nitrogen removal (BNR) reactions. The oxidation of ammonia to nitrite and then the oxidation of that nitrite to nitrate is nitrification. Denitrification bacteria need an electron donor (BOD) to get rid of nitrate (NO_3^-) which is an electron acceptor. Oxygen competes with nitrate as an electron acceptor.

Enzymes (reductases) used by denitrifying bacteria are repressed in the presence of oxygen impacts assimilation process. Biological nutrient removal is done in this process where removal of nutrients is greater than metabolic amounts. The primary objective of BNR plant operation is to achieve regulatory compliance consistency.

Single sludge system removes biological oxygen demand and ammonia in the same reactor with only one set of clarifier and a thickener. Single sludge system are common because many times denitrification is incorporated into the design which would require the mixing of sludge or the addition of the carbon source. Clarifier and sludge thickener are used for sedimenting small particles (sludge) and can be used for the preparation of methane in addition to sludge digestion reactor [4]. Belt filter is used for solid/ liquid separations to dewater the sludge. MLE system reduces the alkalinity demand and is limited by the amount of nitrate returned in recycled activated sludge.

MATERIALS AND METHOD:

Modified Ludzack - Ettinger (MLE) process along with other equipment has been used to remove the wastewater effluent and nitrogen, efficiently. This plant consists of a generic procedure, an anoxic reactor, two aerobic reactors, a clarifier, a thickener, a belt filter, three flow splitter, a 2 way and a 5 way mixer as shown in Figure 1 and components are mentioned in Table 1.



S.No	Components	Description
1	FSS (Fixed Suspended Solids)	Inorganic particles suspended in the liquid (undissolved salt crystals and silt particles)
2	TDS (Total Dissolved Solids)	Combined content of all inorganic and organic substances
3	X-Vss-h	Active volatile solid
4	X-Vss-i	Inert volatile solid
5	X-Vss-n	Nitrifiers
6	Domestic waste	Disposed water from home, offices and industry

Clarifiers are used to remove the TDS (Total Dissolved Solids), FSS (Fixed Suspended Solids) and other solid particles. Water leaving the clarifiers is split into two as liquid effluent and sludge. The sludge is again split into two as one is being recycled back to anoxic reactor and the other is fed into second aerobic reactor [6]. It works with 10g/l of particulate concentration in sludge and 0.001 kg/ms of liquid viscosity.

Nitrification takes place in the presence of dissolved oxygen in the aerobic reactor, subsequently, the stream is fed into thickener. Sludge thickener is used to increase the solid concentration and decrease the sludge volume [7]. It removes the solid particles when set at 30g/l of particulate concentration in sludge and 0.001 kg/ms of liquid viscosity. It is split into two using flow splitting and one is fed into the belt filter and the other is recycled back to anoxic reactor. The sludge effluent is passed out and the remaining is recycled to denitrification [1]. The flow per unit belt width is around 16.67 L/min-m and the solids in cake is set as 15%.

RESULTS AND DISCUSSION:

In Generic Procedure (P-1/GBX-101), only liquid influent is given to act as a storage tank. The feed cannot be given inside immediately, so it is stored and also to reduce the pressure. In this process, only domestic waste is focused, so FSS (Fixed suspended solids) is removed manually. In anoxic reaction (P-3/AXR-101), when domestic waste is dumped for a period of time, it may lead to the production of methane or ammonia gas. In this, ammonia is produced as toxic when released in the environment. Ammonia and domestic waste combine together along with oxygen is reacted. Oxygen has been added the amount little higher than those combination. Ammonia completely break down into nitrogen and hydrogen. Hydrogen combines with oxygen to form water and remaining carbon in the domestic waste combines with oxygen and form carbon dioxide. Remaining solid parts in domestic wastes is present as volatile suspended solids X- Vss –h. In nitrification process, ammonia has been converted to nitrate. Hydrogen removed combines with oxygen and form water. Comparing it with before process volume of oxygen reacted has been raised thrice and carbon dioxide is also produced. Remaining solid parts in domestic waste present as volatile suspended solids X-Vss-n. In denitrification process, unreacted domestic waste and nitrate produced in nitrification process react together then break the nitrate into nitrogen and oxygen. Nitrogen comes out as gas. Oxygen reacts with hydrogen and carbon that has been produced from domestic waste produces water and carbon dioxide. Remaining suspended solids X-Vss-n has been reduced 5 times when compared to the domestic waste. In X-Vss-h decay, volatile suspended solids X-Vss-n produced in the nitrification process and denitrification process reacts with oxygen. Similarly, carbon dioxide and water has also been produced. In this very small amount of FSS is removed due to the presence of bacteria. As bacterial growth will affect the sludge, it is completely removed. In the vent, carbon dioxide is released which may lead to the production of excess heat (exothermic reaction). It also increases the reactor temperature, so it has been released in the form of heat. In Flow splitting (P-7/FSP-103), 95% of water supplied from top stream S- 107 and 5% of water sent for mixing. It has been recycled because when process starts from generic procedure most of the solids starts to settle down leads to clogging in bioreactors and pipeline. In Clarification (P-5/CL-101), mainly the liquid effluent (94.38%) has been removed completely. In this suspended solids, ammonia all those present in recycle stream and fumes of carbon dioxide, nitrogen comes out in the vent. In the recycle stream, 25% of water comes out from clarification. The influent and effluent concentration is measured in Table 2 for Ammonia, Nitrate, Nitrogen and Water.

Components	Influent	Effluent
Ammonia	0.01986	0.000001
Nitrate	0.00248	0.000303
Water	939.14536	993.99899
Nitrogen	-	0.000419

Table 2: Concentration of main components (g/l)

In flow splitting (P-10/FSP-101), 25% of water is passed into splitting among which, 95% supplied for recycling and 5% remained in the sludge. In mixing (P-11/MX-102), the stream S-114 water content is less and sludge is higher. From the thickening recycled water is used for mixing to avoid clogging. In Aerobic Bio-oxidation (P-8/AB-103), along with sludge, water is also added. There may be presence of suspended solids in the waste. In the vent, carbon dioxide and nitrogen is removed. In Thickening (P-6/TH-101), 50% of water is present in the sludge and another 50% sent to mixing stream RAS 2 to reduce clogging. In Flow splitting (P-12/FSP-102) the stream S-120 only 0.5% of moisture is present and remaining is sent into mixing for recycling through stream S-122. In Belt filtration (P-9/BF-101) process, cake has been formed and the air is used for agitation to make sludge into powdered form. In the activated sludge process, all the nutrients is used as manure for the field. The overall component balance for all components measured is mentioned in Table 3 in terms of Kg/batch.

Table 3: Overall component balance (Kg/batch)

Component	Initial	Input	Output	Final	In-Out
Ammonia	0.00	0.04	0.00	0.00	0.04
Carbon Dioxide	0.00	0.10	0.19	0.00	0.09
Domestic Waste	0.00	0.20	0.00	0.00	0.20
FSS	0.00	0.12	0.12	0.00	0.00
Nitrate	0.00	0.01	0.07	0.00	0.07
Nitrogen	0.00	8.48	8.48	0.00	0.00
Oxygen	0.00	2.57	2.53	0.00	0.04
TDS	0.00	0.58	0.58	0.00	0.00
Water	0.00	1,891.50	1,891.53	0.00	0.03
X-Vss-h	0.00	0.10	0.12	0.00	0.02
X-Vss-i	0.00	0.10	0.10	0.00	0.01
X-Vss-n	0.00	0.01	0.00	0.00	0.00
TOTAL	0.00	1,903.80	1,903.73	0.00	0.07
Overall Error : 0.004%					

CONCLUSION:

Residence time in aerobic basin can be extended for more than 6 hours. By-product from sludge effluent like methane gas can be used as a source to run the entire system. Nitrifying bacteria like Nitrosomonas can also improve nitrate production which in turn enhances nitrogen removal efficiency.

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**A REVIEW ON EXTRACTION OF PHYTOCHEMICALS SUCH AS
TANNINS, PHENOLS, TERPENOIDS, CAROTENOIDS AND
ALKALOIDS FROM PLANTS**

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Abstract: *Phytochemicals are the natural compounds produced by plants through primary or secondary metabolism. The phytochemicals exhibit biological activity as a defensive agent and has a role in plant growth. The phytochemicals have been used as traditional medicine, for example salicin extracted from the bark of the white willow tree has been used as pain reliever. However, phytochemicals have also been used as poison, for example tropane alkaloids from A. belladonna have been used as poison by early humans. Nowadays researches are carried out on the use of phytochemicals as antibiotics as microorganism had become resistant to antibiotics. Thus this review article discusses the extraction of such phytochemicals from plants. Extraction of phytochemicals such as tannins, phenols, terpenoids, carotenoids and alkaloids were discussed in this review article.*

Key Words: *Antibiotics, Extraction, Defensive Agent, Phytochemicals and Traditional Medicine.*

INTRODUCTION:

Phytochemicals are the natural chemical compounds produced by plants. Plants produce these chemical compounds through primary or secondary metabolism [1]. The phytochemicals play a role in plant growth and is defensive against pathogens [1]. General phytochemical present in plants are alkaloids, glucosinolates, carotenoids, lectins, polyphenols, albumin compounds, polyacetylenes, terpenes, polysaccharides and betalains [2]. Phytochemicals produced by plants are used in pharmaceutical industry to treat chronic degenerative diseases like diabetics and cancer. Taxol a phytochemical belonging to taxoid family was known for its anti-tumour activity in breasts, lungs and prostate [3]. Phytochemicals such as carotenoids, terpenoids, steroids and phenolic compounds are found to reduce the risk of cancer [4]. Curcumin is a phytochemical derived from *Curcuma* species is used traditionally for their anti-inflammatory and anti-oxidative properties against psoriasis and atherosclerosis [5]. (6)-Gingerol, a phytochemical extracted from the root of the *Zingiber officinale* known for significant inhibition of skin papilloma formation in mice [6]. Capcaisin is a phytochemical used as a carcinogen [7]. Genistein an isoflavnone derived from soyabean is used in dietary supplements of osteoporosis, cardiovascular disease, and cancers [8, 9]. Resveratrol, a common phytochemical present in grapes, peanuts and mulberries are known for its inhibition in initiation, formation and progression of skin tumour [10, 11]. The phytochemicals, bixin and norbixin from *Bixa orellana* seeds, capsanthin and capsorubin from *Capsicum annum* fruit, crocin and crocetin from *Crocus sativus* petals are used as colouring agent and phytochemicals such as carotenes from carrot root and palm oil, lutein and zeaxanthin from marigold petals and lycopene from tomato fruit are used in dietary supplement [13]. The phytochemicals present in plants have been used in many aspects. Phytochemicals are extracted in specific solvents based on their chemical nature. Isolation of phytochemicals are required as they are more efficient in its pure form. Thus this review article describes extraction of general phytochemicals such as tannins, phenols, terpenoids, carotenoids and alkaloids.

EXTRACTION OF TANNINS:

Ishak & Elgailani has extracted tannins from *Acacia nilotica*, *Acacia seyal* and *Acacia senegal* using water, 70% acetone and 80% methanol as solvent. Among these solvents 70% acetone was proved to be the best solvent for extraction of tannins [14]. Hagerman has extracted tannin from leaves with aqueous acetone and acidic methanol. A large amount of tannin was extracted with aqueous acetone and the amount yielded was dependent on maturity of leaf and the method by which it was preserved [15]. Mailloa et al has extracted tannins from Guava leaves with solvents

such as ethanol and acetone at three different concentration 30, 50 and 70% among which 30% yielded a large quantity of tannins ^[16]. Barros et al has extracted tannins from sorghum brans with solvents such as 50 and 70% ethanol, aqueous acetone and acidic methanol but ethanol was proven to be efficient solvent ^[17].

EXTRACTION OF PHENOLS:

Generally phenols are extracted with solvents such as water, acetone, ethyl acetate and alcohols. Khoddami et al has extracted phenols from different plants. A high quantity of phenols was extracted from sorghum leaf with water, while in wheat bran with 80% aqueous ethanol, in *Potentilla atrosanguinea* with 50% aqueous ethanol, in *Vitis vinifera* with pure methanol and in sunflower meal with 80% aqueous acetone ^[18]. Li et al has extracted phenols from citrus peels of Yen Ben lemon, Meyer lemon, grapefruit, mandarin and orange with ethanol and aqueous solvent. A high amount of phenols was extracted with ethanol as solvent at a temperature of 80 °C ^[19]. Fatiha et al has extracted phenols from *Mentha spicata* with pure solvents of methanol, ethanol and acetone and with 50 and 70% of their aqueous mixture. 50% of aqueous ethanol has yielded a large amount of phenols ^[20].

EXTRACTION OF TERPENOIDS:

Terpenoids are of polar and non-polar in nature. The non-polar terpenoids may be volatile or non-volatile based on number of carbon atoms it possess. A terpene compound with 15 carbon atoms or less is volatile. Jiang et al had stated that the non-volatile terpenoids can be extracted with a mixture of non-polar organic solvents such as hexane and ethyl acetate in the ratio of 85:15 (v/v) respectively while volatile terpenoids can be extracted by molecular trapping techniques where organic solvents such as hexane or mixture of hexane and ethyl acetate are used to extract the trapped terpenoid molecule. Whereas polar terpenoids cannot be extracted with non-polar solvent, hexane due to their high polarity. Solvent such as methanol was used to extract polar terpenoids ^[21]. Harman-Ware et al has extracted terpenoids with mixture of solvents such as hexane (H), hexane:acetone, hexane:diethyl ether and hexane:ethyl acetate in the ratio of 1:1 (v/v) and concluded that hexane:acetone yielded a large amount of terpenoids ^[22].

EXTRACTION OF CAROTENOIDS:

Generally solvents such as hexane, diethyl ether, dichloromethane and chloroform are used to extract carotenoids. In recent days solvents such as methanol, hexane and acetone are used to extract carotenoids on the basis of environmental and safety issues ^[23, 24]. Rebecca et al has extracted carotenoids from different vegetables such as carrot, red capsicum, yellow capsicum, beetroot and broccoli with mixture of solvent, hexane and acetone in the ratio of 1:1 with addition of acetone in regular intervals. The extracted carotenoids were separated by addition of mixture of equal volume of distilled water and 10% NaCl solution where carotenoid gets separated into upper layer ^[25]. Zaghdoudi et al has extracted carotenoids from kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and apricot (*Prunus armeniaca* L.) with mixture of solvents methanol and tetrahydrofuran. A large amount of carotenoid was extracted when methanol and tetrahydrofuran was used in the ratio of 20:80 (v/v) ^[26].

EXTRACTION OF ALKALOIDS:

Alkaloids can be extracted using surfactant as an extracting agent. In general the materials containing alkaloids are acidified with dilute acids such as sulphuric acid, hydrochloric acid or acetic acid and extracted with water. As extraction of a plant material yields alkaloids together with other plant components such as sugar, starch, tannins, pigments and proteins an additional extraction step with polar solvent was required. Polar solvents such as chloroform, diethyl ether and isopropyl ether have been used among which chloroform has been proved to be the best solvent. Djilani et al has extracted alkaloids from *Hyoscyamus muticus*, *Datura stramonium* and *Ruta graveolens* where Mayer's reagent had been used to precipitate alkaloids. The precipitated alkaloid was acidified (pH 3-4) with sulphuric acid and then basified (pH 9-10) with sodium carbonate solution and further lipophilic, acidic and neutral materials have been removed by extracting with petroleum ether and diethyl ether and final extraction of alkaloids was done with chloroform ^[27].

CONCLUSION:

This review article described the solvents for extraction of phytochemicals such as tannins, phenols, terpenoids, carotenoids and alkaloids. Tannins are extracted with acetone and ethanol, phenols are extracted with aqueous ethanol, terpenoids are extracted with mixture of hexane and ethyl acetate, carotenoids are extracted with mixture of hexane and acetone and alkaloids are extracted with chloroform. Yield of phytochemicals depends on the solvent with which it is extracted.

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UPDATES ON MODEL PREDICTIVE CONTROL OF INDUSTRIAL ALCOHOL FERMENTATION

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Abstract: Ethanol fermentation has known to be a process of great commercial importance in the food and fuel industries. Several different optimization techniques have been employed till date in order to maximize the process yield. Yet there still remains a lot of scope in this area to establish a proper consensus on the kind of control processes that does the job under the given conditions. This is largely due to the dynamical nature of the biological systems involved in the process and also the nature of the field requiring expertise from multiple areas of specialization like Microbiology, Chemical and biochemical engineering, Control systems and Statistical optimization. This article which reviews the nature of the use of Model Predictive Control as a Control Scheme will also briefly discuss the emerging importance of Machine Learning and stochastic population based optimization techniques in contrast to classical numerical modeling and is intended to combine the needed expertise to form a set of rationale to abide by, in establishing control over these processes.

Key Words: Ethanol fermentation, Statistical optimization, Process control, Model predictive control, Neural Networks, Genetic Algorithm.

INTRODUCTION:

Ethanol fermentation is known to be one of the well-studied and most valuable of processes in the bioprocess industries. Many bacteria and yeast are known to produce ethanol as a major fermentation product from carbohydrates and other sources [1]. Though *Zymomonas mobilis* produces and yields ethanol from glucose and sucrose, *Saccharomyces cerevisiae* along with other yeast is the main species used in current ethanol fermentation due to its properties such as low pH and high ethanol tolerance. [2]. *S. cerevisiae*, *Z. mobilis*, and *E. coli* have been extensively studied for improved ethanol fermentation through metabolic engineering [3-7]. Apart from these methods for maximizing ethanol production, maintaining an optimum environment through control of process variables is an area of active research. Though biological processes are complex and dynamical, a few parameters have been found to have a significant effect on these processes. pH, temperature and dissolved oxygen are the most widely measured and controlled parameters [8]. In production, only significant parameters are usually measured to maintain quality, yield and control of process parameters; however, in practice, less of suitable sensors and tools for online monitoring made this idea impossible to be widely implemented [9]. Advanced control methods like Adaptive control, Hierarchical control, intelligent control, Robust control have been effectively employed for this type of industry. However, recently only model-based control strategies have been implemented for biological processes. The ensemble of more than one type of structure in a mixture form was indicated to perform well for bioprocess control applications [10]. The major control schemes used in the bioprocess industries till 2006, have been briefly reviewed by Zulkaflee et.al [11]. This paper is intended to advance the scope of the review to contemporary techniques employed, in particular to techniques used for modeling from Artificial Intelligence and Evolutionary computation and thereby present a set of policies that could be adopted depending upon the type and constraints imposed by these processes.

An introduction to various Control Schemes used in Bioprocess Industries:

Adaptive control is the class of control methods used by a controller in a system where parameters may vary, or are initially irrelevant. Adaptive control can be contrasted from robust control through the fact that it does not require a prior information about the bounds over these time-varying or uncertain parameters; robust control can perform effectively in conditions where the bounds are well within the defined model, whereas Adaptive control's function is to find these models to suit the widely differing constraints. Optimal control generally refers to open-loop control, while

MPC usually employs a closed-loop control. In both these methods, a model is used to predict the behavior of the system. In optimal control a sequence of input signals that drive this system is initially computed. When this is applied to a real system, there is usually a deviation between the predicted and actual behavior due to system-model mismatch and other disturbances. We either have to account for this deviation or combine optimal control with a closed-loop method. In MPC, the correction is built in, because we re-compute the optimal sequence after periodically resetting the initial conditions in the MPC problem to the true state of the system. Zulkeflee et.al [11] did a detailed review of the most effective control schemes used in bioprocess industries such as Predictive Control [12-16], Neural Network [17-20], Adaptive Control [21-26] and Fuzzy Control [27- 31] as summarized in the Table 1. Even though there are different kinds of techniques described in control theory, they all contain two essential stages as inferred from the above studies: A modeling and a control stage. The main differences in the above studies exist in the modeling stage where different modeling techniques are used under different cases. The main reason for the implementation of different techniques could be the specific nature of the technique suiting the process or the increasing amount of techniques that have become available for use recently. The former can be eliminated from our scope considering the near par capabilities of all the techniques varying only to a little extent while the latter will be the main focus of this article.

Model predictive control

Model predictive control (MPC) algorithms have been widely employed in industrial processes in the recent years. They are more sophisticated than their earlier counterparts in Proportional Integral Derivative Controller (PID) and Linear Quadratic Regulator (LQR) by having predictive and repetitive time slot-based optimization capabilities respectively. Model Predictive Control (MPC) utilises the process dynamic model, history of the control and cost function optimization to calculate the optimum control moves for multivariate control. [33] MPC is based on iterative, finite-horizon optimization of an industrial model. At time 't' the present plant state is sampled and a cost minimizing control criteria is assumed (via a numerical minimization algorithm) for a comparatively short time horizon in the after-time: $[t, t+T]$.

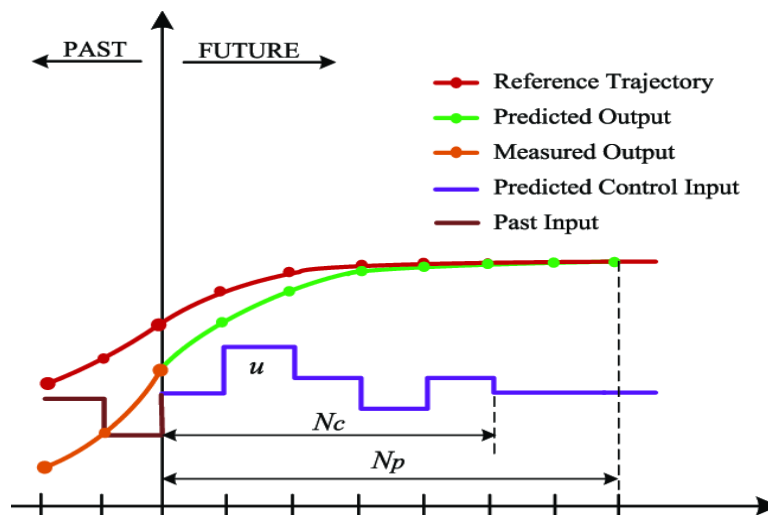


Figure 1: Model Predictive Control Visual Representation [35] where u is the discrete manipulated variable; N_c and N_p are the control and predictive horizons respectively.

A few MPC implementations were discussed in Zulkeflee et. al [11] have been performed well under different conditions of non-linear and dynamic operating regimes [12-15]. Campello et. al. [16] and Alvarez, L et al, [36] reported that combination of MPC with fuzzy model was found to be robust in its operation even in the presence of strong disturbances. ŁawryńCzuk (2007) briefly discussed two MPC-NN combinations with each of their advantages and disadvantages. Also, the implementation of a hybrid MPC algorithm with Nonlinear Prediction, Linearization and Nonlinear optimization (MPC-NPL-NO) was discussed. [37] An extensive review of Model Predictive Control can be found in the works of Zheng, T. (Ed.) and Morari, & Lee (1999) [38, 39]. A survey of commercially available packages has been provided by Qin and Badgwell (2003) [40]. In the next two sections, we will explore the role that techniques from Artificial Intelligence and Evolutionary computing can play in building robust data-based models to extend support to MPC

Artificial Intelligence in Model Predictive Control

Artificial Intelligence has become a disruptive area of active research with the growth of technologies mainly from Computer Science and hence its role in Control processes has gained more relevance than ever. The fields like Machine Learning, Deep Learning and Fuzzy Logic are capable of building Data-based models where no prior knowledge on the subject is needed. This property has made the building of models where the data is often difficult to

interpret, easier by requiring little or no domain specific knowledge. This can be exploited in the predictive modeling of the bioprocesses. Various techniques are discussed in this section.

Machine Learning

Machine learning (ML) is the scientific study of algorithms and statistical models that computer systems use to effectively perform a specific task without using explicit instructions, relying on patterns and inference instead. It is generally classified into Supervised and Unsupervised learning where the former deals with Classification and Regression problems and the latter with Clustering. Predictive Modeling requires the use of Supervised Learning, where the algorithm is allowed to learn a model from a given set of Training data and test its effectiveness on newer datasets through prediction. A whole array of different algorithms exists with different background theory including Logistic Regression, Decision Trees, Random Forest, Gradient boosting, Naïve Bayes Classifier, Support Vector Machines and k-nearest neighbors, where Gradient boosting, Random Forest and Support Vector Machines are known to be better performing at the cost of greater computational time. [41] Other factors that could determine the choice of algorithm are the Size and nature of the data, the Quality and degree of correspondence of the data, the Computational time and the urgency of the task, Data Science goals and the interpretability of the algorithms.

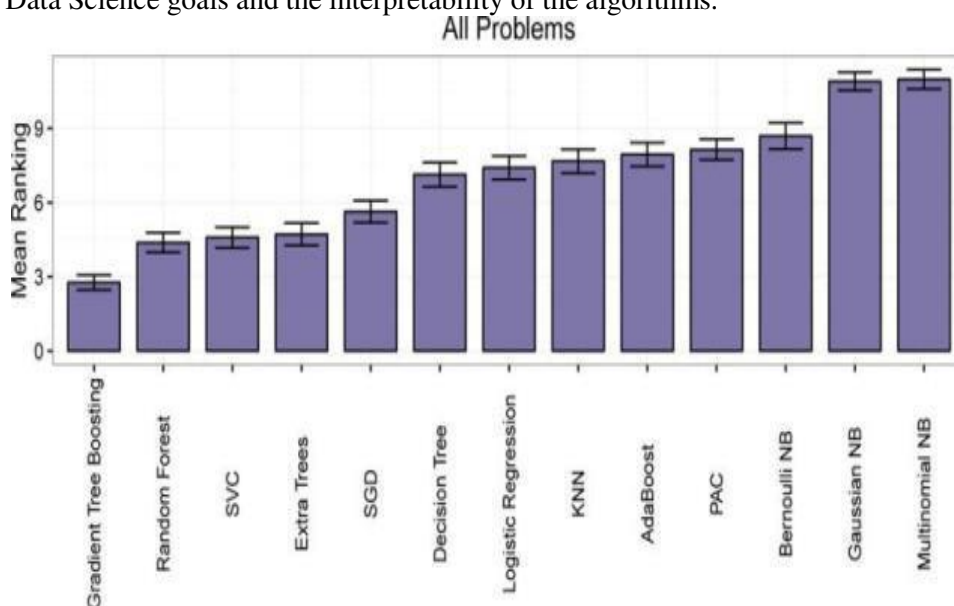


Figure 2: Average ranking of the ML algorithms over all datasets. Error bars indicate the 95% confidence interval. [41]

Deep Learning

Deep learning (also known as deep structured learning or hierarchical learning) is part of a broader family of machine learning methods based on learning data representations, as opposed to task-specific algorithms. Deep learning architectures such as deep neural networks, deep belief networks and recurrent neural networks have been known for their capabilities in finding highly complex patterns and building non-linear models from the given data. Fig. 4 is a good visual representation of the types of architectures in existence. D. R. Baughman, YLiu in their book “Neural Networks in Bioprocessing and Chemical Engineering” have offered a deeper review of the application of Deep Learning techniques to Bioprocesses [42]. Others works have found to have successfully implemented Neural Networks to their models. [43]

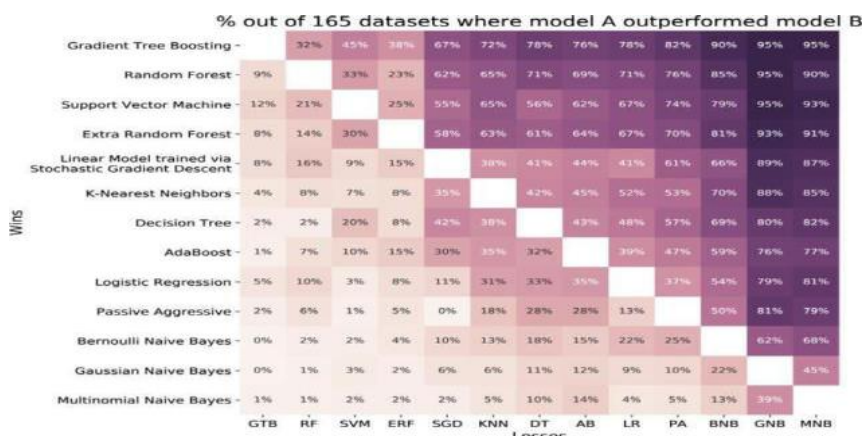


Figure 3: Heat map showing the percentage out of 165 datasets a given algorithm outperforms another algorithm in terms of best accuracy on a problem.

Fig. 3. algorithms are ordered from top to bottom based on their overall performance on all problems. Two algorithms are considered to have the same performance on a problem if they achieved accuracy within 1% of each other. [41]

Fuzzy Logic:

Fuzzy logic is a form of many-valued logic in which the truth values of variables may be any real number between 0 and 1 inclusive. It is employed to handle the concept of partial truth, where the truth value may range between completely true and completely false. By contrast, in Boolean logic, the truth values of variables may only be the integer values 0 or 1. Steps to be followed during the implementation are:

- Fuzzify all input values into fuzzy membership functions.
- Execute all applicable rules in the rule base to compute the fuzzy output functions.
- De-fuzzify the fuzzy output functions to get "crisp" output values.
- Fuzzy Control in bioprocesses is well studied [27-29] and few implementations are listed in the Table 1. [30, 31]

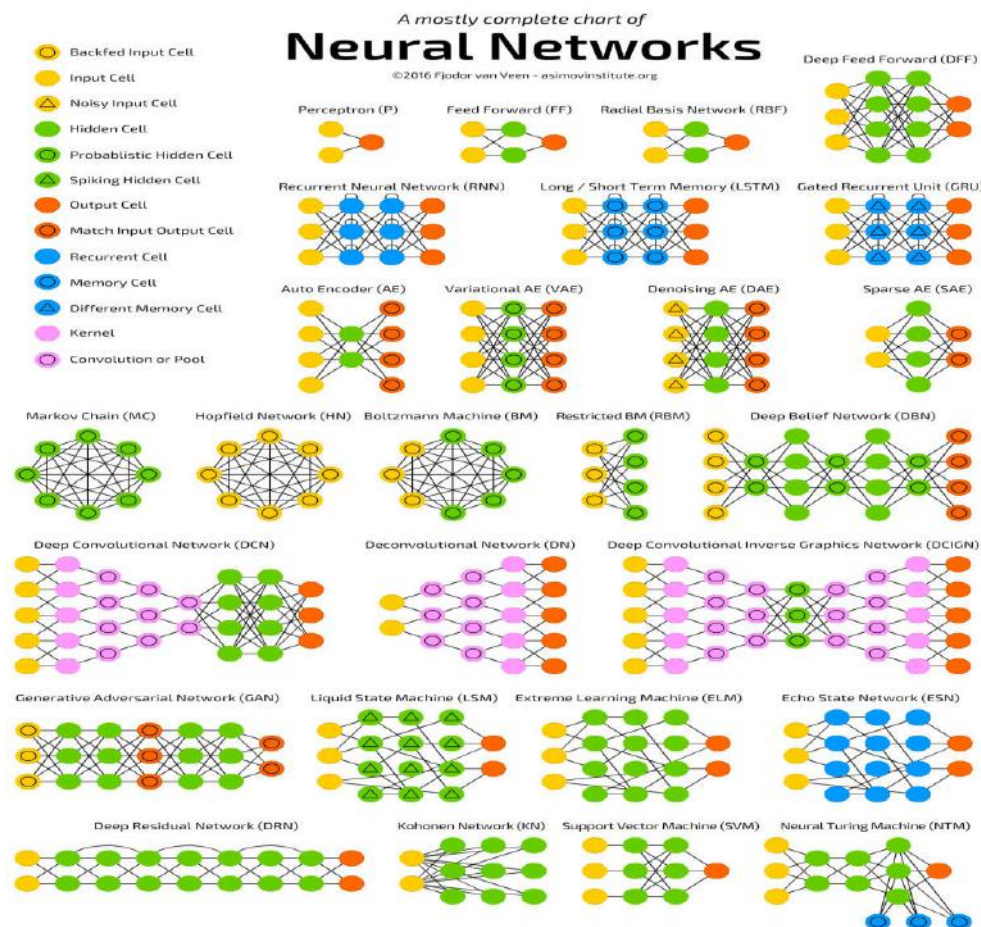


Figure 4: Visual Representation of various Neural Network Architectures [44]

Stochastic Nature Inspired Optimization techniques:

This includes the most recent growths in the field with a set of techniques that intersect with Evolutionary computation and derives inspiration from nature and follows a set of heuristics over a population in the search through a random solution space. Genetic Algorithm and Programming, Particle Swarm, Ant Colony, Bee hive, Grey Wolf, Cuckoo Search, Bat echolocation, Firefly, Fish Swarm and Wave based Optimization algorithms are a few popular brand of these techniques. Of all these algorithms, Genetic algorithm is the most popular and widely applied algorithm since its inception in 1989 and is discussed here.

Genetic Algorithm

A genetic algorithm (GA) is a metaheuristic inspired by the process of natural selection that belongs to the larger class of evolutionary algorithms (EA). Genetic algorithms are commonly used to generate high-quality solutions to optimization and search problems by relying on bio-inspired operators such as mutation, crossover and selection. An Introduction to Genetic Algorithms by Mitchell Melanie is a good starting point to understand the nature of these algorithms. [45] A generic flowchart of the algorithm can be found in Fig. 5. The paper by L.J. Park et. al., [46] explains

the application of a genetic algorithm (GA) to the problem of estimating parameters for a kinetic model of a biologically reacting system. It was demonstrated that the GA is a powerful tool for quantifying the kinetic parameters using kinetic data. As the operation of the GA does not depend on the form of the model equation, it can be applied to the wide spectrum of kinetic modeling problems without any complex formulation procedure.

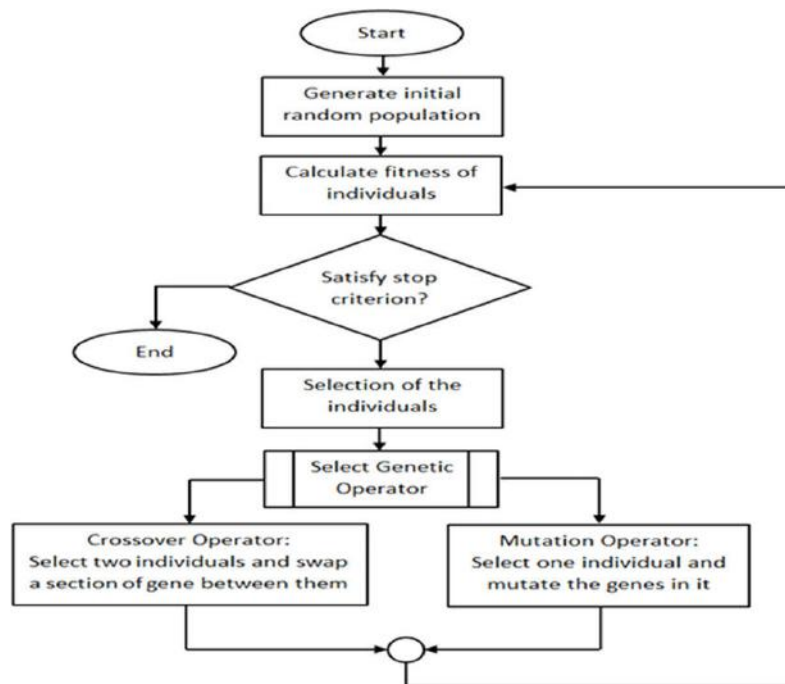


Figure 5: A generic flowchart of the Genetic Algorithms [47]

CONCLUSION:

This article emphasized the growing importance and potential of predictive modelling techniques like Machine Learning and Deep Learning, Fuzzy Logic and Evolutionary Computation to Model Predictive Control of bioprocesses. This was intended to gather and combine the efforts of many from various fields to provide the range of concepts needed, so that appropriate policy can be adopted for specific Ethanol fermentation process based on their constraints.

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