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## *From Editor's Desk*



The Oil Technologists' Association of India had chosen the city of Taj as the venue of its 69<sup>th</sup> Annual Convention. At the backdrop of picturesque Taj Mahal, in Hotel Raddison Blue, the bigwigs of the Oils and Fats fraternity in India and abroad gathered together during November 14-16, 2014 to discuss on various issues related to this particular area of science. The major event was the International Conference on Sustainable Technologies and Futuristic Trends: Oils Processing and Surfactants & Expo 2014. This year the primary thrust was given to mustard oil. The major problems faced by the mustard oil industry, particularly in the areas of extraction efficiency, contaminations of undesirable products and the production of crops were discussed at length and the possible road map was drawn. Experts from industries threw light on emerging processing technologies for the production of nutritionally rich edible oils. In the new era of food safety and nutrition, a manufacturer cannot afford to stick to the age old process technologies. The importance of instrumentation and process automation in improving yield and quality of the product was also discussed. The role of enzymes in various stages of oil processing and fat modifications was also described. In recent times, sustainability model for industry is a burning topic for any oils and fats forum and deliberations were made by different academicians and industry managers. It was decided to look forward to greener options without compromising the quality of the product. A large number of research papers and posters were presented by the research students coming from various parts of the country. All the young brains were bubbling with enthusiasm and looked to be very serious in their attempts to negotiate the nuances of oil technology for a better future. The abstracts of all the research papers are printed once again in this issue for the benefit of our members and readers.

On behalf of the CEC Members and on my personal behalf, I congratulate all the OTAI Award Winners of this year.

Happy reading!!



(PRADOSH PRASAD CHAKRABARTI)

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### ADVERTISEMENTS / ANNOUNCEMENTS

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# A Simple Process for the Enzymatic Synthesis of Phytosterol Esters of Alpha-Linolenic Acid

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## ABSTRACT

In the present study phytosterol esters of alpha-linolenic acid were prepared using immobilized *Rhizomucor miehei* lipase. Linseed oil (LSO) was saponified using potassium hydroxide (KOH) to obtain linseed oil fatty acids (LSOFA). The LSOFA was fractionated using urea adduct method at room temperature to increase the ALA content (70-75%). Phytosterol (PS) mixture (80-85%) was prepared from rice bran oil deodorizer distillate (RBO DOD). The phytosterol composition of the crystallized product was  $\beta$ -sitosterol (38.2%), stigmasterol (34.9%) and campesterol (9.5%) and others (17.4%). The standardized conditions for the preparation of phytosterol ester of ALA was substrate ratio (1:1 for ALA:PS), enzyme concentration (10%), incubation period (24 hrs) and temperature (50°C). The fourier transform infrared spectroscopy (FT-IR) analysis of phytosterol ester of ALA showed a sharp peak at 1740  $\text{cm}^{-1}$  indicating the formation of an ester bond. The hydroxyl and carboxyl carbonyl group that has the absorption at 3300-3600  $\text{cm}^{-1}$  and 1680-1800  $\text{cm}^{-1}$  for phytosterol and FFA were absent in the phytosterol esters of ALA.

**KEYWORDS:** *Alpha-linolenic acid, linseed oil fatty acids, phytosterols from RBO DOD, rhizomucor miehei, phytosterol esters of ALA.*

## INTRODUCTION

Plant sterols or phytosterols that are structurally similar to cholesterol, regulate membrane fluidity and other physiological functions in plants<sup>1</sup>. Nearly 250 different types of phytosterols are present, and the most common phytosterol present in the edible vegetable oils are  $\beta$ -sitosterol, campesterol and stigmasterol. They are present either in free or esterified form in the oil, and they constitute a significant part in the unsaponifiable matter. The phytosterols are esterified to fatty acids to improve their solubility and incorporation

into food products<sup>2-3</sup>. Among the phytosterol esters, phytosterol esters of omega 3 fatty acids are gaining importance, because of their combined hypolipidemic and hypocholesterolemic activity. The combined hypolipidemic activity of phytosterol esters of fish oil was well documented<sup>4-6</sup>. Fish oil esters of plant sterols differ from phytosterols esters derived from vegetable oils in lowering triglyceride level<sup>7</sup>. Avery Sengupta and Mahua Ghosh studied the hypolipidemic effect of phytosterol esters of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and phytosterol esters of alpha linolenic acid (ALA)<sup>8-9</sup>.

Lipase mediated esterification reaction requires absence of moisture and a non-polar solvent. Avery Sengupta et al<sup>10</sup> prepared fish oil and linseed oil fatty acid phytosterol esters using immobilized *Thermomyces lanuginosus*. Yuji Shimada et al<sup>11</sup> used polyunsaturated fatty acids (PUFA) such as DHA, EPA, gamma-linolenic acid and linoleic acid for the preparation of phytosterol esters of (PUFA). Xinxin Pan<sup>12</sup> and his co-workers used four different lipase enzymes such as *Candida antarctica*, Lipozyme from *Thermomyces lanuginosus* (TLIM), Lipozyme from *Rhizomucor miehei* (RMIM) and *Candida* sp for the preparation of phytosterol esters of oleic acid. Nikolaus Weber<sup>13</sup> and his colleagues used *Candida rugosa* enzyme for the preparation of sterol esters with long chain fatty acids. Marie Jonzo<sup>14</sup> and his colleagues immobilized *Candida rugosa* for the preparation of cholesterol oleate.

In all the above studies commercially available phytosterols were used, but in the current study phytosterol mixtures prepared from Indian rice bran oil deodorizer distillate (RBO DOD) was used. The primary objective of the study was to prepare phytosterol esters of alpha linolenic acid using immobilized *Rhizomucor miehei* lipase. Phytosterol obtained from Indian RBO DOD, and ALA obtained from linseed oil (LSO) was used for the preparation of phytosterol esters of ALA. In this study optimization of various parameters such as substrate ratio (ALA:PS), enzyme concentration,

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incubation period, temperature were performed. The retention of enzyme activity after repetitive use was also checked.

## MATERIALS AND METHODS

### Materials

A P Solvex Private Limited, Dhuri, Punjab supplied RBO DOD. Mixed fatty acid methyl ester standard,  $\beta$ -sitosterol, campesterol, stigmasterol (phytosterol standards) and  $\alpha$ -tocopherol standard, were purchased from Sigma Chemical Co. (St Louis, USA). Novozymes (Bengaluru) supplied immobilized *Rhizomucor miehei* lipase enzyme. From the local market in Mysore, linseed was purchased. Solvents and other chemicals used were of analytical grade.

### Methods

*Preparation of LSOFA:* Soxhlet apparatus was used to extract LSO in the laboratory. To LSO (100g), Potassium hydroxide (KOH) (24 g) dissolved in water (24 mL) was added. At 250 rpm for 30 min the mixture was stirred with a mechanical stirrer, followed by incubation at 37°C for 48hrs. After the incubation period, the soap was dissolved in alcohol, deacidified with 2N hydrochloric acid (HCl) and the LSOFA was recovered. Ethanol (10%) solution was used to wash the LSOFA to make it free of acid. The hexane fraction was desolventized in vacuum flash evaporator to obtain LSOFA.

*Physicochemical characteristics:* Free fatty acid value (FFA) and peroxide value (PV) of the LSOFA were determined according to the procedure of AOCS official method<sup>15</sup>.

*Determination of fatty acid composition:* According to the procedure of Van-Wijngaarden<sup>16</sup> the fatty acid compositions of the samples were determined. The fatty acids were identified using standard fatty acid methyl esters (FAME) mixture. The following conditions were used to operate the gas chromatography (GC), column oven temperature was 120°C (initial) and raised 5°C/minute and finally held at 220°C for 10 min. The detector and injector temperature was 250°C and 230°C respectively. GC (model GC-2010, Shimadzu Corporation, Kyoto, Japan) and Rtx-1 fused silica column having length (30m), internal diameter (0.32 mm ID) was used.

*Urea fractionation of LSOFA:* The obtained LSOFA were urea fractionated to increase the ALA content. On a boiling water bath urea (40 g) and ethyl alcohol (320 mL) was heated till the urea dissolved completely. Followed by, LSOFA (10g) was added, and the samples were incubated at three different temperatures RT, 5°C, and -20°C for overnight crystallization. After the incubation period, under vacuum ethanol was filtered

and desolventized in vacuum flash evaporator to get the ALA enriched fraction. The fatty acid composition of the ALA enriched fraction was determined according to the procedure of Van-Wijngaarden<sup>16</sup>.

*Preparation of phytosterol mixture:* The procedure described by Sakina Khatoon<sup>17</sup> and her co-workers was used to prepare phytosterol mixture from RBO DOD. The phytosterol composition of the prepared mixture was determined according to the procedure of Holen<sup>18</sup>. High performance liquid chromatography (HPLC) was used (model LC-10AVP, Shimadzu Corporation, Kyoto, Japan) with a kromasil C-8 5 $\mu$ m column (250 mm X 4.6 mm id, supelco) and UV detector set at 206nm. The mobile phase was methanol and water (99:1.0; v/v) and, chloroform was used to dissolve the samples. Standard sterols such as  $\beta$ -sitosterol, stigmasterol and campesterol were used to identify and quantify sterols present in the sample.

*Effect of reaction conditions on the preparation of phytosterol esters of ALA:* For the preparation of phytosterol esters of ALA, phytosterols (80-85%) and ALA (70-75%) obtained from RBO DOD and LSO. The esterification reaction was carried out in Julabo water bath (Model No-SW22) and RPM (100) was maintained throughout the study. Ethyl acetate was used to bring fluidity to the reaction.

*Effect of substrate ratio (ALA:PS and PS:ALA):* To optimize the ratio of ALA:PS, ALA was taken in the increasing concentrations of 1:1, 2:1, 3:1 and 4:1. Similarly PS were made in the increasing concentrations of 1:1, 2:1, 3:1 and 4:1 to optimize the ratio of PS:ALA. The other reaction parameters such as enzyme concentration (10%), incubation period (24 h), and temperature (60°C), were kept constant.

*Effect of incubation period:* The effect of incubation period was analyzed by incubating the samples for different time intervals such as 6, 12, 18 and 24 h. The other reaction parameters such as ALA:PS ratio (1:1), temperature (60°C), and enzyme concentration (10%) were maintained the same.

*Effect of enzyme concentration:* With increasing concentrations of 2, 4, 6, 8, 10, and 12%, the immobilized *Rhizomucor Miehei* lipase enzyme was taken. The other parameters such as ALA:PS ratio (1:1), temperature (60°C), and incubation period (24 h) were kept constant.

*Effect of temperature:* The effect of temperature was optimized by incubating the samples at different temperatures such as 40°C, 45°C, 50°C, 55°C and 60°C respectively. The ALA:PS ratio (1:1), incubation period (24 h), and enzyme concentration (10%) were kept the same.

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*Effect of repetitive use of enzyme on esterification yield:* The substrates remained semisolid when mixed, and in order to dissolve the substrates ethyl acetate was used. The esterification reaction was performed under the following conditions, ALA:PS ratio (1:1) incubation period (24 h), enzyme concentration (10%) and temperature (50°C). The same enzyme was reused for 11 times under similar conditions as mentioned above, to find out the effect of repetitive use of enzyme on esterification yield.

*Separation of free and esterified sterols:* After the esterification reaction, the presence of excess FFA was removed by alkali treatment. The silica gel column chromatography method was used to separate phytosterol esters of ALA and free sterols (FS) present in the sample<sup>19</sup>. In chloroform, the sample was dissolved, and the first solvent system consists of hexane:ethyl acetate (85:15) to elute phytosterol esters of ALA. The second solvent system consists of diethyl ether:hexane:ethyl alcohol (25:25:50) to elute FS present in the column. The collected fractions were desolventized to get the respective extracts.

*Thin layer chromatography (TLC):* The TLC plates were activated by drying in the oven at 105°C for 2 h and transferred to the desiccator. On the TLC plate, the samples were spotted in the following sequence, groundnut oil (GNO), ALA, phytosterols, mixture of ALA and phytosterols and phytosterol ester of ALA. The developing solvent consists of hexane-diethyl ether-formic acid (80:20:2) and the lipid spots were identified by exposing to iodine vapors in the iodine chamber.

*FT-IR analysis:* FT-IR analysis was performed for the following samples, ALA, phytosterols, mixture of free and esterified sterols (after removing the FFA by alkali treatment), phytosterol esters of ALA and FS (separated by silica gel column chromatography).

*Analysis of phytosterol esters of ALA:* The phytosterol esters of ALA (5 g) was saponified and unsaponifiable matter was extracted according to the procedure of AOCS<sup>15</sup>. The obtained unsaponifiable matter was further purified according to the procedure of Sakina Khatoon<sup>17</sup> to get pure phytosterols. The composition of the obtained phytosterol mixture was determined according to the procedure of Holen<sup>18</sup>. Followed by the extraction of unsaponifiable matter, the soap was treated with hydrochloric acid (HCl 2N) to release the fatty acids. The fatty acids (dissolved in hexane) were washed with distilled water to make it free of acid. The hexane fraction was desolventized, and the fatty acid composition of the fatty acids was determined according to the procedure of Van-Wijngaarden<sup>16</sup>.

*Statistical analysis:* In order to analyze the mean standard deviations, all experiments and analysis were carried out in duplicates. Data was analyzed using the statistical program-GraphPad InStat Demo [DATASET1. ISD]. A two-tailed p value was determined to show the significant differences and an important difference value was considered when the p value  $\leq 0.05$ .

## RESULTS AND DISCUSSION

*Physico-chemical characteristics of LSOFA:* The linseed was ground nicely into a fine powder and extracted with hexane in soxhlet apparatus. Hexane was desolventized in vacuum flash evaporation to obtain LSO. The LSO was saponified using alkali treatment (KOH) to obtain LSOFA. The purity (as FFA content) and peroxide values of the LSOFA were 98.5% and 8.9 Meq O<sub>2</sub> /kg. The fatty acid composition of LSO and LSOFA did not vary significantly (Table 1).

*Urea fractionation of LSOFA:* At three different temperatures (RT, 5°C and -20°C) the urea fractionation of LSOFA were carried out. The ALA content in the ALA enriched fraction was 74.2%, 84.7%, and 84.6% respectively. The recovery of ALA was high at RT when compared with 5°C and -20°C, and that is the reason for choosing same as a source of ALA (Table 1).

*Preparation of phytosterols mixture:* The unsaponifiable matter and hexane were mixed in the ratio of 1:10 with 10% moisture and incubated at -20°C for 72 h. Followed by the incubation period the contents were filtered under vacuum to get the crude phytosterol mixture. To the crude phytosterol mixture, hexane and moisture were mixed in the same ratio and incubated at 5°C for 4 h. The contents were filtered under vacuum to get pure phytosterol mixture having 80-85% purity. HPLC analysis of the phytosterol mixture showed the following sterol composition,  $\beta$ -sitosterol (38.2%), stigmasterol (34.9%), campesterol (9.5%) and others (17.4%) (Table 2).

*Effect of substrate concentration (ALA:PS and PS:ALA) :* The schematic representation for the preparation of phytosterol ester of ALA was shown in fig 1. The esterification percentage was calculated based on the amount of FFA consumed during the reaction. The esterification percentage was 83.0, 71.5, 79.8, and 82.8%, when taken in the increasing concentrations of 1:1, 2:1, 3:1 and 4:1. The esterification percentage was same at all concentrations and did not differ significantly (Fig 2, No.1). Thus 1:1 ratio was chosen as an ideal concentration for ALA:PS.

*Effect of PS:ALA:* To find out the optimum concentration of PS:ALA, phytosterol was taken in the increasing concentrations of 1:1, 2:1, 3:1 and 4:1. The esterification percentage was 83.0, 67.2, 58.5 and 61.7%. The

**TABLE 1**  
**Fatty Acid Composition of LSO, LSOFA and ALA Enriched Fraction**

Fatty acids	LSO	LSOFA	FAC of ALA enriched fraction obtained after urea fractionation at different temperature		
			RT	5°C	-20°C
Lauric acid (C12:0)	0.1 ± 0.05 <sup>a</sup>	0.1 ± 0.05 <sup>a</sup>	0.1 ± 0.05 <sup>a</sup>	ND	ND
Myristic acid (C14:0)	0.1 ± 0.05 <sup>a</sup>	0.1 ± 0.05 <sup>a</sup>	1.1 ± 0.05 <sup>a</sup>	ND	ND
Palmitic acid (C16:0)	5.3 ± 0.3 <sup>a</sup>	4.9 ± 0.4 <sup>a</sup>	0.5 ± 0.2 <sup>c</sup>	0.4 ± 0.05 <sup>c</sup>	0.4 ± 0.03 <sup>c</sup>
Stearic acid (C18:0)	4.3 ± 0.1 <sup>a</sup>	3.1 ± 0.2 <sup>b</sup>	0.2 ± 0.01 <sup>c</sup>	0.2 ± 0.05 <sup>c</sup>	0.2 ± 0.05 <sup>c</sup>
Oleic acid (C18:1)	15.7 ± 0.4 <sup>a</sup>	14.0 ± 0.5 <sup>a</sup>	7.4 ± 0.07 <sup>c</sup>	2.0 ± 0.1 <sup>d</sup>	2.4 ± 0.1 <sup>d</sup>
Linoleic acid (C18:2)	13.7 ± 0.2 <sup>a</sup>	13.0 ± 0.3 <sup>a</sup>	14.2 ± 0.12 <sup>a</sup>	12.8 ± 0.1 <sup>a</sup>	12.3 ± 0.1 <sup>a</sup>
Linolenic acid (C18:3)	60.7 ± 0.1 <sup>a</sup>	65.0 ± 0.4 <sup>b</sup>	74.2 ± 0.9 <sup>c</sup>	84.7 ± 0.3 <sup>d</sup>	84.6 ± 0.1 <sup>d</sup>

Values given in column followed by different alphabetical superscript are significantly different at P ≤ 0.05.

LSO-Linseed oil, LSOFA-Linseed oil fatty acids, FAC-Fatty acid composition, RT-Room temperature. ND-Not Determined, LSOFA were obtained by saponification of LSO. The obtained LSOFA were urea fractionated at three different temperature (RT, 5°C and -20°C) to obtain ALA enriched fraction.

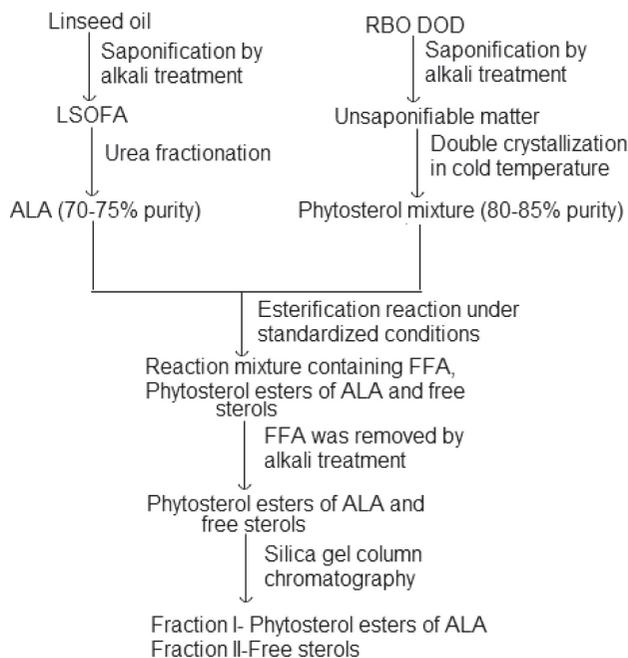
**TABLE 2**  
**Phytosterol Composition Obtained from RBO DOD**

Phytosterols	(%)
Campesterol	9.5 ± 0.07
Stigmasterol	34.9 ± 1.05
β-Sitosterol	38.2 ± 1.07

esterification percentage was high at 1:1, later the esterification percentage decreased as the ratio of phytosterol was increased. As a result the optimum ratio of PS:ALA was decided as 1:1 (Fig 2, No 2).

*Effect of incubation period:* The samples were incubated at different time intervals such as 6, 12, 18 and 24 h while keeping other reaction conditions constant. The esterification percentage was increasing with the increase in incubation period 53.8, 71.9, 81.5 and 83.6% respectively. The esterification percentage at 18 and 24 h were equivalent, still 24 h was taken as the optimum incubation period to obtain maximum esterification yield (Fig 2, No 3).

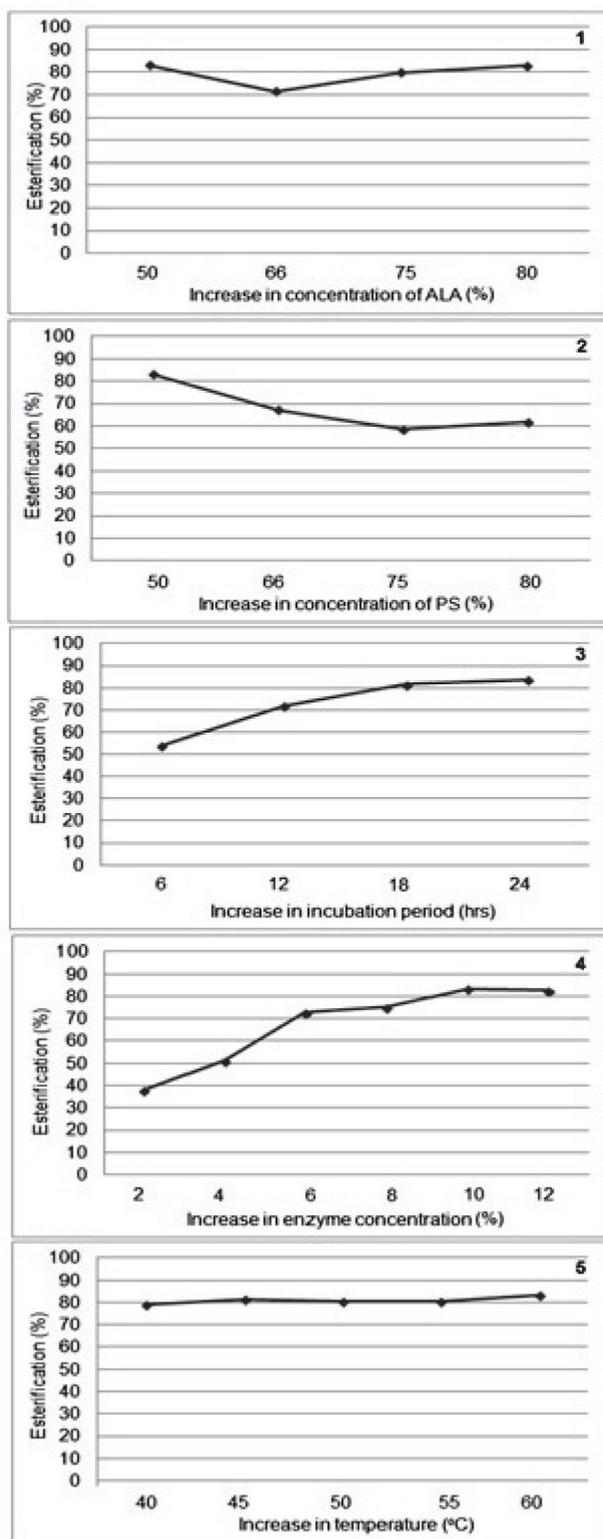
*Effect of enzyme concentration:* Immobilized *Rhizomucor miehei* lipase enzyme was taken in the increasing concentration of 2, 4, 6, 8, 10, and 12%, to find out the effect of enzyme concentration. The esterification percentage was in the increasing order of 37.9, 51.2, 72.7, 75.2, 83.9, and 82.6% respectively. At 10% and 12% enzyme concentration, the esterification percentage was comparable. Thus, 10% enzyme



**Fig. 1: Flow sheet for the preparation of phytosterol esters of ALA**

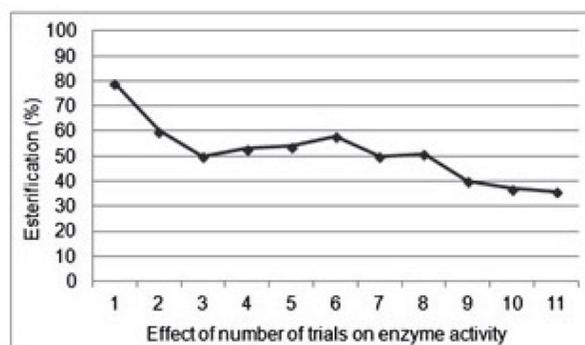
concentration was decided as the optimum enzyme concentration (Fig 2, No 4).

*Effect of temperature:* With the increase in temperature, the samples were incubated at 40°C, 45°C, 50°C, 55°C and 60°C while keeping other reaction conditions same. The esterification percentage was 79.0, 81.6, 80.6, 80.4 and 83.2%. The optimum temperature was taken as 50°C, as the esterification percentage was same at all the temperatures (Fig 2 No 5).



**Fig. 2:** Optimization of reaction conditions,  
 1.Effect of ALA:PS ratio  
 2.Effect of PS:ALA ratio,  
 3.Effect of incubation period,  
 4.Effect of enzyme concentration,  
 5.Effect of temperature

*Effect of repetitive use of enzyme on esterification yield:*  
 The effect of repetitive use of enzyme on esterification yield was studied by using the same enzyme for 11 consecutive trials under similar conditions (Fig 3). The esterification percentage was 79% in the first trial, and later the activity decreased to 50-60% from 2<sup>nd</sup> to 8<sup>th</sup> trail. The enzyme lost 24-36% of activity from 2<sup>nd</sup> to 8<sup>th</sup> trail. The esterification percentage was further reduced to 36-40% between 9<sup>th</sup> to 11<sup>th</sup> trial, and the enzyme lost further 49.3-54.4% of activity.



**Fig. 3 :** Effect of repetitive use of enzyme on esterification yield

*Thin layer chromatography (TLC):* A single spot near the solvent front at the top of the TLC plate corresponds to phytosterol esters of ALA (No 5) (Fig 4). ALA and PS showed a single spot (No 2 and 3) and the mixture of ALA and PS showed two spots (No 4). The phytosterol ester of ALA showed no band corresponding to either ALA or PS, and this reveals that the esterification had taken place.



**Fig. 4:** Thin layer chromatography 1.Groundnut oil, 2.ALA, 3.Phytosterols from RBO DOD, 4. Mixture of phytosterols and ALA, 5. Phytosterol esters of ALA.

**FT-IR analysis:** The carboxyl carbonyl group present in the fatty acids has absorption in the range 1670-1800  $\text{cm}^{-1}$  and free OH group present in phytosterols has a broad absorption in the range of 3300-3600  $\text{cm}^{-1}$ . The reaction mixture (after FFA removal) showed peak at 3300-3600  $\text{cm}^{-1}$  and 1740  $\text{cm}^{-1}$  indicating the presence of free OH group in phytosterols and formation of an ester bond. The FT-IR analysis of phytosterol esters of ALA (after separation by column chromatography) showed a sharp peak at 1740  $\text{cm}^{-1}$  indicating the formation of an ester bond. The hydroxyl group and carboxyl carbonyl group that has the absorption at 3300-3600  $\text{cm}^{-1}$  and 1670-1800  $\text{cm}^{-1}$  were absent in the phytosterol esters of ALA. These results reveal that the enzyme had carried out the esterification reaction between fatty acids and phytosterols. The FS (after separation by column chromatography) showed broad absorption in the range of 3300-3600  $\text{cm}^{-1}$  corresponding to OH group.

**Analysis of phytosterol esters of ALA:** The phytosterol and ALA used in this study consist of a mixture of different types of sterols and fatty acids. The phytosterol ester of ALA were saponified to extract the unsaponifiable matter (phytosterol will be part of unsaponifiable matter). The phytosterol composition of the phytosterol esters of ALA was  $\beta$ -sitosterol - 50.47  $\pm$  0.66, stigmasterol - 34.7  $\pm$  0.35, campesterol - 8.41  $\pm$  1.48 and the minor sterols constitutes remaining 6%. HCl (2N) was used to neutralize the soap, and the fatty acids were recovered by dissolving in hexane. The fatty acid composition of the obtained fatty acids was determined by converting into FAME. The fatty acid composition of the phytosterol esters of ALA was  $\alpha$ -Linolenic acid - 78.5  $\pm$  0.34, linoleic acid - 17.1  $\pm$  0.2, oleic acid 3.28  $\pm$  0.2, palmitic and lauric acids were present in minor quantities (less than 1%).

This is probably the first study that reports the esterification of phytosterol obtained from Indian RBO DOD. Avery Sengupta<sup>10</sup> and her team used commercially available phytosterol for the preparation of phytosterol esters of ALA. They used *Thermomyces Lanuginosus* enzyme to carry out the transesterification reaction of phytosterol with linseed oil and fish oil. They used higher temperature (170°C) under vacuum to separate the product (esterified sterol) from excess oil and free sterols. In the present study, ALA (70-75% purity) was used for esterification with phytosterol (80-85% purity) obtained from Indian RBO DOD. In the current method, the presence of excess FFA and excess sterols were removed by FFA neutralization

and simple column chromatography method without using any higher temperature or vacuum. The above discussed issues are the added advantage of the current method.

DOD is a by-product fraction collected from the deodorization process, which is a complex mixture of free fatty acids, monoacylglycerol, diacylglycerols, triacylglycerols, sterols, steryl esters, tocopherols, hydrocarbons, broken down products of fatty acids such as aldehydes, ketones, and oxidized products<sup>20</sup>. India one of the largest producer of rice bran oil, produces vast quantities of RBO DOD, which is being considered as waste and sold to soap industry, inspite of DOD being rich in nutraceuticals such as phytosterols, tocopherols, tocotrienols and squalene. The RBO DOD contains phytosterol (4-8%) and represents one of the richest sources of phytosterol available currently. As the substrates remained semisolid at room temperature, ethyl acetate was used to bring fluidity to the reaction mixture. Ethyl acetate had trace amounts of acetic acid, and acetic acid-free ethyl acetate was prepared by washing the ethyl acetate with distilled water till it is free of acid. The solvent was later dried over anhydrous sodium sulphate to remove the moisture. Molecular sieve was used to absorb moisture generated during the reaction. Molecular sieve at different concentrations such as 5, 10, 15 and 20% of the total substrate weight was used to optimize the ideal concentration of molecular sieve required. The esterification yield was same (78.5, 78.6, 80.3 and 78.9%) at all concentration, and molecular sieve (10%) was taken as the optimal concentration to absorb moisture generated during the reactions.

## CONCLUSION

In the present study phytosterol esters of alpha-linolenic acid were prepared using immobilized *Rhizomucor miehei* lipase. RBO DOD and LSOFA were used for the preparation of phytosterols (80-85% purity) and ALA (70-75% purity). The optimum substrate ratio (ALA:PS), enzyme concentration and incubation period were 1:1, 10% and 24 h respectively. The optimum temperature was decided as 50°C. India is one of the largest producers of RBO and no clear data is available regarding the amount of DOD produced every year. If utilized properly DOD can be a good source of nutraceuticals like phytosterols, tocopherols and tocotrienols.

## ACKNOWLEDGEMENT

The authors are thankful to Prof. Ram Rajasekharan, Director, CSIR-CFTRI, Mysore for his keen interest and providing the infrastructural facilities

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to carry out the work. The author R G Raja Rajan is thankful to Indian Council of Medical Research (ICMR) for the award of Senior Research Fellowship (SRF).

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# Antioxidative Role of Lutein Esters Extracted from Indian Marigold Flower on Soybean Oil during Heating

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## ABSTRACT

Soybean oil is being long used as frying oil for variety food stuffs. The oil is rich in linoleic acid, a polyunsaturated fatty acid (PUFA) which is susceptible to oxidation during heating/frying. The present study attempted to protect the oil during heating by adding a natural anti-oxidant, lutein-ester, isolated from Indian marigold flower by solvent extraction. Two sets of experiment were carried out by taking 1 L of soybean oil. In one set, 500 mL of soybean oil was heated for 24 h (8 h daily for 3 consecutive days) and in the second set, lutein ester of 0.01g/kg was added to the remaining 500 mL of soybean oil and the oil was heated for 24 h in the similar manner. Heated oil samples were collected in every 4 h and the protective effects of lutein ester were assessed by monitoring the color, viscosity, acid value, peroxide value, p-anisidine value and 4-hydroxy-2-trans-nonenal (HNE) content of the heated oil samples. The content of HNE, which can be used as an index to monitor the overall progression of PUFA towards oxidation, showed a value of  $10.09 \pm 0.1 \mu\text{M/g}$  in case of control soybean oil and value of  $1.11 \pm 0.02 \mu\text{M/g}$  in experimental soybean oil, after 24 h of heating. All the other parameters also confirmed that lutein ester can act as effective antioxidant to protect the oil during frying/heating.

**KEYWORDS:** Antioxidants, 4-hydroxy-2-trans-nonenal (HNE), Lutein Ester, Polyunsaturated fatty acid (PUFA), Soybean oil.

## INTRODUCTION

Oils and fats have key commercial role in frying of foods. During deep-frying, fats and oils are exposed at elevated temperatures in the presence of atmospheric oxygen and receive maximum oxidative and thermal damage. Heating in presence of air causes partial conversion of fats and oils to volatile chain-scission products, nonvolatile oxidized derivatives and dimeric, polymeric or cyclic substances<sup>1</sup>. Apart from this, the nutritional value of frying fats is affected by loss of

natural antioxidants and polyunsaturated fatty acids (PUFA), which supplement the essential fatty acids requirement in human metabolism.

When subjected to heating at elevated temperatures, oils and fats undergo oxidation that result in changes in physical and chemical characteristics<sup>2, 3</sup>. Some of the reactions that occur in heated oils include lipid peroxidation, hydrolysis, polymerization, cyclization and pyrolysis. The rate at which these reactions occur is dependent on time and temperature. At typical frying temperatures in the presence of air, lipid peroxidation reactions proceed very quickly to form hydroperoxides that undergo further decomposition to yield a wide variety of secondary lipid peroxidation products. Highly unsaturated oils are frequently used for frying, especially in home applications, because of availability and cost; however these oils are especially susceptible to oxidation at higher temperature because of their fatty acid composition. It has been reported that both frying and thermal oxidation of oils result in degradation of linolenic acid<sup>4</sup>.

Soybean oil (SBO) is the most important vegetable oil used throughout the world because of its high quality and low cost. SBO, because of its high PUFA content, is considered to be superior than many other vegetable oils and hydrogenated fats from a nutritional standpoint, but it is inferior in thermal stability at high temperatures. It has been reported<sup>5,6</sup> that the thermal oxidation of SBO, which contains about 58% linoleic acid and about 6% linolenic acid, results in the formation of 4-hydroxy-2-trans-nonenal (HNE), an  $\alpha$ -,  $\beta$ -unsaturated aldehyde and a secondary oxidation product of linolenic acid that has been shown to have cytotoxic and mutagenic effects<sup>7</sup>. It is also associated with the incidence of atherosclerosis<sup>8, 9</sup>; stroke; Parkinson's; Alzheimer's and Huntington's disease<sup>10-15</sup> and can damage DNA by inducing gene mutation and can alter the structure and function of cancer related proteins<sup>16</sup>. During frying these thermally oxidized products are incorporated into the fried food also by absorption. Studies have shown that the percentage of natural antioxidant, tocopherols, present in soybean oil also gets reduced on heating<sup>17</sup>.

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Lutein (C<sub>40</sub>H<sub>56</sub>O<sub>2</sub>), i.e 3,3-dihydroxy-β-carotene, is one of over 600 known naturally occurring carotenoids found in green leafy vegetables and also in many flower petals etc and is employed as an antioxidant. Lutein occurs naturally in the acylated form as lutein esters (LE), with one or two fatty acids bound to the two hydroxyl-groups and is more stable against heat and UV light<sup>18</sup>. The beneficial effects of lutein ester are mainly related to their antioxidant activity, which protects the human body from free radicals and decreases the incidence of many chronic diseases<sup>19-21</sup>. Studies have shown that in animal models, it prevents colon<sup>22, 23</sup> and breast cancer<sup>24, 25</sup>. Lutein has been shown to enhance antibody production in response to T-dependent antigens in spleen cells in vitro, as well as in mice in vivo. It is also known to enhance recovery of cells oxidative change by stimulating DNA strand break repair<sup>26</sup> and has proposed protective functions in the eye as an antioxidant and blue light filter<sup>27</sup>.

Lutein is a lipophilic molecule and is generally insoluble in water. As early as 1996, lutein has been incorporated into dietary supplements. While no recommended daily allowance currently exists for lutein as for other nutrients, positive effects have been seen at dietary intake levels of 6-10 mg/day<sup>28</sup>. Previous study reported from our laboratory established that lutein ester extracted from Indian marigold flowers possesses strong *in-vitro* antioxidant capacity<sup>29</sup>.

The present work aimed to study the influence of lutein ester (LE) extracted from Indian marigold flowers on soybean oil stability under heating at frying temperature during different exposure periods (0, 4, 8, 12, 16, 20, 24 h). Marigold flower petals are excellent sources of lutein as they contain high levels of lutein and no significant levels of other carotenoids<sup>30</sup>. Several quality parameters viz. colour, viscosity, acid value, peroxide value, p-anisidine value and HNE content were determined.

## EXPERIMENTAL

### Materials

The marigold flower samples were collected from local flower market. The petals were separated, sun-dried, powdered and stored in a refrigerator at -20° C. The fine powder of the petals of marigold flower was extracted with methanol in a Soxhlet apparatus independently. The extract was filtered and concentrated to dryness in a rotary evaporator under reduced pressure at 40°C and stored in an amber colour container at -20° C<sup>29</sup>.

Refined soybean oil, with brand name of "Fortune", manufactured by Adani Wilmar Ltd., Haldia, India was purchased from local market. 1L oil was divided

equally in two parts. In one part 5 mg of LE, extracted from marigold flower, was added. The other part of soybean oil was kept as control.

A nonstick vessel (uncovered) of 2 L capacity was used for carrying out the heating operations.

2, 4-Dinitrophenol hydrazine (DNPH) and all other chemicals used were of AR grade and procured from E-Merck India Ltd.

### Methods

*Heating of oil:* The oil with added LE (500 mL) (experimental SBO) was heated at 180-190°C (frying temperature) for 8 h daily for 3 consecutive days. Oil samples of about 50 mL were collected after every 4 h and kept in refrigerator (-20 °C) for further analysis after cooling under nitrogen atmosphere. The volume of oil was not replenished during frying operations. The same experiment was performed with control soybean oil.

Three sets of replicate studies were performed for both the control and experimental set.

*Oil Analysis:* The following oil quality parameters were determined for the oil samples collected at different times.

*Colour:* Colour was measured using the Lovibond Tintometer [AOCS official method Cc13b-45].

*Viscosity:* Viscosity was measured in centipoises using Brookfield Viscometer at 25 °C.

*Acid Value:* For determining acid value, oil sample weighing 5 g was taken in a 250 mL conical flask. Freshly neutralized 50 mL ethyl alcohol was added. The mixture was boiled for 5 min and titrated as hot as possible against N/10 NaOH solution using 1 to 2 drops of phenolphthalein indicator [AOCS official method Te 1a-64].

*Peroxide Value:* For determination of peroxide value, 2.00±0.05 g of sample was taken in a glass stoppered flask. To it, 10 mL of chloroform was added and the flask was shaken until the sample dissolved in the solution. Then 15 mL of acetic acid was added and the flask was swirled. Saturated potassium iodide solution (1mL) was added and the flask was shaken vigorously for 1min, with the stopper on. The flask was then kept in dark for 5 min and then 75 mL of distilled water was added. It was titrated against standard sodium thiosulphate solution until the yellow colour almost disappeared. Then about 0.5mL of starch indicator solution was added. Titration was continued until the blue colour just disappeared. Blank was also determined under similar conditions [AOCS official method Ja 8-87].

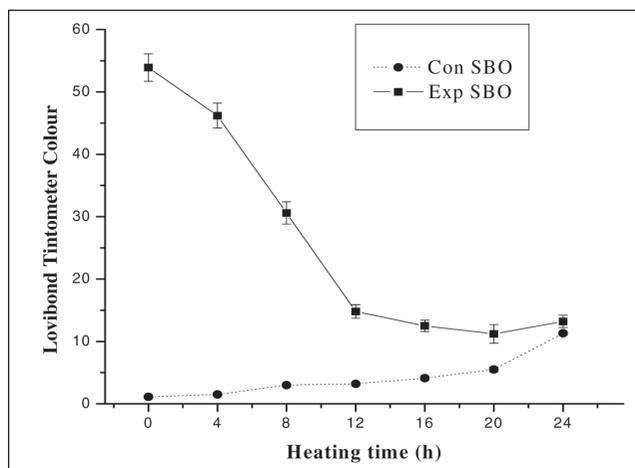
*p*-Anisidine Value: *p*-anisidine value of the heated oils was done according to the method given by AOCS. For determination of *p*-anisidine value, 0.5g of the sample was taken in a stoppered test tube and diluted to volume with iso-octane. The absorbance of the solution was measured at 350 nm in a cell with the spectrophotometer, using the reference cell filled with solvent as a blank. Then exactly 5 mL of the fat solution was pipetted out into one stoppered test tube and exactly 5 mL of the solvent into another stoppered test tube. Exactly 1mL of *p*-anisidine reagent was added to each tube and shaken vigorously with the stopper on. After exactly 10 min, the absorbance of the solution containing the oil was measured at 350 nm, using the solution from the test tube not containing the oil, as a blank in the reference cell [AOCS official method Cd 18-90].

*Estimation of HNE by DNPH derivatization*<sup>31</sup>: Standard HNE-DNPH derivative was prepared by the reaction of HNE and DNPH. An HNE–water solution was mixed with an equal volume of DNPH solution (3.5 mg of DNPH dissolved in 10 mL of 1 M HCl) and stored in the dark at room temperature for 2 h. HNE-DNPH was extracted twice from the mixture with CH<sub>2</sub>Cl<sub>2</sub> and concentrated under nitrogen. HNE-DNPH was separated from the reaction mixture by TLC using CH<sub>2</sub>Cl<sub>2</sub> as developing solvent. The HNE-DNPH band was scraped off and extracted with methanol. The extract was filtered, dried under a nitrogen stream, and redissolved in methanol. The concentration of HNE-DNPH was measured by spectrophotometer (UV-VIS Shimadzu 1700) at 370 nm and calculated by using the molar absorptivity  $\epsilon_{370\text{ nm}} = 28,000\text{ M}^{-1}\text{cm}^{-1}$ .

*Statistical Analysis*: All experiments were done in triplicate and the results were expressed as the mean value  $\pm$  standard error mean (S.E.M) of three measurements. One way ANOVA was computed using Origin software.

## RESULTS AND DISCUSSION

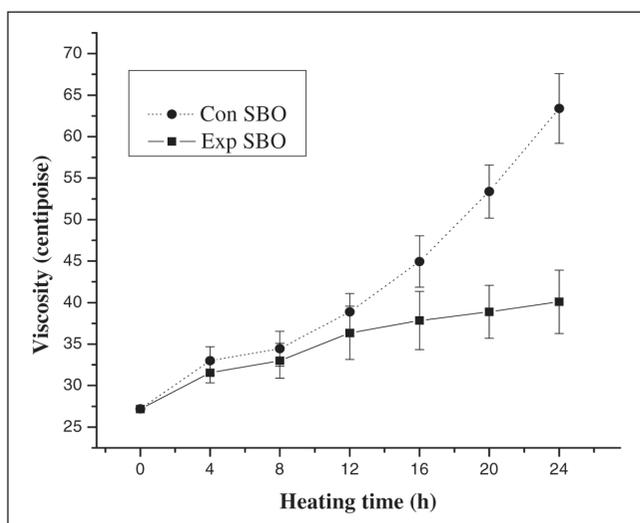
*Evaluation of physical characteristics of heated oil*: The physical characteristics viz. colour and viscosity of the control SBO and experimental SBO, collected at different time intervals, were determined. **Fig 1**. Changes in colour (Y+5R) of control soybean oil (Con SBO) and experimental soybean oil (Exp SBO), during heating at 180°-190° C for 24 h. The change in colour was visible to the naked eye and this was supported by the tintometer values obtained as shown in Fig. 1. The initial tintometer reading for the control SBO indicated total colour (Y + 5R) value of  $1.1 \pm 0.1$ . After every 4 h of heating the change in yellow value was significant and finally after 24 h of heating the value was found to be  $11.3 \pm 0.3$ . However, the



**Fig 1 :** Changes in colour (Y+5R) of control soybean oil (Con SBO) and experimental soybean oil (Exp SBO), during heating at 180°-190° C for 24 h

experimental SBO showed a reverse trend. The initial tintometer indicated value of  $53.9 \pm 2.2$ . After every 4 h of heating the decrease in yellow value was significant and finally after 20 h of heating the value was found to be  $11.2 \pm 1.5$ . The gradual darkening of the colour of the control SBO after heating may be attributed to the polymerization reactions at high temperatures. In experimental oil with LE, the dark colour at 0 h of heating can be attributed to the orange colour of the lutein ester. As the duration of heating increased, the colour pigments degraded and the colour of the oil became lighter. But oil sample collected at the end of the experiment i.e. after 24 h showed increase in the yellow value from the one at 20 h. The reason behind this could be the usual factors of polymerization reactions at higher temperatures. **Fig 2**. Changes in viscosity (in centipoises) of control soybean oil (Control SBO) and experimental soybean oil (Exp SBO), during heating at 180°-190° C for 24 h.

As evident from Fig. 2, the viscosity of the oil increased with the duration of heating in both the cases. But the control SBO showed a marked increase in viscosity with time than the experimental one. The control SBO showed an increase in value from  $27.19 \pm 0.1$  at 0 h to  $63.39 \pm 4.2$  at 24 h. The viscosity of the SBO with LE sample collected after 24 h heating showed a value of  $40.09 \pm 3.8$  which is much less than the viscosity of the control oil sample. The increase in oil viscosity for both the oils during heating has also been attributed to polymerization and the concomitant formation of high molecular weight compounds via carbon- to -carbon and/or carbon-to-oxygen-to-carbon bridges between fatty acids<sup>32</sup>. In control SBO, the increase in viscosity with time is more pronounced due to greater oxidation of the oil



**Fig 2 :** Changes in viscosity (in centipoises) of control soybean oil (Control SBO) and experimental soybean oil (Exp SBO), during heating at 180°-190° C for 24 h

when compared with the experimental one. The lutein ester present in the experimental SBO has prevented SBO from accelerated oxidation at frying temperature.

*Evaluation of chemical characteristics of heated oil:* The chemical parameters such as acid values, peroxide values, p-anisidine values and content of HNE of both control oil and experimental SBO have been determined at different intervals of heating.

The acid values of both the control SBO and SBO with LE after heating are given in Table 1. In the experimental oil, the readings showed an acid value of  $0.27 \pm 0.05$  at 0 h while the value increased to  $0.84 \pm 0.12$  after 24 h of heating. However when compared with the control one, it was found that the

acid value of experimental oil was lower. In control SBO, the oil collected after 24 h heating showed an acid value of  $2.78 \pm 0.16$ . The increase in acid value in both the oils is due to the oxidative and thermal degradation taking place in unsaturated fatty acids. In addition, an increase in free fatty acid has been known to result from hydrolysis of triglycerides (by absorbing moisture from the atmosphere) and its oxidation. The lesser acid value in the experimental SBO indicated that antioxidant property of lutein ester has retarded the oil deterioration. In order to assess the oxidative changes in control and experimental SBO, peroxide value was determined and presented in the Table 2. Detection of peroxides gives the initial evidence of development of rancidity in unsaturated fats and oils. Significant differences were observed in peroxide value at different time intervals. Peroxide value increased from  $1.4 \pm 0.04$  meq/kg in control SBO oil to  $56.1 \pm 1.00$  meq/kg in 24 h heating. But, the increase in PV was less in case of experimental oil which showed a value of  $39.5 \pm 0.88$  meq/kg in 24 h heating. The lesser value can be attributed to the antioxidant property of lutein. The p-anisidine value, which is a more reliable parameter to measure oil quality changes against exposure time, also showed a higher reading in control SBO than the experimental one, as in Table 3.

The process of lipid peroxidation is a free radical-mediated deterioration of FA in the presence of air. During lipid peroxidation, hydroperoxides are formed, which then undergo decomposition and yield FA-oxy free radicals and hydroxyl radicals via  $\beta$ - cleavage. Subsequently, this chain cleavage leads to the formation of a wide variety of secondary lipid peroxidation products, including aldehydes, ketones,

**TABLE 1**  
**Changes in Acid Values of Control Soybean Oil (Con SBO) and Experimental Soybean Oil (Exp SBO), during Heating at 180°-190° C for 24 h**

Heating time (h)	Con SBO	Exp SBO
0	$0.26 \pm 0.02$	$0.27 \pm 0.05$
4	$0.83 \pm 0.12$	$0.56 \pm 0.10$
8	$1.95 \pm 0.14$	$0.56 \pm 0.12^a$
12	$1.96 \pm 0.15$	$0.55 \pm 0.12^a$
16	$2.50 \pm 0.08$	$0.56 \pm 0.10^a$
20	$2.62 \pm 0.09$	$0.83 \pm 0.14^a$
24	$2.78 \pm 0.16$	$0.84 \pm 0.12^a$

Values are expressed as mean  $\pm$  SEM (n=3).

The superscript letters represent statistical significance at  $p < 0.05$ .

<sup>a</sup>Comparison between control soybean oil (Con SBO) and experimental soybean oil (Exp SBO)

**TABLE 2**  
**Changes in Peroxide Values of Control Soybean Oil (Con SBO) and Experimental Soybean Oil (Exp SBO), during Heating at 180°-190° C for 24 h**

Heating time (h)	Con SBO	Exp SBO
0	1.4 ± 0.04	1.2 ± 0.02
4	18.8 ± 0.42	18.6 ± 0.14
8	32.5 ± 1.12	20.4 ± 1.05 <sup>a</sup>
12	45.8 ± 1.02	22.3 ± 1.02 <sup>a</sup>
16	50.9 ± 2.04	32.2 ± 1.24 <sup>a</sup>
20	55.5 ± 1.18	34.7 ± 0.86 <sup>a</sup>
24	56.1 ± 1.00	39.5 ± 0.88 <sup>a</sup>

Values are expressed as mean ± SEM (n=3)

The superscript letters represent statistical significance at p<0.05.

<sup>a</sup>Comparison between control soybean oil (Con SBO) and experimental soybean oil (Exp SBO)

**TABLE 3**  
**Changes in *p*-Anisidine Values of Control Soybean Oil (Con SBO) and Experimental Soybean Oil (Exp SBO), during Heating at 180°-190° C for 24 h**

Heating time (h)	Con SBO	Exp SBO
0	1.1 ± 0.02	1.3 ± 0.04
4	18.1 ± 0.06	12.8 ± 0.42 <sup>a</sup>
8	30.7 ± 1.02	26.2 ± 1.12 <sup>a</sup>
12	33.6 ± 2.12	28.9 ± 1.18 <sup>a</sup>
16	48.8 ± 3.02	41.5 ± 1.16 <sup>a</sup>
20	69.5 ± 1.16	56.7 ± 2.12 <sup>a</sup>
24	83.3 ± 3.12	58.5 ± 2.42 <sup>a</sup>

Values are expressed as mean ± SEM (n=3)

The superscript letters represent statistical significance at p<0.05.

<sup>a</sup>Comparison between control soybean oil (Con SBO) and experimental soybean oil (Exp SBO)

**TABLE 4**  
**Changes in HNE (µM/g) Content in Control Soybean Oil (Con SBO) and Experimental Soybean Oil (Exp SBO), during Heating at 180°-190° C for 24 h**

Heating time (h)	Con SBO	Exp SBO
0	0.02 ± 0.01	0.02 ± 0.01
4	2.90 ± 0.04	0.09 ± 0.02 <sup>a</sup>
8	3.11 ± 0.12	0.11 ± 0.01 <sup>a</sup>
12	3.55 ± 0.14	0.30 ± 0.02 <sup>a</sup>
16	9.38 ± 0.16	0.53 ± 0.02 <sup>a</sup>
20	9.75 ± 0.12	0.54 ± 0.04 <sup>a</sup>
24	10.09 ± 0.16	1.11 ± 0.02 <sup>a</sup>

Values are expressed as mean ± SEM (n=3)

The superscript letters represent statistical significance at p<0.05.

<sup>a</sup>Comparison between control soybean oil (Con SBO) and experimental soybean oil (Exp SBO)

and other carbonyl-containing compounds. Of particular interest is the group of aldehydes known as  $\alpha$ -hydroxyalkenals, some of which have been shown to be cytotoxic and mutagenic. The toxicity of these compounds arises from the high reactivity of their three main functional groups: the aldehyde group, the  $\alpha$ ,  $\beta$  C=C double bond, and the hydroxyl group. Reaction of the  $\beta$ -carbon with thiol (SH) and amino (NH<sub>2</sub>) groups results in protein and DNA modifications. The toxic aldehyde 4-hydroxy-2-*trans*-nonenal (HNE) is an oxidation product of linoleic acid and is formed during the thermal oxidation of SBO at frying temperature<sup>33</sup>. Therefore, HNE can be considered as an important parameter to determine lipid peroxidation. The HNE content in control SBO and experimental SBO are represented in Table 4.

The HNE value showed a marked increase from 0.02±0.01  $\mu$ M/g to 10.09±0.16  $\mu$ M/g in case of control oil. However, the SBO with LE showed far resistance to lipid peroxidation even after 24 h of heating at frying temperature. Therefore, amount of HNE present in oil could be taken as a marker for assessing oil quality.

The detection of peroxide values, p-anisidine values and content of HNE showed greater oxidative stability of the oil with added LE when compared with the controlled one. This may be attributed to the antioxidant property of the LE as it possesses pronounced free radical scavenging ability due to its polarity and number of conjugated double bonds<sup>26, 34</sup>.

Studies had confirmed that antioxidants like butylated hydroxyl anisole (BHA) and tertiary butyl hydroquinone (TBHQ) did not prevent deterioration of SBO during frying<sup>1</sup>. However, the natural antioxidant viz LE showed a greater oxidative and thermal stability at frying temperature even after 24 h of heating of the oil.

Analysis of quality parameters, physical as well as chemical during heating of SBO, revealed that the quality of heated oil started deteriorating with time, the rate of deterioration being more in control SBO than the oil with added LE. The amount of HNE present in oil could be taken as an important marker to assess the quality characteristics of heating medium. The result suggested that lutein can be successfully employed in fortification of SBO to ensure lesser oxidation of the oil at during cooking.

#### ACKNOWLEDGEMENT

We sincerely acknowledge the contribution of Late Dr. Santinath Ghosh, Associate Professor, Dept. of Chemical Technology, University of Calcutta, for his never forgettable inspiration and contribution.

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# Lipid Classification and Characterization of *Terminalia Belerica* Seed Oil From Tripura

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## ABSTRACT

*Terminalia belerica* fruit is known for its medicinal properties and is currently exploited by manufacturers of Indian traditional ayurvedic medicines. Apart from its abundance in some states of India, organized farming of this tree has been started in some places. The seed of this fruit was found to be a good source of oil (38%) and is not commercially exploited. In this study, attempts were made to extract oil from the *Terminalia belerica* seeds and analyze the oil for the physico-chemical characteristics. *Terminalia belerica* fruits were collected from the forests of Tripura and the seeds were dried and powdered after the separation of pulps. The Soxhlet extraction of seeds yielded 38.0% of lipids. Various physico-chemical properties like acid value (AV), iodine value (IV), saponification value (SV), unsaponifiable matter (USM) and phosphorous content etc were determined following standard methods and was found to have AV of 0.9, IV of 71.2, SV of 185.8 USM of 1.7% and 181.6 ppm of phosphorus. The total lipids were separated into neutral lipid (NL), glycolipid (GL) and phospholipids (PL) fractions by using column chromatography and found to contain 98.9%NL, 0.4%GL and 0.7%PL respectively. Among the phospholipids PE was obtained as the major phospholipid along with PC and PI. The triacylglycerol (TAG) composition was also determined. The molecular species with effective carbon number (ECN) C48 and C46 were found to be the major ones. Fatty acid composition of the oil extracted from the seed was determined. The oil was found to contain oleic acid (43.1%), palmitic acid (28.3%), linoleic acid (17.0) and stearic acid (10.6%) as major fatty acids. Unsaponifiable fraction of seed oil was analyzed for sterol composition and found to have  $\beta$ - Sitosterol as the major sterol. The total tocopherol content was found to be about 660 ppm. The fruit of *Terminalia belerica* is generally regarded as safe for human consumption.

The lipid composition of seed oil also did not show any unusual fatty acids. These data obtained may help for commercial exploitation of the *Terminalia belerica* seed oil.

**KEYWORDS:** *Terminalia belerica*, physico- chemical characteristics, fatty acid composition, molecular species, phospholipid composition.

## INTRODUCTION

*Terminalia belerica*, known as vibhitaki in traditional literature in India, is one of the most common herbs for medicinal uses<sup>1</sup>. This is used for controlling cough and nourishment of lungs, throat, voice, hair and eyes. Another important use of the fruit is in treatment of liver disorders<sup>2,3</sup>. The fruit also has purgative properties<sup>4</sup> and it was used as cardiac depressant<sup>5</sup>. The fruit contains hosts of antioxidants and nutraceuticals like gallic acid, ellagic acid, ethyl gallate, beta-sitosterols, rhamnose etc<sup>6</sup>. The uses of fruit as anthelmintic, antiseptic, astringent and expectorant are also known in traditional Indian medicine<sup>7</sup>. In recent years, researchers have focussed their interest and are thoroughly studying the medicinal aspects of this particular fruit. Jadon et al. has studied the protective effect of this fruit extract against the carbon tetrachloride induced damage in albino rats<sup>8</sup> and concluded that the damage caused by carbon tetrachloride exposure could be revised to normal condition by *Terminalia belerica* fruit extract in combination with gallic acid. The ayurvedic medicine in India has also experienced paradigm shift in recent past and lots of scientific studies being done in various laboratories worldwide to understand the working mechanism of these medicines and on isolation of bio-active molecules from these medicinal plants. Organized farming of these plants and herbs have started in a big way as the sales of ayurvedic medicines are having a steady rise. This tree is available in huge quantities in Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Punjab, Maharashtra and almost all the North-eastern states. However, no data is available on the total production of these

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herbs in India. Though the fruits are being studied thoroughly and many literature reports are available on its uses, no significant data is available in literature on the seed and oil obtained from this seed. The seeds are not exploited for any commercial benefits. The biodiesel industry in India is facing short comings of raw materials and recently some researchers have utilized the oil extracted from the '*Terminalia belerica* seeds as raw materials for biodiesel preparation<sup>9,10</sup>. However, detailed studies on the physico-chemical properties of the oil was not performed. In this present study, the oil was collected from a forest of Tripura, a north-eastern state. The fatty acid compositions of the oil and various lipid fractions obtained from the oil were determined. The oil obtained was totally analyzed for its physic-chemical properties. The molecular species present in the triglycerides were estimated. The oil was also analyzed for phosphorous contents and the phospholipids compositions also determined. These data may be useful for various types of commercial exploitation other than biodiesel preparation.

## MATERIALS AND METHODS

### Materials

*Terminalia belerica* (TB) fruits were collected from the forests of Tripura and the seeds were dried after the separation of pulps. Standards of Fatty acid mixtures and triglycerides were purchased from M/s Sigma Chemicals, St Louis, USA. Standard tocopherol and tocotrienols were procured from M/s Sigma Chemicals, St Louis, USA. Silica gel (60–120 mesh) for column chromatography was purchased from Acme Synthetic Chemicals, Mumbai, India. Pre-coated thin layer chromatography (TLC) plates (silica gel 60 F<sub>254</sub>) were procured from Merck, Darmstadt, Germany. Reference samples of phosphatidylethanolamine (PE), phosphatidylcholine (PC), Phosphatidylinositol (PI), cardiolipin (CL) were purchased from M/s Sigma Chemicals, St Louis, USA. HPLC grade solvents were procured from M/s Merck, Mumbai, India. All other analytical grade reagents and solvents were purchased from M/s S.d.fine Chemicals, Mumbai, India.

### Methods

*Extraction of oil from Terminalia belerica Seed:* The dried *Terminalia belerica* seeds were crushed to get powder and the oil was extracted using soxhlet extractor with hexane as solvent. After 6-8 hr of extraction, the lipid extract was concentrated using a rotary evaporator and dried completely under reduced pressure to obtain the seed oil.

*Physico-Chemical Characteristics of oil:* Different physico chemical characteristics like acid value<sup>11</sup>, iodine value<sup>12</sup>, peroxide value<sup>13</sup>, moisture & volatiles<sup>14</sup>, saponification value<sup>15</sup>, Unsaponifiable matter<sup>16</sup>,

refractive index<sup>17</sup>, tocopherol content<sup>18</sup> were determined according to AOCS Official methods and phosphorous content was determined by IUPAC method<sup>19</sup>. The density of the oil was determined by using Automatic Density Meter (Anton Parr, DMA 4500) according to the method prescribed by ASTM D4052<sup>20</sup>.

*Separation of lipid classes:* Total lipids of *Terminalia belerica* seed oil (10 g) was separated in to neutral, glyco- and phospholipids by silica gel column chromatography<sup>21</sup> with the elution of chloroform, acetone and methanol respectively. The results of class of lipids were taken in average of triplicate column results. Glycolipids and phospholipids were qualitatively identified by developing TLC using solvent system chloroform / methanol / water (65:25:4 v/v/v) and spraying with  $\alpha$ -naphthol and ammonium molybdate<sup>22, 23</sup> as spray reagents respectively.

*HPLC analysis of phospholipids:* The phospholipid fraction obtained from column chromatography was qualitatively analyzed by normal phase HPLC equipped with a quaternary pump and an evaporative light scattering detector (ELSD 2000, Alltech, Deer field, IL, USA). The drift tube temperature of the ELSD was set at 50°C and the nebulizing gas (nitrogen) flow rate was 1.5 lit/min, with impactor 'on' mode. The concentration of the sample 1 mg/ml was injected to a SunFire™ Prep Silica column (5 $\mu$ m, 4.6 x 250 mm; Sun Fire columns, Waters, Ireland) at a mobile phase flow rate of 0.5 ml/min. A binary gradient solvent system composed of eluent A [chloroform/ methanol/ ammonium hydroxide (80:19.5:0.5, v/v/v)] and eluant B [chloroform/methanol/ammonium hydroxide/water (60:34:0.5:5.5, v/v/v/v)] was used for elution following the method described by Avalli and co-workers<sup>24</sup>. The Identification of phospholipids was carried out by comparing the retention times of the respective commercial standards.

*Preparation of fatty acid methyl esters:* Fatty acid methyl esters (FAME) of total, neutral, glycolipid and phospholipids fractions of the oil were prepared according to the methods described by Christie<sup>25</sup>. The oil (10-20 mg) was taken in 15 ml of 2% H<sub>2</sub>SO<sub>4</sub> in methanol reagent and refluxed for about 4 hours. After complete conversion as monitored by TLC, solvent was partially removed and the remaining mixture was extracted with ethyl acetate and the combined ethyl acetate layers were washed with water until neutral. Then the ethyl acetate extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure on a rotary evaporator to obtain FAME.

*GC analysis:* The fatty acid composition was determined with a Agilent 6890N series Gas Chromatograph

equipped with a flame ionization detector (FID) on a split injector. A fused silica capillary column (DB-225MS, 30 X 0.25mm i.d., J & W Scientific, USA) was used for separation. The oven temperature was programmed at 160°C for 2 min, increased to 230°C at 5°C/minute and hold for 20 minutes at 230°C. The injector and detector temperatures were maintained at 230 and 250°C respectively. The nitrogen gas used as carrier at the flow rate of 1 ml/minute. Identification of fatty acids was carried out by comparing them with the retention time of respective commercial standards.

**Molecular species determination by HPLC:** Triglyceride molecular species were estimated by High performance liquid chromatography (HPLC) equipped with a quaternary pump and an evaporative light scattering detector (ELSD 2000, Alltech, Deerfield, IL, USA). The drift tube temperature of the ELSD was set at 50°C and the nebulizing gas (nitrogen) flow rate was set at 1.5 L/min. The concentration of sample in acetone solution was 1 mg/ml and was injected on a reversed phase silica column (X Bridge™ C18, 5µm, 4.6 x 250 mm). The molecular species were eluted with mobile phase acetone (100%) at a flow rate of 1 ml/min. The molecular species of *Terminalia belerica* seed oil was tentatively identified by their equivalent carbon numbers (ECN).

**Regiospecific analysis of Terminalia belerica seed oil:** Positional distribution of fatty acids was carried by porcine pancreatic lipase mediated hydrolysis as described by Christie<sup>26</sup>. 20 mg of the oil was mixed with tris buffer (pH 8.0, 4 ml), calcium chloride solution (0.4 ml, 2.2%) and bile salt solution (1 ml, 0.05%). To this 10 mg of pancreatic lipase was added, the mixture was incubated for 3 min at 40°C. The reaction was then stopped by adding ethanol followed by addition of 1.5 ml of 6 N HCl. The hydrolysis product was extracted with diethyl ether and washed with water until neutral and dried completely under reduced pressure. The hydrolyzed product was separated on thin layer chromatography (TLC) with a mobile phase of hexane/ethyl acetate/acetic acid (70/30/1, v/v/v). The bands corresponding to 2-monglyceride and free fatty acids were separated. The fractions were converted in to fatty acid methyl esters using 2% sulfuric acid in methanol reagent. The mean composition of each fatty acid in 1 and 3 positions is calculated from the intact triacylglycerols and in sn-2 position by following the equation

$$\text{Position 1 and 3} = [(3 \times \text{TAG}) - \text{SN-2}] / 2$$

## RESULTS AND DISCUSSION

Oil was extracted from the *Terminalia belerica* seed collected from Tripura using hexane and found to contain 38.0% of lipids. Physico chemical properties

of the oil were determined and are shown in Table 1. The analytical data was comparable with the data reported earlier<sup>9</sup>.

**TABLE 1**  
**Physico-Chemical Characteristics of**  
***Terminalia belerica* Seed oil**

Characteristics	Values
FFA (%)	0.45
Iodine value (g/100 g)	71.2
Peroxide value(ppm)	0.5
Moisture & volatiles (%)	0.12
Saponification value	185.8
Density at 40°C (g/cm <sup>3</sup> )	0.89410
Phosphorous content (ppm)	181.6
Unsaponifiable matter (%)	1.7
Refractive index at 30°C	1.3081
Viscosity at 40°C (cst)	35.6
Triglycerides (%)	97.0
Diglycerides (%)	0.25
Monoglycerides (%)	0.20
Tocopherol (ppm)	660
Oxidation stability at 110°C ( hr)	23.8

The *Terminalia belerica* seed oil after extraction appeared as light yellow in colour with liquid form at room temperature. The analytical data revealed that, the oil is having free fatty acids content about 0.45%, which is low compared to earlier reported data<sup>10</sup>. The iodine value observed 71.2, which tells about the unsaturation of the T B seed oil. From the iodine value, the oil can be considered as non-drying oil and also having the combination of saturated and unsaturated fatty acids. The peroxide value of the oil was found to be 0.5 ppm. This clearly indicates the quality of the seed oil after extraction was good low level of deterioration.

The oil was also found to contain the phospholipids 181.6 ppm, which are most important in forming emulsions. The result indicated that, the oil contained 1.7% of unsaponifiable matter, which contains mostly tocopherols and sterols apart from hydrocarbons. Among all the sterols β-sitosterol was found to be the major and campesterol, stigma sterols were observed in low level. The total tocopherol content as considered as vitamine E was around 660 ppm in the seed oil. γ-tocopherol (584.7 ppm) is the major tocopherol and α-tocopherol (42.0 ppm), δ-tocopherol (33.2 ppm) were observed in minor quantities. Almost the similar composition of tocopherol

content was observed compared to sunflower oil<sup>27</sup>. The saponification value of the oil was estimated as 185.8 mg/KOH and that comparable with other vegetable oils. The seed oil contained triglycerides almost 97.0%, diglycerides 0.25% and monoglycerides 0.2%. The oxidative stability of the seed oil was obtained as 23.8 hr, which was higher in compared with earlier reported data<sup>10</sup>. The seed oil contains the tocopherols and phospholipids; this could be the reason for the T B seed oil oxidatively stable. According to *Kotake-Nara et al*, the synergetic effect between tocopherols and phospholipids influence the stability of the oils<sup>28</sup>. The viscosity (35.6 cst) and density (0.894 g/cm<sup>3</sup>) of the oil was found to be in lower ranges compared to earlier report<sup>10</sup>.

The fatty acid composition of the seed oil was determined by using gas chromatography after conversion in to fatty acid methyl esters and the results shown in Table 2. The positional distribution of fatty acids of *Terminalia belerica* seed oil was also given in Table 2.

The total oil was separated in to neutral, glyco- and phospholipids fractions with the elution of chloroform, acetone and methanol respectively. Further, the phospholipid fraction was analyzed for individual class of lipids by using HPLC. The results observed that, phosphatidylethanolamine (80.1%) were present as the major phospholipid followed by phosphatidylcholine (16.7%) and phosphatidylinositol (3.2%).

**TABLE 2**  
**Fatty Acid Composition and Positional Distribution of *Terminalia belerica* seed oil Triacylglycerols**

Fatty Acid	Wt%		
	Total	Sn 1, 3	Sn-2
16:0	28.3	40.7	3.5
16:1	0.5	0.1	1.3
18:0	10.6	15.0	1.8
18:1	43.1	35.7	58.0
18:2	17.0	7.8	35.4
18:3	0.1	0.1	0.1
20:0	0.2	0.3	0.1
22:0	0.2	0.3	0.1
Saturates	39.3	56.3	5.5
Unsaturates	60.7	43.7	94.5

The result showed that, oleic acid is the major fatty acid (43.1%), followed by palmitic acid (28.3%) and stearic acid (10.6%) in total seed oil. The oil contained total saturated fatty acids about 39.3%

and unsaturated fatty acids about 60.7%. Oleic acid distributed 58.0% in sn-2 position of triacylglycerols and 35.7% distributed in sn-1 and sn-3 positions. Linoleic acid were located 35.4% in sn-2 position whereas 7.8% were located in sn-1 and sn-3 position of triglyceride. In case of palmitic acid 40.7% was distributed in sn-1 and sn-3 positions and 3.5% distributed in sn-2 position. It can be observed that, the saturated fatty acids mostly located at sn-1 and 3 positions of triglyceride and unsaturated fatty acids dominated at sn-2 position of triacylglycerols of T B seed oil as usual normal vegetable oils.

**TABLE 3**  
**Molecular Species of *Terminalia belerica* Seed Oil Triacylglycerides**

ECN	Expected Molecular species	% of Triacylglycerol composition
C42	LLL	2.3
C44	LLO	3.2
C46	OOL/POL	26.4
C48	OOO/POO/POP/OSL	58.3
C50	OOS/SOP	9.7

L- Linoleic, O- Oleic, P- Palmitic, S- Stearic acid.

The triacylglycerols compositions as molecular species were determined by using reversed phase HPLC and the results obtained were shown in Table 3. The molecular species are reported as effective carbon number (ECN) of triglyceride. The results revealed that, the molecular species of the *Terminalia belerica* Seed oil obtained primarily in 5 types which were in the range of triglyceride having ECN C42-C50. The species of triglyceride of the seed oil having C48 is the major component, followed by C46, C50 and others.

### CONCLUSIONS

The oil obtained (38%) from *Terminalia belerica* seeds collected from forest of Tripura was thoroughly analysed for its physico-chemical characteristics. No unusual fatty acid was identified and this oil was found to have oleic acid as the major fatty acid (43.1%) followed by palmitic (28.3%), linoleic acid (17%) and stearic acid (10.6%). The oil was found to have 660 ppm of tocopherol. The major phospholipid present in the oil was phosphatidyl ethanol amine. The fruit of this tree is regarded as safe and has many medicinal uses. The data generated about the oil obtained from the *Terminalia belerica* seed may help in further commercial exploitation of the herb.

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## ACKNOWLEDGEMENT

This work was carried out with the financial grant provided by Council of Scientific and Industrial Research, Ministry of Science and Technology, Govt. of India under the project PEOPLE HOPE (CSC 0112).

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**Report on 69<sup>th</sup> Annual Convention of OTAI  
& International Conference on  
SUSTAINABLE TECHNOLOGIES AND FUTURISTIC TRENDS:  
OILS PROCESSING AND SURFACTANTS & EXPO 2014  
November 14-16, at Hotel Radisson Blue, Agra, India**



Lighting of lamp by Dr. Manoj Pandey, Hon. Minister, Science & Technology, Govt. of U.P.

The Oil Technologists' Association of India (OTAI), Central Zone, Kanpur, supported by Council of science & technology (UP) and Science and Technology Entrepreneurs Park (STEP-HBTI), Kanpur jointly organized an International Conference on Sustainable Technologies and Futuristic Trends: Oilseeds – Oils processing And Surfactants & Expo-2014 during November 14-16, 2014 at Hotel

Radisson Blu Agra, India. The Conference was organized by OTAI Central Zone on the occasion of the 69<sup>th</sup> Annual Convention of OTAI.

As a part of the conference, Oils Processing and Surfactants Expo-2014 was also organized at the convention centre. Many oleo-chemical based industries, vegetable oil industries, project and process engineering companies/ consultants,



Address by Prof. R. K. Trivedi, Conference Chairman & Convener and President OTAI (CZ)

plant & machinery manufacturers, specialty chemical manufacturers, analytical equipment manufacturers/dealers have put their stalls and participated in the exhibition.

The programme was inaugurated by Dr. Manoj Pandey, Hon. Minister, Science & Technology, Govt. of U.P. Dr. Pandey in his inaugural address talked about the Vegetable oil processing as one of the largest industry segment in India. Dr. Manoj Pandey also mentioned about the recent trends in global oleo-chemical industry and how India should re-orient to cope up with the demand. Shri S.K. Roy, President, OTAI in his welcome address, thanked delegates for actively participating in the conference. Shri Roy elaborated on various activities of OTAI. Prof. R. K. Trivedi, Conference Chairman & Convener and President OTAI (CZ), explained about salient features of the conference and how different sessions were designed. During inaugural ceremony, OTAI Lifetime Achievement awards were presented to Dr. M. S. Saxena, former President, OTAI CZ and Shri P. K. Tiwari, former Hon. General Treasurer (both former faculty members of HBTI Kanpur), for their outstanding contributions to OTAI. Dr S. Hussain Zaheer award was presented to Dr N.B.L. Prasad of Oil Technological Research Institute, Anantpur, A.P.

Dr M.S.L. Karuna from IICT, Hyderabad received Dr. S.D. Thirumala Rao memorial Award. Dr Santinath Ghosh Memorial Research Award was presented to Mr. Sreenu Madhumanchi. J.G. KANE MEMORIAL AWARD-2014 was conferred upon Dr. B.V.S.K. Rao of IICT Hyderabad. Mr. Umesh Chandra Sharma, Conference Co-convener and Hon. Secretary, OTAI CZ, proposed vote of Thanks. Chief Guest, Dr. Manoj Pandey later inaugurated the Oilseeds-Oils processing and Surfactants & Expo-2014.

After the inaugural session, Technical session I on the theme Oil Seed Processing-Futuristic trends and sustainability was chaired by Mr. D. Mathur, Managing Director, Farelabs Pvt. Ltd and president OTAI (NZ). Keynote address was given by Mr. V.K. Mathur from Nav Krishak Enterprise titled "The Queen of Oils – Mustard Oil". Mr. S.K. Bajaj General Manager, Mother Dairy Fruit and Vegetables Pvt. Ltd. and Mr. A K Jain of Vibrandt Project Consultants Pvt. Ltd delivered lectures on oil seed processing. At the end, a panel discussion was held having eminent panelists from Industry



Release of Seminar souvenir. From left to right Shri R.K. Srivastava, Hon. General Secretary, OTAI, Shri S.K. Roy, President, OTAI, Dr. Manoj Pandey, Hon. Minister, Science & Technology, Govt. of U.P., Shri S.K. Rathore, Chairman Steering Committee, 69<sup>th</sup> Annual Convention of OTAI, Prof. R. K. Trivedi, Conference Chairman & Convener and President OTAI (CZ), Mr. Umesh Chandra Sharma, Conference Co-convener and Hon. Secretary, OTAI CZ.

namely Mr. Dinesh Rathore, Mr. Bharat Bhagat and Mr. Kumar Goel.

The session II was on Theme : Oil processing-Futuristic trends and sustainability. The session was chaired by Dr. R.P. Singh, Former President, OTAI. Mr. Piyush Patnayak of Cargill India delivered keynote address titled "Health and Nutrition in Oil Refining-New Frontiers in Food Safety". Mr. K.C. Rajput from Ruchi Soya Industries in his plenary lecture talked on yield improvement in refining & fractionation. Mr. Rajan Skhariya of Mecpro Heavy Engineering Ltd. delivered the fourth plenary lecture of the day on "Recent research and improved Process Technology in the Oil and Fats". The fifth plenary lecture was delivered by Mr. Javed Husain of Muez-Hest India Pvt. Ltd. on "New Method of Degumming of Rice Bran Oil Using Water and Enzyme at Two Different Stages Followed by Bleaching".



Address by Dr. Manoj Pandey, Hon. Minister, Science & Technology, Govt. of U.P.

Dr Sambasivrao J. of Shirdi Sai Nutraceuticals delivered Sixth Plenary lecture on "Value Addition in Vegetable Oil Refining Through Enzyme Based Solutions". Mr. M.C. Pandey from JVL Agro delivered Seventh Plenary on modern refinery and its operation with new automation and tools. At the end of session II, a panel discussion was held amongst the speakers as panelists.



Awardees with Dr. Manoj Pandey, Hon. Minister, Science & Technology, Govt. of U.P. (Left to right standing) Shri R.K. Srivastava, Hon. General Secretary, OTAI; Shri S.K. Roy, President, OTAI; Dr. B.V.S.K. Rao, IICT Hyderabad; Shri S.K. Rathore, Chairman Steering Committee, 69<sup>th</sup> Annual Convention of OTAI; Dr. Manoj Pandey, Hon. Minister, Science & Technology, Govt. of U.P.; Prof. R. K. Trivedi, Conference Chairman & Convener and President OTAI (CZ); Dr. M.S. Saxena; Sri P.K. Tiwari; Mr. Umesh Chandra Sharma, Conference Co-convener and Hon. Secretary, OTAI CZ. (Left to right sitting) Dr M.S.L. Karuna from IICT, Hyderabad; Dr N.B.L. Prasad of Oil Technological Research Institute, Anantpur, A.P.; Mr. Sreenu Madhumanchi and A. Samaiah from IICT Hyderabad.



Inauguration of Expo 2014 by Dr. Manoj Pandey, Hon. Minister, Science & Technology, Govt. of U.P.

There was a parallel research session where researchers and students presented their papers. At the end of the day, Dr. JG Kane Memorial Lecture was delivered by Dr. B.V.S.K. Rao. This was followed by Cultural Programme and Dinner.

The Third technical session held on the next day was on Surfactants- Futuristic trends and sustainability which was chaired by Dr. R.K. Trivedi, Professor Oil & Paint Technology, HBTI Kanpur. Dr Uday S Racherla, Professor IME Department, IIT Kanpur delivered keynote address on Innovation and Sustainability. Mr. Pranjal Goswami from Novozymes India delivered his plenary lecture on "House Hold Care Industry and Sustainability". Mr. Amit Kumar from Oxford Instruments delivered Ninth plenary lecture on Rapid Analysis of Oils and Fats with Benchtop NMR. The Tenth plenary lecture was by Dr. Upendra Khurana from Agilent Technology on Solutions for Oil Testing. Eleventh plenary lecture was delivered by Mr. Kekin Doshi of Paramount Minerals on polymers for enhancing performance in HPC products. The 12<sup>th</sup> Plenary lecture was delivered by Mr. J Dordi from Godrej Industries on "Mixed Surfactant Systems-A Sustainable and Green Option". At the end of session III, a panel discussion was held amongst the speakers as panelists.



Shri S.K. Roy, President, OTAI with Ms. Norashikin Ahmed from Malaysian Palm Oil Board, Malaysia and other delegates.

The last technical session of conference was on theme Oleo-chemicals – Future Trends and Sustainability was chaired by Shri AK Sharma of Pegasus Technologies. The keynote address was delivered by Ms. Norashikin Ahmed representing Malaysian Palm Oil Board, Malaysia on di-hydroxystearic acid and palm kernel cake derived from sustainable for personal care products applications. The 14<sup>th</sup> Plenary Lecture was delivered by Mr. Rajvinder Singh of SPEC engineering on usage of methyl ester in oleo-chemicals. Mr. Subhas Srivastava from K.Tech delivered 15<sup>th</sup> Plenary lecture on sustainability in application of paints and coatings, role of newer innovative coating additives. Last plenary lecture of the conference was delivered by Dr. K.K. Saxena of Anuvi Chemicals on polymer system for sustainable growth of printing and packaging industry. At the end of session, a panel discussion was held amongst the speakers as panelists.

At the end of conference, certificates of participation along with mementos were given to all speakers/presenters of Oral Research Session (16) as well as Poster session participants (52) by OTAI President Shri SK Roy and convener of conference, Prof. RK Trivedi. The organizing team members were also felicitated by them.

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**Abstracts of Oral Presentation-Research Session/Posters presented in  
69th Annual Convention of Oil Technologists' Association of India  
International Conference on  
Sustainable Technologies and Futuristic Trends**

*(Reprinted from Annual Convention Souvenir)*

**OP-1**  
**Solvent and Enzyme-Assisted Extraction of  
Banana Peel (*Musa Sapientum*) and Its  
Antioxidant Activity in Refined Vegetable  
Oils**

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The present study aimed to evaluate and compare the phyto chemical content and antioxidant activity of banana peel. The conventional extraction technique and the enzyme -assisted extraction were carried out. The extraction of banana peel with solvents, methanol, ethanol, water, methanol-water, ethanol-water was carried out. In enzyme-assisted extraction, pre-treatment of banana peel with enzymes celluclast BG, ultrafo XL and alcalase 2.4 LFG followed by extraction with ethanol was undertaken. The banana peel extracts were subjected to in vitro free radicals scavenging activity assay like DPPH. The total phenolic content and flavanoid content in the solvent extracts of banana peel ranged from 1.35-41.86 mg/g of extracts and 4.38-28.97mg/g of extracts. While the DPPH radical activities for solvent banana peel extract ranged from 2.2-36.2%. The ethanolic extract obtained from the pre-treatment of banana peel with enzyme followed by extraction showed total phenolics in range of 344-1235mg/ml of extract and flavanoid content in range of 94.6-686 mg/ml of extract. The DPPH% inhibition for the enzyme pre-treated banana peel followed by extraction varied from 13.7% to 79.33%. The temperature played an important role in extracting the bioactive compounds from the banana peel extracts. The enzyme assisted extraction yielded the extract with higher antioxidant activity than the solvent extracts. The effect of banana peel extract was studied on the oxidative stability of sunflower and soybean oil. The oxidative stability of both the oils was studied by rancimate at 110°C and schaal oven test at 60°C. The oxidation properties of banana peel oleoresin in sunflower and soybean oil were compared to other synthetic antioxidants such as TBHQ and BHA. It has been observed that banana peel

oleoresin showed thermal stability equivalent to that of BHA but lower than that of TBHQ.

**KEYWORDS:** Banana peel, DPPH, total phenolic content, total flavanoid content.

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**OP-2**

**Production of Medium Chain Fatty Acid  
(MCFA) Containing Structured  
Diacylglycerols (DAGs) By Enzymatic  
Esterification Reaction**

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With the advancement of science and technology in all spheres of human life in recent times, there is simultaneous progression of varied life style related anomalies such as obesity, hypertension, osteoarthritis, type 2 diabetes, coronary heart disease, stroke and dyslipidemia. Food habit and life style are being the two major causative agents for such health related diseases. Dietary oils constitute a major portion of food and it consists of bulk amounts of fatty acids namely, long-chain fatty acids (LCFAs), which are molecules containing more than 12 carbon atoms. But after extensive research, medium chain fatty acids (MCFAs) have attracted attention as being part of a healthy diet, because they are absorbed directly into the portal vein, transported rapidly to the liver for  $\beta$ -oxidation and thus increase diet-induced thermogenesis. Extensive research work has already been carried out regarding MCFAs containing triacylglycerols (TAGs). The literature studies have revealed that not much work has been executed with the MCFA containing structured diacylglycerols (DAGs) as a regular lipid substitute in diet.

The objective of the present study is to produce MCFA containing DAGs and the main purpose of such production lies in the fact that, it might play a beneficial effective role in protecting human well-being from severe long term pathophysiological damages. Eventually,

MCFA containing structured DAGs might aid, with its nutritional aspects, as a proper healthy lipid substitute in daily diet. Therefore, the present study deals with the standardization of the reaction parameters for the preparation of structured DAGs by enzyme catalyzed esterification reaction of MCFA and glycerol. Caprylic acid (C:8), Capric acid (C:10) and Lauric acid (C:12) were the three major MCFAs that were reacted individually with glycerol under optimized experimental conditions and parameters. The immobilized lipases *Rhizomucor meihei* enzyme (RMIM-1,3specific) and *candida antarctica*, commercially known as Novozyme 435 (non-specific) enzymes were utilized as catalysts to prepare different isomers of DAGs. MCFA containing structured DAG isomers were successfully obtained in significant yield by this simple enzymatic esterification process.

**KEYWORDS:** Medium chain fatty acids (MCFAs), medium chain triacyl glycerols (MCTs), structured diacylglycerols (DAGs), enzymes, esterification.

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OP-3

### ***In situ* Transesterification Process and Reactor Development for Biodiesel Synthesis from Sunflower Seed Lipids**

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Biodiesel is an eco-friendly, renewable Biofuel and manufactured commercially by base catalyzed transesterification of refined oil. However triglycerides based on refined oil are expensive feedstocks hence a commercial feasible process or cheaper feedstocks are required to be developed for reducing the cost of production of biodiesel. Aim of present investigation was to combine extraction of oil from sunflower seeds, refining of crude oil and base catalyzed transesterification of oil in one step through *in situ* transesterification of lipids in sunflower seeds in stirred tank batch reactor (SBR) using NaOH as catalyst and packed bed reactor (PBR) system formed by seed powder and anhydrous sodium carbonate catalyst. In SBR, fine seed powder particle size (< 0.5 mm) and low moisture content were found to be the key parameters. The biodiesel yield was limited to 16% due to establishment of mass transfer equilibrium in SBR. Moisture content below 2%, anhydrous sodium

carbonate catalyst loading at 16%, sunflower seed lipid to methanol molar ratio at 1:190, provision for release of pressure due to methanol vapours and uniform distribution of packed bed of sunflower seed powder with minimum channelling were the key parameters for maximization of efficiency of combined leaching and transesterification process in PBR system. Thus the *in situ* transesterification route based on PBR presents a feasible technique suitable for the production of biodiesel at industrial scale.

**KEYWORDS:** Biodiesel, sunflower seeds, *in situ* transesterification, stirred tank batch reactor, packed bed reactor.

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OP-4

### **Turbidity Studies on the Mixed Surfactant Systems in Hard Water for Hardness Estimation by the Synthesized Sugar Based Alkyl Polyglucoside Surfactant**

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The objective of this research is to evaluate the new type of surfactant based on renewable materials for estimation of water hardness. By applying green chemistry approaches we aim to minimize the adverse environmental effect created by the existing methods for water hardness estimations. The sugar based do decyl polyglucoside has been synthesized on laboratory scale and their structure has been confirmed by various spectroscopic and chromatographic techniques. Water hardness tolerance of anionic surfactant sodium do decyl sulphate and the sodium salt of linear alkyl benzene sulphonate have been investigated with the digital Nephelo -turbidity meter. Lime soap dispersing ability of these surfactants has been studied on the effect of incorporation of biodegradable do decyl polyglucoside. The incorporation of the non-ionic surfactant significantly improved the hard water tolerance of linear alkyl benzene sulphonate. At certain range of the surfactant concentration in the pre micellar region, the turbidity has been found to vary linearly with the degree of hardness of water. This linear relationship between the turbidity and the hardness has been used effectively for the estimation of the hardness caused by calcium and magnesium ions.

**KEYWORDS:** Water hardness, mixed micelles, lime soap, turbidity.

OP-5

## Surface Studies of Dimeric Surfactant in the Presence of Different Sodium Salts

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In the present study, an ionic dimeric surfactant has been synthesized and its micellization behaviour in presence of various sodium salts, *i.e.*, NaBr, NaI, Na<sub>2</sub>SO<sub>4</sub>, NaNO<sub>3</sub> and NaSal was studied using tensiometric methods. The surface parameters of dimeric surfactant viz. critical micelle concentration (CMC), surface pressure at the CMC ( $\Pi_{CMC}$ ), efficiency of surfactant ( $C_{20}$ ), maximum surface excess ( $\Gamma_{max}$ ) and minimum surface area ( $A_{min}$ ) in the presence of different concentration of various salts have been investigated. All the salts significantly reduce CMC values of the an ionic dimeric surfactant and followed the sequence as NaSal>NaI>NaBr>NaNO<sub>3</sub>>Na<sub>2</sub>SO<sub>4</sub>.

**KEYWORDS:** Anionic dimeric surfactant, surface activity, salt effect.

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OP-6

## Optimization of Enzymatic Clarification of Pine Apple Pulp with Multi Enzymes using Response Surface Methodology

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Mohammed I. Talib**

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of Chemical Technology, North Maharashtra  
University, India.

Pineapple (*Ananas comosus*) an abundant source of vitamins (A, B & C), and minerals (Ca, Mg, K, Fe, Zn) is one of the major fruit crop in the world. Clarified pulp and juices of pineapple are always in high demand as used in the preparation of ready to serve drinks, nutritional carbonated beverages, cordials, jelly and concentrate. The production of clarified pulp from pineapple through membrane Filtration, sedimentation and stabilization are reported to have low yield and clarity due to presence of plant cell wall polysaccharides (cellulose, xylan and pectin). However the clarification with enzyme is showing good results and the performance of treatment can be further made more effective and economical if multi-enzymes are used in appropriate combination. The application of Response Surface Methodology (RSM) can be proved beneficial in this context as it helps to decrease the number of

experimental trials in determining proper interaction combination of multiple parameters. The present investigation was carried out with the aim to prepare good quality clarified pineapple juice with high yield by optimizing the combination of enzymatic treatment (cellulase, xylanase and pectinase) using Response Surface Methodology (RSM). Optimization of treatment temperature was also the objective of the study. The 30 trial run for the treatment was designed using design expert (9.0.1) software with Central Composite Rotatable Design (CCRD) to analyze the effects of four independent variables xylanase, pectinase, cellulase and temperature on juice yield and clarity. As per the designed runs the clarified pineapple juices were prepared by treating pulp with various level of cellulase (0.15, 0.30, 0.45, 0.60 and 0.75 %), xylanase (0.15, 0.30, 0.45, 0.60 and 0.75 %), and pectinase (0.1, 0.2, 0.3, 0.4 and 0.5 %) in combination at different temperature (35, 40, 45, 50 and 55°C) for treatment period of 1 hr. Juice clarity was measured by measuring the transmittance (%T) of juice at 650 nm using UV Spectrophotometer. The results of the trials were statistically analyzed again using design expert (9.0.1) software. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R<sup>2</sup> and its statistical significance was checked by Fishers F- test. The level of significance was given as p-value. The optimized condition achieved for clarified pineapple pulp with 83.9 % clarity and 91.2% yield was treatment of pulp with 0.6% xylanase, 0.6% cellulase and 0.2 % pectinase enzymes in combination at 45°C for 1hr.

**KEYWORDS:** pineapple, xylanase, cellulase, pectinase, response surface methodology.

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OP-7

## Comparative Study of *in vitro* Digestion and Gastrointestinal Absorption of Medium Chain Fatty Acid Rich Rice Bran and Rice Bran Oil

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In our present study, we worked on the preparation of different medium chain fatty acid (MCFA) rich rice bran oils and compared their *in vitro* digestion and *in vivo* intestinal absorption pattern in the single-pass perfusion rat model. Rice bran oil is well known for its

beneficial micronutrient components which render it high antioxidative property. MCFA have a better absorption capacity in comparison to long chain fatty acids. We tried to combine both the benefits in single oil by preparing MCFA rich rice bran oil using enzyme catalysis technique which resulted in substitution of long chain fatty acids in rice bran oil with MCFA. *In vitro* digestion study showed that MCFA rich rice bran oil was better digested in comparison to native rice bran oil. *In situ* absorption efficiency of the oils was measured in laboratory acclimatized adult Sprague – Dawley rats. A comparative study of lipid absorption from MCFA rich rice bran oil and native rice bran oil of similar dilution level, in cannulated small intestine of rats, at time gradient, has been done. Subsequent analysis (e.g., percent volume absorption, percent lipid absorption) have shown that the MCFA rich rice bran oil significantly enhanced the absorption of lipids from the emulsion system, in the small intestine of the rats. This finding about absorption of MCFA rich rice bran oil could be useful for treating the infants, patients with low absorption or reduced absorption capacity.

**KEYWORDS:** Rice bran oil, medium chain fatty acids, *in vitro* absorption, *in vitro* digestion study, *in vitro* absorption study.

**Acknowledgement:** This research is funded by Dr. D. S. Kothari Post doctoral Fellowship, UGC.

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#### OP-8

### Rice Bran Oil Oleogel: A Novel Technology for Structuring Edible Oils

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Substitution of *trans* fatty acid rich fat is an emerging research interest of food scientists worldwide. Hence, recently, novel kinds of products are being developed in the name of oleogel using liquid oils and appropriate oleogelators. The present investigation reports on the preparation of one edible oleogel using rice bran oil (RBO), a healthy oil of India. Palm stearin (PS) and cetylolate (CO) were used as gelator on 20% weight basis. The parameters in terms of the ratio of PS and CO in gelator and temperature and time required for the preparation of gel were optimized through the study. Characterization of the oleogels regarding their structure and suitability for edible usages were also

studied. The optimized gel sample was prepared by using oleogelators PS and CO in 2:1 molar ratio with 1 hour stirring at 60°C to obtain desired texture and thermal and rheological properties. The study of gel samples with penetrometer showed positive result in terms of hardness and consistency. The cryogenic SEM images revealed that the optimized sample exhibited a large number of small crystals with no gaps between the gel networks, indicating the formation of building blocks by not allowing the diffusion of oil from the gel matrix. The appearance and texture of the experimental samples were analyzed for a period of 90 days at 4°C at room temperature and no drastic change was observed. RBO based oleogel can be used as a shortening agent instead of the conventional fat and shortening products. Therefore, structured solid fat can be successfully prepared from liquid oil like RBO by utilizing the crystalline properties and suitable edible gelators.

**KEYWORDS:** Rice bran oil, palm stearin, cetylolate, oleogel, penetration, cryo-SEM.

**Acknowledgement:** This research is funded by Department of Science and Technology (DST) sponsored by INSPIRE fellowship.

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#### OP-9

### Synthesis and biological activity of fatty-chain containing heterocyclic rings

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Oleo-chemicals by virtue of their economic and ecological advantages are now competing with petrochemicals. A variety of new industrially and biologically useful products can be prepared by taking advantage of the inherently present functional groups in fatty acids and the functionalities of the alkyl chain or derivatization of the chain groups could provide a host of new fatty chemicals. Thus fatty acids have increasingly being found utilizable as explicit raw material for the up coming chemical industry. They have also found applications in metal catalyzed metathesis and ring-opening reactions, polymerization, oxidation etc. Fatty esters are used as antifogging agents and found very useful for plasticizer in biodegradable plastic materials and also known to be good alternative fuel (biodiesel).

Heterocycles play an important role in all spheres of life including pharmaceuticals, natural resources, veterinary, agriculture products, analytical reagents and

dyes. The development of new approaches for the synthesis of heterocycles decorated with unique functional groups forms the basis of extensive research activity in synthetic organic chemistry. Justification of much of the chemistry directed to the synthesis of the compounds, possessing nitrogen at the ring fusion is due to the application of compounds having interesting biological properties in the field of medicinal chemistry.

In this paper, variety of heterocyclic moieties such as triazole, oxazole, thiaziazole, imidazole etc were introduced in fatty acids. All the compounds prepared were characterized by modern spectroscopic analysis data. Most of the compounds were screened for *in vitro* antibacterial activity against the representative panel of Gram-positive and Gram-negative bacteria. Synthesized compounds were also tested for their inhibitory action against trains of fungi. The newly formed compounds are found to be active against some bacteria and fungi.

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OP-10

### Studies on the synthesis and tribological performance of IL based anti-wear additives blended green lubricants

**Ponnekanti Nagendramma, Gananath D. Thakre, P. K. Khatri, Neeraj Atrey and Suman L. Jain**

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At present mineral oil based lubricants are widely being used for all types of practical applications, but the increased environmental awareness has forced to look into the alternatives for replacing mineral oils. Synthetic polyol esters with their good lubrication properties offer an option. Keeping this in view, new generation biodegradable polyol ester has been synthesized by using Polyol alcohols, dibasic acids and mono alcohols as end capping agent with indigenous ion exchange resin as catalyst. The synthesized product was evaluated for its physico-chemical properties such as kinematic viscosity, viscosity index and Flash point. The pour point measurements were also made to study the low temperature properties. Further, the synthesized ester was blended with aspartic acid and glutamic acid derived ionic liquids possessing anti-wear performance characteristics, at a recommended dosage of 2% concentration by weight. The thus obtained formulation was evaluated for its tribological performance on a four ball tribo-tester. The anti-friction and anti-wear

performance characteristics were thoroughly investigated. The results indicate that the selected IL additives have compatibility with the synthesized polyol esters also. The ionic liquids were found to be efficient anti-wear and friction reducing additives for polyol esters. On the basis of physico-chemical characterization and tribological performance the products were found to have good potential for use as biodegradable lubricants. In this paper we present the studies carried out on the synthesis, characterization and performance evaluation of the polyol esters and the suitability of this ester as green lubricants for the industrial applications. This study also presents a systematic approach to improve the wear property of synthetic polyolester base fluids with ionic liquids as anti wear additives.

**Key words:** Synthesis, polyol esters, characterization, Ionic liquid, performance evaluation, Ionic liquid.

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OP-11

### Study of Erucic acid based polyol esters as industrial gear oils

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The growing environmental concern increased the exploitation of renewable materials for the development of bio based lubricants. Synthetic and vegetable oil-based esters offer the best choice in formulating environment-friendly lubricants. In the present study trimethylol propane esters (TMP) and pentaerythritol esters (PE) of erucic acid were prepared and characterized using IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectral techniques and evaluated for lubricant properties namely kinematic viscosity, viscosity index, oxidation stability, wear, weld load and copper corrosion value. The products were compared with extreme pressure type of industrial gear oils of VG-68 and VG-100 grade. The products were found to have good potential for use as abase stock for formulation as extreme pressure type of industrial gear oils.

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POSTER I/1

**Isolation and Characterization of Oil from  
*Caesalpinia sappan***

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The seeds of *Caesalpinia sappan* were analyzed for physicochemical properties and chemical composition for the first time. The results showed that the oil content was 13.5 % (dry w/w), moisture and volatiles (10.0 % v/w), protein content (18.8 % on dry basis) and carbohydrate content (48.7 % on dry basis). The physico-chemical properties of the extracted oil showed acid value (1.9), iodine value (100.9 mg/g), peroxide value (1.958), saponification value (197.7), density at 40 °C (0.9263 g/cm<sup>3</sup>), specific gravity at 40 °C (0.9336), Kinematic Viscosity (39.7 Cst). The unsaponifiable matter was 3 % and the phosphorous content was 692.3 ppm. The major fatty acids present in *Caesalpinia sappan* oil were palmitic (11.5 %), stearic (4.9 %), oleic (15.9 %) and linoleic (66.2 %) acids.

**Nature and scope of work:**

The aim of the investigation was to screen the newer or lesser known oil seeds of Indian forest origin.

**Conclusion:**

*Caesalpinia sappan* seed was identified as a newer source for oil from Fabaceae family. This is the first report on the oil composition studies from seeds of *Caesalpinia sappan*. Complete analysis of the seed and oil from *Caesalpinia sappan* showed that oil content was about 13-14% and all the physico-chemical properties were similar to other vegetable oils.

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POSTER I/2

**Production of Co-Extruded Products for  
Food Uses from De-Oiled Tamarind Seeds  
Kernel Flour and Edible Soy Flour**

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The work is based on utilization of Tamarind Seed Kernel Flour (TSKF) in food products. Research has shown that tamarind seed is an under-utilized seed in food industry; its use has been found in textile and paper industries and as adhesives due to its high gelling / gum like property, popularly known as 'jellose'. In rural areas it has been consumed by roasting after de-hulling, which resembles ground nut in terms of flavor as well as appearance.

Based on the research, TSKF has been studied for its nutrient content. Two mesh sizes of TSKF were selected in this study; 22mm mesh and 100 µm mesh. Since TSKF is a good source of starch and other nutrients it has been utilized in preparation of extruded food along with a protein rich material like soy Flour in various compositions. The TSKF based extrudates have been utilized in preparation of snack, breakfast cereal and soup powder. Proximate composition analysis and sensory evaluation of the extrudates showed acceptable result. The extrudate was subjected to proximate composition analysis. Moisture was found to be low in all the products, ash content was found to be highest in TSKF and Soy Flour (3:2) of 22µm mesh based product. Carbohydrate content was found to be very good in all the products, the highest being in TSKF and Soy Flour (1:1) of 100 µm meshes and lowest being in TSKF and Soy Flour(7:3) of 22 µm meshes. Protein content of all the products was good. Fat percentage was low in all the products and fibre content was found to be higher in products of 22 µm mesh and lower in 100 µm meshes, due to higher retention of Fibre in them. Sensory analysis revealed that products made from TSKF of 100 µm mesh and Soy Flour were more acceptable in terms of taste, colour, texture and overall acceptability.

The extruded food products were assessed in terms of physical properties like water holding and oil holding capacity which revealed that they have very high water holding capacity. Colour reading of the products has indicated that product made from Tamarind seed and Soy Flour (1:1) of 22 µm mesh has the highest L\*value indicating lightest among all the other products; a\*value of all the products were found to be nearly 6 for 22 µm mesh products and 3-4 in 100 µm

mesh products; b\* value also has similar data for all the products of 22 µm mesh which is near about 20, where as in case of 100 µm mesh products it ranged

between 17-19; the c\* indicated the Chroma and was found to be highest in TSKF and Soy Flour (3:1) of 22 µm mesh.

The hue value of all the products ranged with in 90 indicating that they belong to the first quadrant of colour.

The proximate composition analysis and sensory results indicated that the extrudates can be utilized as snack food, breakfast cereals to be consumed with milk and as soup powder. TSKF should be explored as a raw material as it can have potential use in food processing industries.

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**POSTER I/3**

**New Process Technology for Producing at Low Cost Nutritionally Health Quality Non-Dairy Paneer from Edible Soy Flour and Antioxidant Rich Vegetable Oils**

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Paneer is a nutritious heat acid coagulated indigenous milk product, which occupies an important place in the Indian diet. However, the high cost of Paneer has restricted its popularity particularly among the middle class and the low income group. Milk fat is costly and is a major contributive factor for the increasing occurrence of coronary complications. Hence, there is a considerable interest to reduce or replace the milk fat in Paneer. This requires the production of Paneer like products utilizing low fat meal from non conventional food solids like Soybean, which are not only cheap but can also be converted to a product closely similar to the nutritional and textural qualities of Paneer. Soybean is highly nutritious, low priced, conventional, convenient and one of the richest sources of vegetable protein.

The primary purpose of the present study is to develop an appropriate process technology for producing at reduced cost compared to Milk Paneer, some non-dairy Paneer products with better composition of both macro and micro constituents to provide distinct nutritional advantages by utilizing the edible quality oil seed Flour(s) and refined vegetable oil(s) enriched in specific antioxidant and known for the cholesterol lowering properties.

The process involved the conversion of edible Soy Flour to Soy milk by the methodology developed by us. The soaked Soy de-oiled cake (DOC) was mixed with five volumes of distilled water and blended in a blender for 15 min and later boiled and cooled to destroy trypsin inhibitor and for improving flavour. The resultant Soy milk was next homogenized in a homogenizer and pasteurized at 80 °C for 15 min. It was then cooled down to 40 °C for the addition of Rice Bran Oil and Sesame Oil, respectively. Rice Bran Oil was blended with Sesame Oil in the ratios of 20:80, 50:50 and 80:20. After addition of blended oil to pasteurized Soy milk in the desired proportion, the mixtures of Soy milk and Oil(s) were homogenized again in a homogenizer until the oil mixture were mixed properly throughout the Soy milk and coagulated with 2% citric acid/ lactic acid/ tartaric acid. The coagulation was left for 8 min to allow the Soy milk to forma gel. This gel was then transferred into the mould and compressed with a 600 g (or 900 g) load for 1 hr. After removing the load, the Paneer was allowed to remain standing in the mould and left over night at room temperature.

The products with proximate composition similar to the commercially available products display very good antioxidant activity due to the micronutrients like to copherol, tocotrienol, oryzanol and much better amino acid profile compared to dairy Paneer. The microbial status of these samples conformed to the accepted standard.

A novel process technology could be successfully developed based on the use of edible Soy Flour and blending of oil providing unique functionality such as smooth texture, pleasant flavour, antioxidant activity and abundance of nutritive value of superior quality Paneer. This process has much potential for industry along with in favour of the present process technology for producing superior quality Paneer products enriched in antioxidants.

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**POSTER I/4**

**Isopropanol Fractionation of Coconut oil and Use of Its Olein and Stearin Fractions in Margarine Formulation**

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Coconut oil, traditional edible oil in India, has been receiving importance for its medium-chain triglycerides (MCT) content that confers health and nutritional benefits. In fact, the use of MCT rich oil or fraction in products like health margarines and in blending with other cooking oils especially in oils with long chain fatty acids demands for development of an appropriate process technology to produce very effectively MCT rich fraction from coconut oil. The process that appears feasible is the adaptation of the solvent fractionation process. Margarine consists of a water-in-fat emulsion with tiny droplets of water dispersed uniformly throughout a fat phase which is in a stable crystalline form.

The present study deals with the fractionation of coconut oil by using isopropanol as solvent for the first time and incorporation of the obtained Olein and Stearin fractions in margarine production. Fractionates (Olein and Stearin) obtained were characterized in terms of melting properties, iodine value, saponification value and fatty acid composition. Coconut oil was fractionated with isopropanol in different ratios (1:3, 1:4 and 1:5 w/v) at different temperatures (10 °C, 15 °C and 20 °C).

The Olein and Stearin fractions displayed a distinct variation in the fatty acid compositions. Both caprylic acid (C<sub>8:0</sub>) and capric acid (C<sub>10:0</sub>) content increased in Olein fractions from original coconut oil whereas decreased in corresponding Stearin fractions. Coconut Olein and Stearin further converted to DAG, as well as RBO DAG enzymatically prepared were also incorporated in margarine formulation to make it nutritionally better. The effect of RBD Palm oil, Fish oil containing EPA and DHA was also studied depending on physical and chemical characteristics.

Coconut Olein margarine is low in calorie and has high MCT to prevent cholesterol production. In terms of firmness Coconut Stearin incorporated margarines are much more stable than Coconut Olein incorporated margarines. Coconut Olein based margarines are satisfactory in terms of firmness, spread ability and phase stability. Nutritionally the margarines may be claimed superior due primarily to both medium chain fatty acids and long chain PUFA as well as due to antioxidants like oryzanol from RBO which can be shown to reduce blood cholesterol level to maximum extent (17%) compared to other edible liquid oils. Products made from coconut Olein DAG ( $\beta$ -phase) yield less satisfactory result as there was a phase separation seen during storage and to overcome the problem incorporation of Palm Stearin ( $\beta$ -phase) in higher percentage may be effective.

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POSTER I/5

### Vegetable Seeds Production

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Vegetables are an integral part of human diet and known as protective foods, which contribute required minerals, vitamins and other nutrients of medicinal and therapeutic values. The most important and feasible approach to enhance the productivity of vegetable crops would be the production of quality seed and making its availability. There are four classes of seeds –Nucleus seed, Breeder seeds, foundation seeds, certified seeds. The length of time for which a seed remains viable depends upon a number of factors like kinds of seeds, Proper maturity, drying to a desired low moisture content and storage condition. Now a day the advance technologies are used in agriculture sector for production of good quality seeds. In this paper, we described the different seed production technology of various vegetable crops like Tomato, Brinjal Chili and Sweet Pepper, Cucurbitaceous vegetables Radish, Carrots and Onion. This information meets the demand and fulfils the requirement for practical guide to the Student, Industries and farmer engaged in the vegetable oil productions.

**KEYWORDS:** Vegetable Crops, Quality of Seeds, Technology of Oil Seed Production.

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POSTER I/6

### Safety Evaluation of Vegetables Cultivated Using Karanja Seed Cake

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Karanja cake is a by-product of biodiesel industry. This will be available in huge quantities when karanja oil based biodiesel industry will run in full swing. The overall economy of the biodiesel industry will be improved if the de-oiled cake is also commercially utilized. Karanja cake has high N, P, K values and is traditionally being used as bio-fertilizer. However, no scientific study was reported on the safety of the crops grown with karanja cake as manure. In this investigation, both expelled and de-oiled karanja cakes were used as fertilizer for cultivation of three different vegetable crops, namely, onion – an underground crop, amaranthus – a leafy vegetable crop and tomato – a crop that grows over the ground. No adverse effect was observed in these crops during cultivation having dosages up to 1000kg per hectare. After harvesting, the above three crops were dried by lyophilisation and then the dried samples were analyzed for presence of anti nutritional components like karanjin using GC-MS-MS technique. The presence of karanjin was found to be around 2 to 5 ppm for onion and tomato and around 45 to 60 ppm for amaranthus. Acute toxicity was tested for these crops by feeding experimental animals by following standard methodologies. No adverse effects and no mortality were observed. The results indicated that the vegetables grown with the given dosages of karanja cake as manure were safe for consumption. Further studies for long term effects are in progress.

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**POSTER I/7****Production of MCFA Rich Mustard Oil Using Packed Bed Bioreactor****Sohini Mukherjee\*, Susmita Roy and Mahua Ghosh**Department of Chemical Technology,  
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Medium chain fatty acids (MCFAs) have primarily fatty acids that contain chain lengths of 6-12 carbons. Because of their saturation, they are stable to oxidation. They have low viscosity and melting points and are generally liquid at room temperature. Their smaller molecular size and relatively high solubility in water contribute to different digestive and absorptive properties compared to long chain fatty acid (LCFA). Mustard oil is widely used edible oil in Eastern and Northern parts of India. It is rich in mono unsaturated fatty acid (MUFA), erucic acid (EA, C<sub>22:1</sub>, n-9) and some amounts of polyunsaturated fatty acids (PUFA). The minor component viz. tocopherol present in the oil may also

provide beneficial effects to reduce risk factors for cardio vascular diseases (CVD). But erucic acid sometime leads to myocardial lipidosis and fibrosis and therefore low erucic mustard/rapeseed are now on demand. Presently the genetical modification of the crop is the only way to produce low erucic mustard/rapeseed oils.

In the present study attempt was made to produce low erucic, high MCFA mustard oil using biocatalysis reaction. To facilitate the large scale production of the structured lipid, we tried to carry out the reaction in a packed bed bioreactor. Caprylic acid (C<sub>8:0</sub>) was taken as a model MCFA and three enzyme varieties (*Thermomyceslanuginosus* TLIM, *Rhizomucormeihei* RMIM and *Candida antartica* NS 435) were tried to obtain the optimum incorporation in optimum reaction time. Analysis of fatty acid composition of the products was determined by gas chromatography to determine the amount of incorporation of MCFA in mustard oil. The study showed the incorporation of caprylic acid in mustard oil was found to be highest in packed bed bioreactor when NS 435 lipase was used rather than TLIM and RMIM along with less migration of acyl groups. This research work is funded by DVVOF, Govt. of India.

**KEYWORDS:** Medium chain fatty acid, caprylic acid, mustard oil, enzymatic interesterification, packed bed reactor.

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**POSTER I/8****Oil Obtained from Genetically Modified Crops****Vrushali K. Rithe\*, Swati R. Rane, Rohit P. Dhongade**Department of Oil Technology, University Institute of  
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Genetically modified oil (GMO) crops are plants, the DNA of which has been modified using genetic engineering techniques. In most cases the aim is to introduce a new trait to the plant which does not occur naturally in the species. The aims behind genetic modifications of oil crops are resistance to certain pests, diseases, or environmental conditions, reduction of spoilage or resistance to chemical treatments or improving the nutrient profile of the crop. There is no analytical technique which distinguishes between oils derived from GMO and non-GMO crops. The use of

GMO crops includes production of pharmaceutical agents, fatty acids, biofuels, biodiesel, cosmetic and other industrially useful goods. Farmers have widely adopted GM technology. There is a broad scientific consensus that oil available in the market derived from GM oil crops possesses no greater risk to human health than conventional oil crops. GM crops also provide a number of ecological benefits.

**KEYWORDS:** Oil crops, GMO, non-GMO, biofuels, cosmetic, and biodiesel.

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#### POSTER I/9

### Extraction of Antioxidant from Sunflower Cake/Meal for Various Industrial Applications

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Sunflower cake/meal is the by-product of the sunflower oil industry that has low economic value despite its high content of protein and of antioxidants such as phenolic compounds. Recently natural antioxidants have gained increasing interest because natural food and cosmetic ingredients are safer than synthetic ones. In terms of production, it is the 4th most important oil meal after soybean meal, rapeseed meal and cottonseed meal besides low amounts of anti-nutritional compounds and the absence of toxic substances. The aim of present investigation is to evaluate the antioxidant efficacy of solvent extract of sunflower (*Helianthus annuus* L.) seeds meal for showing application scope in cosmeceuticals for anti aging benefits. Sunflower cake/meal was subjected to conventional liquid solvent extraction using different solvents. The optimum solvent extraction conditions of phenols were 180 min using ethanol, at a solvent to sample ratio 5:1 v/w by using stirring at room temperature. Solvent extracts were tested for their antioxidant activity by the DPPH radical scavenging method and folin-cioalteau phenol reagent, also by determination of peroxide value on soybean oil and sunflower oil. The ethanol extract exhibited the highest antiradical activity and no correlation was found between antiradical activity and phenol content. Moreover, the ethanolic extract appeared to be a stronger antioxidant than BHT and BHA by the Rancimat method on sunflower oil and soybean oil. LCMS and GCMS analysis of the ethanolic extract showed that the

predominant phenolic compound was chlorogenic acid and caffeic acid. Various phenolic acids and flavonoids were also identified. Hence the separation of chlorogenic acid was performed by using column chromatography. The results of chromatography separation were confirmed by using retention factor of chlorogenic acid. Phytochemical screening, protein content and FTIR analysis of the sunflower cake solvent extract was a prior necessity to evaluate its potential, to determine the extraction yield and to be controlled qualitatively.

**KEYWORDS:** Sunflower meal, chlorogenic acid, antioxidant activity, Rancimat, LCMS, GCMS, FTIR, column chromatography.

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#### POSTER II/1

### Optimization of Microwave Assisted Ethanolic Extraction Parameters for Orange Peels Using Response Surface Methodology (RSM)

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Microwave assisted extraction methodology was employed for the extraction of bio-oil from orange peels using renewable solvent, ethanol. The extraction parameters, temperature and time were optimized using central composite rotatable design (CCRD) of response surface methodology (RSM) to obtain high yield of bio-oil. The physico-chemical properties and chemical composition of the extracted oil was analyzed using standard methods and GC-MS, respectively. Aqua dratic regression model diagnosed to estimate the optimum conditions for bio-oil recovery was validated in terms of desirability (D) function. The optimum extraction conditions obtained with RSM were extraction temperature (76.5°C) and time (30 min). The predicted yield and desirability achieved with ethanolic extraction at optimized conditions was 7.9% and 0.88, respectively. The true experimental extraction yield obtained at the same conditions was 7.3% and is in good concurrence with the predicted results.

**KEYWORDS:** Orange peels, microwave extraction, bio-oil, response surface methodology, optimization.

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## POSTER II/2

**Non-traditional oils with water soluble substrate as a cell growth booster for production of mannosylerythritol lipids by *Pseudozyma antarctica* (ATCC 32657) with their surfactant and antimicrobial activities.**

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Biosurfactants like Mannosylerythritol Lipid (MEL) are eco-friendly surface active agent, which has various applications like cosmetic and health care products. In addition to surfactant properties, MEL also possesses antimicrobial and skin healing property. In the research work, Non-traditional oils (10%) like jatropha oil, karanja oil and neem oil were used in combination with glucose (10%), honey (10%) and with glycerol (15%) as a newer carbon source for MEL production by using *Pseudozyma antarctica* (ATCC 32657). In the fermentation, non-traditional oils with honey gave higher MEL yield and cell growth as compare to non-traditional oil with glucose and glycerol.

#### Findings

MEL yield was estimated by anthrone methods. Produced MEL structure was confirmed by TLC, FTIR, HPLC, Mass Spectra (LC-MS) and <sup>1</sup>H NMR methods. The MEL found various applications like surface activity and antimicrobial agent. MEL showed surface activity at very low concentrations estimated by Kruss Tensiometer. The various properties were explained on the basis of the structural similarity between MEL and Gemini surfactant. The performance properties of SLS like foaming estimated by Ross-Miles apparatus, emulsification estimated by Subrahmanyam and Acharya (1961) and wetting estimated by Canvas disc method were improved when SLS was partially substituted (5-20%) by MEL. Purified MEL by silica gel chromatography at various concentrations were applied as an antimicrobial agent against cosmetic products allied gram positive, negative bacteria and fungi by deep well dispersion method.

#### Scope

Based on the study, MEL can be used in various cosmetic products like shaving creams, skin creams and lotions where MEL will not only enhance foaming property but also provide moisturizing and healing properties on skin. MEL also can be used in pharmaceutical products as antimicrobial agent.

#### Conclusions

The study has shown that MEL can be produced by using *Candida antarctica* (ATCC 32657) strain grown on glycerol available as by-product from industry and low cost glucose as water soluble substrate to be used along with non-traditional oils. Non-traditional oils with honey instead of glycerol and glucose showed high cell growth which improved the MEL yield. When considering yield of MEL, honey was found to be the best water soluble substrate to be used with oil. However, when considering the cost effectiveness, glycerol was the excellent alternative. Pre-treated neem oil as a substrate shows improvement in MEL yield as well as cell growth. Combination of crude MEL with SLS showed synergistic action and increased SFT reduction, IFT reduction. Additionally it improved various performance properties of SLS like foaming, emulsification and wetting. The synergism for foaming property was due to hydrogen bonding of MEL with SLS. There was also improvement in emulsification due to formation of stable liquid film. This foam stabilizing property of MEL can be used in various formulations like face wash, shampoos and shaving creams where additional properties of MEL like antimicrobial activity are desirable. Purified MEL found to show better inhibition zone as antimicrobial agent against different organisms. Antimicrobial activity of MEL against all micro-organism observed good clearance of zone. This antimicrobial study will be helpful for the formulation of cosmetic product with natural surfactants and also in drug delivery with antibiotics.

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## POSTER II/3

**Production of Fat Replacer from Mango Starch and its Application in Baking**

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Fat replacer is an ingredient that can be used to provide some or all of the functions of fat yielding fewer calories than fat (Schwenk *et al.*, 1997). Fat replacers are either fat substitutes or fat mimetics depending on their role in food products.

Starches of varying sources, types and functional properties are used in fat replacing systems to provide sensory properties of oil, e.g., slippery mouth feel. Fat and calorie reductions continue to be a leading priority in baked product development (Bath *et al.*, 1992). In 1994, 544 new bakery ingredients were introduced, while in 1995, low fat and low-calorie baked goods annual sales reached \$720 million (Shukla, 1995).

Carbohydrate fat-replacers play an important role in mimicking the sensation of fat in baked goods, primarily by binding water and bulking of their solids. Carbohydrate-substitutes also aid in reducing fat because they provide 1-4 kcal/g rather than 9 kcal/g of traditional fats (Lindsay, 1996). Carbohydrate-substitutes include polydextrose, altered sugars, starch derivatives, cellulose, hemicellulose and gums (Glueck *et al.*, 1994).

Sal seed and Mango kernel contains at around 62.7 and 70% starch as major biopolymer, respectively. Its isolation and use in food sector is considered to be important for improving the economy of industry based on these tree born seeds. Utilization of these starches has been very inadequately investigated. It is reported that Mango starch is used as rice substitute in scarcity, but when it comes to Sal starch there is only one report which shows the uniqueness as maize starch substitute in making pharmaceutical tablets. However, there are opportunities of developing processes such as hydrolysis process for making water soluble sugars as fat replacer.

There has been an increased exploitation of organic residues as de-oiled cake from the sector of agricultural industries over the past few decades. However, with increasing emphasis on cost reduction of industrial processes and value addition to agro-industrial residues new applications of de-oiled cakes are highly desired.

Chemical production from carbohydrate rich waste materials has received considerable attention in recent years. Pre-treatment and hydrolysis are required stages during processing the waste materials for the purpose. Present study focuses on the study of enzymatic hydrolysis of mango starch to maltodextrin, evaluating the performance of a maltodextrin gel as a replacement (25, 50, 75, and 100%) for shortening, to assess the effect of the maltodextrin gel on the qualities in the various cake treatments and to assess the effect of the malto dextrin gel on the variations.

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POSTER II/4

## Lipase Catalyzed Interesterification of Rice Bran Oil with Hydrogenated Cottonseed Oil to Produce Trans Free Fat

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Lipase catalyzed interesterification of rice bran oil (RBO) with hydrogenated cottonseed oil (HCSO) was carried out for producing a low trans free fat. The interesterification reaction was performed by varying parameters such as weight proportions of RBO and HCSO, reaction temperatures, time period and lipase concentration. Both non-specific and specific lipases namely Novozym 435 and Lipozyme TL IM were employed for this study. Based on the data generated, the optimum reaction conditions were found to be: weight proportion of RBO and HCSO, 80:20; lipase concentration, 5% (w/w) of substrates; reaction temperature, 60°C; reaction time, 4 h for Lipozyme TL IM and 5 h for Novozym 435. The degree of interesterification, calculated based on the results of solid fat characteristics was used for comparing the catalytic activity of Novozym 435 and Lipozyme TL IM. It was observed that the degree of interesterification (DI) reached a near 100% at the 4th hour for reaction employing Lipozyme TL IM with a rate constant of  $0.191\text{h}^{-1}$  while Novozym 435 catalyzed reaction reached a near 100% degree of interesterification at the 5th hour with a rate constant of  $0.187\text{h}^{-1}$ , suggesting that Lipozyme TL IM has a faster catalytic activity.

Nature and scope of work: The aim of the investigation was to prepare a trans free fat employing enzymatic transformation and therefore, the widely studied lipases (Novozym 435 and 1, 3 specific lipase like Lipozyme TL IM) were selected for the present study.

Conclusion: Interesterification of RBO and HCSO was optimized in terms of weight proportion of substrate mixture, lipase concentration, reaction temperature and time. The study revealed that a weight proportion of 80:20 (RBO:HCSO), lipase concentration of 5% and reaction temperature of 60°C yielded the desired product for both the lipases Novozym 435 and Lipozyme TLIM. Based on the degree of interesterification, our results

indicate that Lipozyme TLIM showed a faster degree of conversion with a reaction rate of 0.191 h<sup>-1</sup> at the 4th hour. Novozym 435 on the other hand showed a reaction rate of 0.187 h<sup>-1</sup> at the 5th hour. Hence, it is concluded that TLIM produces the structured fat at a faster rate and has a higher catalytic activity.

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#### POSTER II/5

### *Moringa oleifera*: A Potential Source of Nutrition and Edible Oil

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*Moringa oleifera*, is one of the best known and most widely distributed and naturalized species of a monogeneric family Moringaceae. Almost all the parts of this plants have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders. Leaves of *M. oleifera* have been reported to regulate thyroid status and possess radio protective and antitumor activities. Pod showed hypotensive and chemo modulatory effects where as seeds have been reported for coagulative, antimicrobial and antitumor activity. *Moringa oleifera* seed oil is pleasant tasting, highly edible and resembles olive oil in its fatty acid composition.

*Moringa oleifera* seeds collected from IIT Delhi campus were analyzed for oil content, oil quality and the fatty acid composition. The oil extracted from seeds and kernels using soxhlet method were 22.4% and 32.6% respectively. The acid value, iodine value, peroxide value, saponification value and unsaponifiable matter were determined following standard method. The result of gas chromatographic analysis of fatty acid methyl ester of moringa oil showed the presence of 73.22 ± 0.50 %, C18:1 *i.e* oleic acid. The percentage of behenic acid, specific to moringa oil was found to be 6.16 ± 0.15%. The induction period of the oil determined by Rancimat was 15.5hrs. High oleic acid vegetable oils have been reported to be very stable even in highly demanding applications like frying. Therefore moringa oil can be used as an excellent source of cooking oil and can also be blended with vegetable oil to increase their stability. To obtain sufficient quantity of moring a seeds and kernels large scale cultivation of moring a should be couraged.

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#### POSTER II/6

### Enhancement of oxidative stability of vegetable oils by using spice extract

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Antioxidants are used in food industries to prevent free radical damage and lipid pro-oxidant formation. Currently various synthetic antioxidant are used such as BHA, BHT, TBHQ. Some of the synthetic antioxidants are not suitable in food industry. Therefore demand for natural antioxidants increasing day by day due to their increasing use in different types of food products as well as increasing consumer awareness about natural antioxidants. Herbs and spices are the good sources of natural anti oxidants.

Ginger (*Zingiber officinale Roscoe*) is a well-known and widely used spice, especially in Asian countries. Ginger contains several interesting bioactive constituents in its essential oils and oleo resins. The average yield of the ginger rhizomes in India is around 7,000 - 10,000 kg/hectare. Ginger extract shows almost equal antioxidant activity to that of BHA and BHT. The major antioxidant compound in ginger oleoresin is [6]-gingerol with more pungency and antioxidant activity than [8]-gingerol and [10]-gingerol. In the present study the attempt has been made to isolate [6]-gingerol in concentrated form by using super critical CO<sub>2</sub> extraction method.

Super critical fluid extraction of ginger was carried out in the lab-scale SFE-RESS unit. The process conditions were optimized to increase the recovery of [6]-gingerol by varying pressure (100-200 bar), temperature (30-40°C) and CO flow rate (20-40 g/min). The CO extracts of ginger were analyzed by HPLC to determine the concentration of 6-gingerol. The ginger extracts were further proceed as an additive in edible oil to enhance their oxidative stability.

Two edible oils like peanut and linseed were chosen for oxidative stability study according to their degree of unsaturation. Because possibility of oxidation increases with increase in unsaturation. Fatty acid profile for oleic acid and linolenic acid were analysed through gas chromatography. The experiments has been performed in rancimat-743 in order to know the antioxidant effect of different concentration of extracted [6]- gingerol (25%, 40%). This extracts were added in

the oil at 100-300 ppm level. Experimental conditions for rancimat-743 was temperatures (110-130°C) at constant air flow rate (20 L/h). In this study SC-CO<sub>2</sub> ginger extract with 40% [6]- gingerol concentration was found to be better antioxidant to enhance the shelf life of edible oils. Obtained results were comparable with commercial synthetic antioxidants.

**KEYWORDS:** *Zingiber officinale*, SC-CO<sub>2</sub> extraction, Antioxidants, Edible oils, Oxidative stability.

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#### POSTER III/7

### Oxidative Stability Studies on Castor and Tapioca based Eri Pupal Oils

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The present study was performed to investigate the effect of synthetic and natural antioxidants like TBHQ, BHT and Oryzanol (at 0.1% level) by direct addition to the crude and refined silkworm oils at 70°C. The control samples and antioxidant added samples were collected for every 24hr time interval from 0hr to 168hr. The progress of the lipid peroxidation was assessed in terms of peroxide value, p-anisidine value, FFA and evaluated for inhibition of oil oxidation (IO) and total oxidation state (TOTOX Value). The peroxide value increased from 3.42 to 17.66 and 3.98 to 19.60 meq. of peroxide/kg in castor based and tapioca based crude silkworm pupal oils respectively during 0 - 168 hr. After addition of antioxidants the peroxide value was found to be 9.5 and 11.0 in castor and tapioca based pupal oils compared to the control samples which showed a peroxide value of 17.66 and 19.60 respectively. Similarly the p-Anisidine value also found to be lower with values of 14.5 and 17.2 compared with control samples which had values of 21.08 in castor based and 24.1 in tapioca based pupal oils respectively. In refined castor and tapioca based pupal oils higher peroxide values were observed even after addition of antioxidants compared to crude pupal oils. It was observed that all the three antioxidants exhibited more or less similar anti oxidative effects based on the analysis performed. In conclusion oxidative stabilities were found to be superior in the case of crude oils

compared to refined oils due to the presence of tocopherols and phospholipids. As the silkworm pupal oils are rich in ω-3 fatty acids, it is essential to add antioxidants to the refined oils to achieve better shelf life and also to protect from auto oxidation.

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#### POSTER III/1

### Novel Glycerol-Based Surfactant: Synthesis, Characterization and Performance Properties

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Production of surfactant from renewable sources is important as this reduces environmental hazards since product is highly biodegradable. The synthesis of novel glycerol based non-ionic Gemini surfactant was carried out in two stages in the present research work. Initially glycerol ester was synthesized by transesterification using cotton seed oil, glycerol and calcium oxide as catalyst at higher temperature followed by reaction with 1, 6-dibromohexane to form the Gemini surfactant. The chemical structure of targeted product was tested by FTIR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectroscopy.

The surfactant properties such as surface tension, critical micelle concentration, effectiveness, foaming power and stability, emulsifying power, wetting power, dispersion capability and contact angle were studied. The effects of this surfactant on the solubilisation of a polar and non-polar solute were studied. The synthesized non-ionic Gemini surfactant can be used as wetting agent, foaming agent and an emulsifier.

**KEYWORDS:** Gemini surfactant, characterization, performance properties, contact angle measurement, solubilisation behaviour.

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#### POSTER III/2

### Synthesis, Surface Properties and Antimicrobial Activity of Sulfosuccinate Monoester Surfactant

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In this study, the low temperature non catalytic process for synthesis of mild anionic surfactant-disodium salts of sulphosuccinate monoester from maleic anhydride and fatty alcohols having variable chain length ( $C_{12}$ ,  $C_{16}$ ,  $C_{18}$ ) is reported. The synthesized surfactant, after purification with petroleum ether, were analyzed for the physico-chemical characterizations such as acid value, hydroxyl value, saponification value, cloud point, melting point, pH, active matter, % sulphite content, % yield, foaming and dispersion power. The chemical structures of the synthesized surfactant we reconfirmed using FTIR and  $^1H$ -NMR analysis. The surface properties of sulpho succinate including surface tension, critical micelle concentration, maximum surface area, minimum surface area, Gibbs adsorption, enthalpy and free energy of micelle formation were also determined and interpreted for the analysis of the micellization process in relation to their chemical structures. Antimicrobial properties were evaluated against following bacteria: *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. The use of synthesized sulphosuccinate monoesters in synthesis of nanoparticles and dispersing agent for preparation of water based pigment concentrate were demonstrated.

**KEYWORDS:** Fatty alcohol, surface activity, critical micelle concentration, antimicrobial activity.

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#### POSTER III/3

### Synthesis, Characterization and Physico-chemical Properties of Ethanol Amide Based Non-Ionic Gemini Surfactant from Renewable Sources

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The syntheses of novel Gemini surfactants were carried out in two stages in the present research. Initially ethanol amines were converted into ethanol amide by using four different fatty acids followed by reaction with 1,2,7,8-Diepoxy to form the Gemini surfactant. The new Gemini surfactants were characterized using chemical analysis like FTIR,  $^1H$ -NMR and  $^{13}C$ -NMR spectroscopy. The surfactants

properties such as surface tension, critical micelle concentration, foaming power and stability, emulsifying power, wetting power, dispersion power, contact angle and solubilisation behaviour were studied. Synthesized Gemini surfactant has generally good physico-chemical properties.

**KEYWORDS:** Gemini surfactant, synthesis, characterization, physico-chemical properties, contact angle, solubilisation behaviour.

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#### POSTER III / 4

### Synthesis, Characterization and Performance Properties of Anionic Sodium Methyl Ester Sulphonate Surfactant from Renewable Sources by Microwave Irradiation

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The transesterification of palm oil was carried out under microwave irradiation to prepare methyl ester of Palm oil (MEPO). MEPO thus obtained was sulphonated at refrigerated condition using chloro sulphonic acid as a sulphonating agent. The resulting product after subsequent purification was neutralized with alkali to yield anionic surfactant, sodium methyl ester sulphonate (SMES). The synthesized surfactant was characterized by FTIR,  $^1H$ -NMR and  $^{13}C$ -NMR spectral techniques. The performance properties of surfactant such as critical micelle concentration (CMC), Kraft point, emulsifying power and foaming power were determined.

**KEYWORDS:** Microwave, anionic surfactant, FTIR, surface tension, CMC, Kraft point.

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#### POSTER III/5

### Synthesis and Properties of Gemini Surfactant Using Palm Oil

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Benzoic ester ether amide non-Gemini surfactant was synthesized by using a one-shot technique at room temperature from diol palm oil fatty amide [HECA a monomer obtained from the aminolysis of renewable resource such as palm oil with diethanolamine and sodium methoxide used as catalyst], 0.1 mole of fatty amide react with 0.1 mole of benzoic acid in the presence of catalyst  $H_2SO_4$ , 50ml of xylene as a solvent to give poly ester amide. The poly ester methoxy amide was synthesized by 0.1 mole of poly esteramide react with sodium methoxide and further reaction of poly methoxy amide react with poly ester amide in presence of conc.  $H_2SO_4$  and toluene to gives the benzoic ester ether amide non ionic Gemini surfactant this reaction followed by claisen condensation reaction. The mode of reaction and structure of non ionic Gemini surfactant was confirmed by surfactant properties, spectral analysis, FT-IR,  $^1H$ -NMR and  $^{13}C$ -NMR. Its surface active properties in water were also reported, including surface tension, wetting ability, foaming properties (LSDR) and emulsifying.

**KEYWORDS:** Gemini surfactant, cotton seed oil, surface tension, wetting ability, foaming properties, emulsifying.

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**POSTER III/6**

**Biosurfactants from Renewable Sources By Using *Candida Antarctica* Lipase-B Enzyme**

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The sorbitol molecule contains six hydroxyl groups able to be esterified with carboxylic acids. Sorbitol esters were prepared by lipase catalyzed controlled esterification reaction between sorbitol and fatty acids. The enzymatic reactions were carried out by the reaction of sorbitol and various fatty acids like oleic, lauric, palmitic at temperature 45-50°C in orbital shaker incubator at 200 rpm for 48h. In this study, enzymatic reactions were performed in mixtures of organic solvents pure DMSO and Tert. Butanol in 80:20 ratio in percentage (v/v). This synthesis strategy gave complete conversion of SMO (92%), SMP (82%) and SML (85%). The purity and chemical structures of synthesized non-ionic biosurfactants were determined by TLC,  $^1H$  NMR,  $^{13}C$  NMR, HPLC and FTIR spectroscopy.

**KEYWORDS:** Biosurfactant, renewable sources, *Candida antarctica* lipase B, characterization.

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**POSTER III/7**

**AMINO ACID BASED GEMINI SURFACTANTS DERIVED FROM LYSINE AND FATTY ACIDS**

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Surfactants are amphiphilic compounds which can reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids and increase the solubility, mobility, bioavailability and subsequent biodegradation of hydrophobic or insoluble organic compounds. Amino acid based surfactants play a important role in the field of novel surfactants research, mainly because of their environment friendly properties. These surfactants have various applications in the area of life sciences as in drug carriers, DNA transfection, anti viral agents and in gene therapy. The hydrophobic group can be attached to amino acids either by the amine or by carboxylic moiety, leading to four types of different surfactants. This work focuses on the study of amino acid based gemini surfactants synthesized by reacting L-lysine with fatty acids. The effect of reaction variables like molar ratio of raw materials and temperature has been studied for the synthesis of these gemini surfactants. In preparation of sodium dilauraminolysine (SDLL) and sodium dimyristaminolysine (SDML) gemini surfactants, fatty acids (lauric/myristic) were reacted with thionyl chloride to obtain respective acid chlorides (lauroyl/myristoyl). These acid chlorides were further reacted with L-Lysine at different reaction conditions to get amino acid based gemini surfactants. The study revealed that 1:2.5 molar ratio of reactants viz. L-lysine and lauroyl/myristoyl chloride and 10-150C reaction temperature resulted in maximum yield of amino acid based gemini surfactants (SDLL and SDML). The structures of synthesized amino acid based gemini surfactants were also confirmed by FT-IR,  $^1H$  NMR and ESI-MS spectral data.

**KEYWORDS:** Gemini surfactant, cotton seed oil, surface tension, wetting ability, foaming properties, emulsifying.

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## POSTER III/8

### Synthesis, Characterization and Physico-chemical Properties of Ethanol Amide Based Non-Ionic Gemini Surfactant from Renewable Sources

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The syntheses of novel Gemini surfactants were carried out in two stages in the present research. Initially ethanol amines were converted into ethanol amide by using four different fatty acid followed by reaction with 1,27,8-Diepoxydetoformthe Gemini surfactant. The new Gemini surfactants were characterized using chemical analysis like FTIR, <sup>1</sup>H-NMR, and <sup>13</sup>C-NMR spectroscopy. The surfactants properties such as surface tension, critical micelle concentration, foaming power and stability, emulsifying power, wetting power, dispersion power, Contact angle and Solubilization behaviour were studied. Synthesized gemini surfactant has generally good physico-chemical properties.

**KEYWORDS:** Gemini Surfactant, Synthesis, Characterization, Physico-chemical properties, Contact angle, Solubilization Behaviour.

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## POSTER III/9

### Syndet bars: Manufacturing and its assessment

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Syndet bars are speciality soaps. The word "syndet" is derived from "synthetic" combined with "detergent". Usually soaps are manufactured by saponification process. But syndet bars are made from synthetic surfactants. These surfactants are made from oils, fats, or petroleum products that are processed in some way other than saponification. These synthetic

surfactants make syndet bars the mildest personal cleansing bars. Dove®, which was launched in 1955, was the first syndet bar produced. Soaps and synthetic toilet bars are used for the same purpose but they are relatively different in formulation types, behavior of the components, and performance of the products.

Syndet bars are produced by melt and cast process. Maximum required temperature for this process is 95°C. All the ingredients are heated and mixed well in a mixer which is equipped with homogeniser. When the soap mass is ready with all the constituents completely mixed, the mass is cast into moulds and it is allowed to solidify. Then after solidification, the mass is taken out from the moulds and then stamping is done. This process gives good surface finish to the soap bar and makes them free from grittiness. Quality of syndet bars is checked by measuring leather volume, foam height, PV, pH, active matter, FFA & moisture.

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## POSTER IV/1

### Chemical, Fatty Acid Composition and Antioxidant Activity of *Momordica tuberosa* Roxb.

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Adavikakara (*Momordica tuberosa* Roxb.) is a highly perishable underutilized vegetable available during August to November every year in Andhra Pradesh, Maharashtra, Tamilnadu and Karnataka. The seed and pulp extracts have been reported to possess hypoglycaemic, antihelminthic and hepatoprotective activity in rats and a flavonoid rutin was found to an extent of 0.27% in the methanolic extract of *M. tuberosa*. In the present study, chemical, fatty acid composition of whole fruit of *M. tuberosa* and antioxidant activity of dehydrated whole fruit, pulp and seeds was investigated. The yield of *M. tuberosa* on dehydration was 21.4% which possessed 21.76% protein and 33.96% fibre. Total polyphenols were observed to be 0.45 g/100 g in dehydrated whole fruits, 0.98 g/100 g in dehydrated fruit pulp and 0.49 g/100 g in seeds. The total lipid of the dried seed (13.3%) was rich in oleic (22.05%) and linoleic acids (25.09%), while linolenic

acid was found to an extent of 6.17%. The ethanolic extract of whole fruit, pulp and seed of dehydrated *M. tuberosa* were screened for their antioxidant activity and compared with that of trolox. IC<sub>50</sub> for 1,1-diphenyl-2-picrylhydrazyl (DPPH) was higher for pulp (6 mg/ml) when compared to whole fruit (14 mg/ml) and seed (20 mg/ml). The ABTS radical inhibition assay was found to be very high with whole fruit (98.4%), followed by pulp (50.76%) and seed (16.14%) at 2 mg/ml. In case of ferric ion reducing power minor variation was observed among all three samples at 10 mg/ml level. The nutritional and antioxidant potential of *M. tuberosa* warrants attention to initiate its processing and value addition.

**KEYWORDS:** *Momordica tuberosa*, *Adavi kakara*, DPPH, ABTS, flavonoidrutin

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#### POSTER IV/2

### Ecological Aspects of Used Lubricating Oils

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A lubricant is a substance applied between two moving surfaces to reduce friction and wear. The subject of lubrication is so important that in Western industrialized nations alone 0.4% of gross domestic product could be saved in terms of energy with proper application of current tribological knowledge (science of friction, wear and lubrication) to lubricated processes. There are three possible ways to classify wide variety of lubricants, namely, a general classification based on the source of lubeoil base stock, e.g., mineral lubricants (oils, greases, solid lubricants), synthetic lubricants (polyalphaolefins, polyglycols, ester oils, silicones), vegetable lubricants and animal lubricants; a second classification based on application, e.g., automotive lubricants (engine oils, gear oils) and industrial lubricants (hydraulic oils, cutting fluids, way lubricants, compress or oils, quenching and heat transfer oils, rust protection oils, transformer oils, turbine oils, chain lubricants, wire rope lubricants) and the third classification based on the type of additive used in the lubricant, e.g., extreme pressure, anti-

wear, friction modifiers, corrosion inhibitors, anti-oxidants, dispersants, detergents, anti-foaming agents, pour point depressant, etc.

The per capita consumption of lubricants in 2004 was highest in North America (24.9 kg per annum) and lowest in Africa (2.1 kg per annum), while the global average was 5.6 kg per annum. It is estimated that 50% of the lubricants sold worldwide are lost because of spillage and evaporation and thus pollute the environment. The ecological aspects of these used as well as lost lubricating oils are poorly understood as only limited data exist on the collection of used lubricants and that too are not readily available. The objective of the present study is to disseminate the information collected from the scarcely available literature on the potential risk of used lubricating oils as potent environmental pollutant.

**Lubricating oils basically pollute in all stages of use:** during shipment to the sites of use, prolonged storage in tank, during use in machines and during collection and recovery after their working life. A variety of microorganism species (microscopic fungi and bacteria) develop during hardware storage and operation of various devices in different climatic regions, both cold and very hot and dry. Heavy metals are absorbed to the lubricants by abrasion of metal parts in engines. Used oils are often utilized as boiler or furnace fuel. Although, it gives a certain saving of fuel and energy resources, but the significant adverse effects are borne by the environment. For each 100 tons of used lubricating oil burned, on the average 1.4 tons of sulphur dioxide is discharged into the atmosphere. Besides, the ash that is formed by the combustion of used oils contains compounds of iron, copper, aluminium, chromium, lead and other metals as well as the various additives that are used in commercial oils, containing barium, magnesium, phosphorous and sulphur that are harmful for the biosphere.

In order to minimize such effects, the industry has shifted its focus towards developing bio-based lubricants and synthetic lubricants. Bio-based lubricants have the potential to reduce the amount of toxic metals found in engine sludge and can increase fuel economy by 3-5% over conventional oils. In addition, a little awareness and self discipline from the end-users can result in unprecedented results. The production of 1 kilolitre (kl) lube oil base stock requires 67 kl lube bearing crude oil to be processed, while re-refining of used lubricating oil requires only 1.6 kl used lubricating oil to produce 1 kl finished lube oil base stock as good as the virgin

lube oil base stock. Thus, re-refining of used lubricating oil is an equally important strategy to mitigate the environmental risks of used lubricating oils.

**KEYWORDS:** Microscopic fungi and bacteria, heavy metals, re-refining, used lubricating oils, environmental risks, bio-based lubricants.

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#### POSTER IV / 3

### Mustard-Estolide Ester Synthesis for Enhancement of Tribological Properties

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Eco-friendly and sustainable resources such as vegetable oils needs to be developed for replacing the fossil feedstock. The aim of present investigation was to improve cold flow properties of easily available renewable mustard fatty acids through chemical transformation into estolide esters of different saturated fatty acids such as lauric and isobutyric acid and thus to meet their acceptance as alternate feedstock for development of industrial lubricants. The estolide synthesis was conducted under microwave, ultrasound and high pressure mode in absence of solvent and different acid through use of catalysts such as methane sulphonic acid and sulphamic acid. The quantitative availability of double bonds and carbonyl group as sites for esterification with saturated fatty acids to yield mustard-estolides (MuE) was monitored through FTIR and GPC analysis. Further capping of residual –COOH groups was carried with branched alcohol-isoamyl alcohol to yield MuE-ester with superior low temperature properties (pour point ranging from -25 to -35°C) and high viscosity index (148-180). The overall influence of variables such as nature and amount of catalyst, mode of synthesis (microwave, ultrasound and high pressure), retention time and reactant molar ratio on tribological properties of MuE-esters have been presented. Techno-commercial evaluation of mustard based bio-lubricants in reference to petroleum oils has been discussed. Use of green and noncorrosive material-sulphamic acid as catalyst and solvent free microwave/high pressure synthesis supports the prospects of commercialization of MuE-esters based bio-lubricants.

**KEYWORDS:** Estolide, mustard oil, bio-lubricant, pour point, viscosity index.

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#### POSTER IV/4

### Utilization of Acid oil for Synthesis of Alkyd Resin

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Acid oil, which is a by-product in vegetable oil refining, mainly contains free fatty acids (FFAs) and acyl glycerols. Alkyd resins from acid oil have been prepared by employing two stage alcoholysis-polyesterification processes. Different types of alkyd resins have been developed from acid oil by varying the amount of phthalic and maleic anhydride. The prepared resins are cured by using methylethyl ketone peroxide (MEKP) as initiator and co-octoate as an accelerator at 120°C. The characterizations of the resins for structure establishment is carried out using Fourier transform infrared (FTIR) technique. The concomitant properties of the cured resins such as acid value, saponification value, viscosity, *etc.* are also evaluated by standard methods. The coating performance of the cured resins was tested by measurement of chemical resistance, pencil hardness, adhesion and gloss, *etc.*

**KEYWORDS:** Acid oil, alkyd resin, FTIR, alcoholysis - polyesterification, initiator, saponification value.

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#### POSTER IV / 5

### Wastewater Treatment in Chicken Fat Extraction Industry for Possible Reuse

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In a bid to generate wealth from waste, some industries are extracting fats from chicken wastes for various industrial uses. In the process, huge amount of wastewater is generated and for a typical industry extracting 2 tons of chicken fat, 10 tons of wastewater is generated. That wastewater is contaminated with protein, fat, blood, feathers, bones and small amount of inorganic matters. Due to presence of all these contaminants, the wastewater samples have very bad odour, dark colour, high TDS, COD and BOD. The present work describes a process for the treatment of

this wastewater for possible reuse. In this investigation, a membrane based approach was adapted to obtain the quality of water that can be reused. The raw wastewater was found to have pH 7.63. The conductivity, TDS and COD were found to be 3040 mg/L, 2103 mg/L and 3600 mg/L, respectively. The wastewater was initially filtered through ordinary filters to remove all suspended solids. Then it was subjected to coagulant treatment. The coagulant dosages were optimized for maximum level of coagulation. The pH of the wastewater was adjusted to 7. Then the water was treated with 0.2% of Powdered Activated Charcoal (PAC). The colour and the odour were reduced significantly. This water was then passed through a MF filtration membrane (0.45 µm, Millipore Corporation, USA) using a dead end type membrane test cell. The permeate of this filtration was then passed through a reverse osmosis membrane (BW30, Film Tec. Corporation, DOW Chemical Company, USA). The permeate was found to be colour less and odour less. The treated water thus obtained had the following characteristics: pH of 6.9, 121mg/L of conductivity, 103 mg/L of TDS and 90 mg/L of COD. This treated water can be reused for various purposes. This process, if implemented in the industry, will definitely help in reducing the environmental pollution significantly.

**KEYWORDS:** Chicken fat, powdered activated charcoal, permeate, filtration membrane, environmental pollution.

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#### POSTER/IV/6

### Novel Route of Sucrose Ester Synthesis under Microwave Irradiation Using Renewable Sources, Characterisation and Study of Their Some Performance Properties

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In modern days, because of the economical causes choosing professional transesterification method for production of non ionic surfactant has become important. Using microwave assisted unit for the transesterification reaction provides high yields of highly pure products within a short time. We form a non-ionic surfactant

from palm methyl ester and sucrose by novel microwave assisted synthesis in presence of sodium methoxide (CH<sub>3</sub>ONa) as catalyst, using DMF as a solvent. The chemical structure of synthesized surfactant was determined by FTIR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR. Performance properties of synthesized surfactant like surface tension, CMC, effectiveness, wetting power, emulsifying power were studied.

**KEYWORDS:** Microwave, non-ionic surfactant, FTIR, surface tension, CMC, emulsifying power, wetting power.

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#### POSTER IV/7

### Synthesis and Lubricant properties of estolides from castor fatty acid esters and dicarboxylic acids

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Castor oil is an attractive feedstock for the preparation of estolide esters due to the presence of 85-90 % ricinoleic acid. The hydroxyl functionality of methyl ricinoleate was esterified with different dicarboxylic acids to prepare a series of a new class of estolides. The products were characterized by <sup>1</sup>H NMR, <sup>13</sup>CNMR, FT-IR, and ESI-MS analysis and evaluated for their lubricant properties namely viscosity, viscosity index (V.I), and pour point. The synthesized products have ISO VG 46 viscosity and exhibited excellent viscosity index. All the products exhibited excellent low temperature properties (-54°C) out performing commercial lubricants. The products are useful as lubricant base stocks for environmentally friendly industrial oils and automotive applications.

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#### POSTER / IV / 8

### Formulation of Water-in-Diesel Micro emulsion as a Biofuel: Phase Study, Interfacial Composition, Effect of Chain Length of Co-surfactant and Viscosity

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The present study is focused on the formulation and characterization of water-in-diesel fuel micro emulsion. The blends of non-ionic surfactants were used and it was found that the hydrophilic surfactant performs dominating role over lipophilic surfactant present in the blend. Further, the chain structure of surfactant also plays an important role in micro emulsion formulation. The interfacial composition of fuel micro emulsion was also investigated by Schulman's method of co-surfactant titration. Alkanols of different chain length (n-butanol, iso-butanol, n-hexanol and n-octanol) were used to determine the effect of co-surfactants on fuel micro emulsion formulation. The distribution of alkanols between interfacial region and oil (diesel) phase was also investigated. It was found that the efficiency of alkanol increases with increasing chain length. Further, a linear chain alkanol gives better performance than that of branched alkanol containing same carbon number. The fuel properties like viscosity, cloud and pour points were also determined and were found to be effective to be used as a fuel.

**KEYWORDS:** Fuel micro emulsion, Blend of surfactant, Co-surfactant, Interfacial composition, Viscosity.

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#### POSTER IV/9

### Soya and Rice Bran Lecithin-based Organogels

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India is the 5th largest producer of soybean oil (107 lakh tons in 2012-13) in the world and produces 3-4 MMT tons of rice bran oil annually. Soybean and rice bran lecithins are thrown into waste obtained during refining of crude oil. These lecithins can gel with water and other solvents to form thermo dynamically stable, biocompatible surfactants which can be used as food and DDS. The present study involved optimization of the ratio of solvents, water and lecithin (soya/rice bran lecithin and their hydroxylated

derivatives) to prepare stable gels. Rice bran lecithin formed as table gel in dimethyl formamide (DMF) and water (0.2:0.2:0.03, wt of lecithin/vol of DMF/vol of water); soya lecithin in hexane and water (0.1:0.25:0.04, wt/vol/vol); terta hydrofuran and water (0.1:0.18:0.03, wt/vol/vol); acetonitrile and water (0.2:0.2:0.03, wt/vol/vol) and Hydroxylated lecithin in hexane and water (0.4:0.2:0.03, wt/vol/vol), cyclohexane and water (0.5:0.2:0.03, wt/vol/vol). The effect of the lecithin and its derivatives on gelation properties like gelation concentration, gelation time and gel melting temperatures were studied. Choosing a particular gelator and a specific ratio of solvent and water, the effect of the concentration on the gelation properties were studied. The thermal studies using DSC revealed the presence of phase transitions corresponding to the premelting and melting of the gels during the heating cycle. Thus lecithin and its derivatives exhibiting gelation ability in a wide range of solvents can be used for making functional gels for various applications.

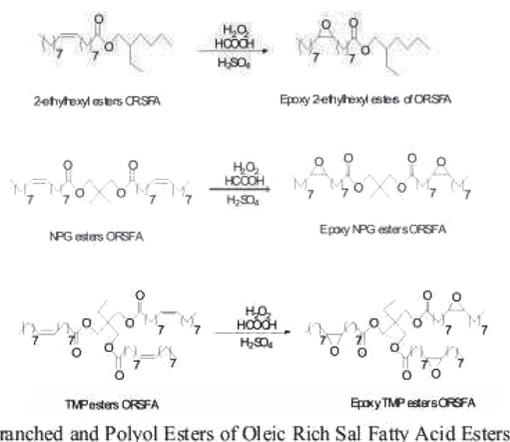
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#### POSTER/IV/10

### Influence of Structural Modification on Lubricant Properties of Sal Fat-based Lubricant Base Stocks

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With an attempt to improve the pour point of the sal fatty acid based lubricant base stocks, oleic rich sal fatty acids (ORSFA, 73.2% by GC) were reacted

with 2-ethylhexanol and polyols namely neopentyl glycol (NPG) and trimethylol propane (TMP) to obtain the corresponding branched mono- and polyol esters. These esters were further epoxidized to their corresponding epoxy esters. All the base stocks were evaluated for lubricant properties and compared with different lubricant specifications. The oleic rich branched mono- and polyol esters of sal fatty acids exhibited low pour points (-9°C to -3°C) and high viscosity indices (178-201). While, the epoxy branched mono- and polyol esters exhibited higher viscosities, high flash points, good thermal and oxidation stabilities compared to oleic rich sal fatty acid-based lubricant base stocks and sal fatty acid-based lubricant base stocks. Overall, all the base stocks have potential to be developed into hydraulic, metalworking fluids and other industrial applications with appropriate formulations.

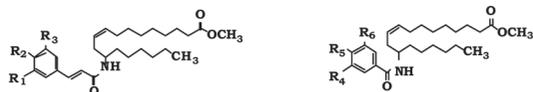
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POSTER IV/11

**Synthesis and Antioxidant Activities of Novel Methyl 12-Amino octadec-9-enoate based Phenolipids**

**Y. Mohinia, K. Shiva Shankera, R. B. N. Prasada, M. S. L. Karuna\*, Y. Poornachandrab, C. Ganesh Kumarb**

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- |  |   |
|--|---|
| 41a) R <sub>1</sub> = H, R <sub>2</sub> =H, R <sub>3</sub> =H                                  | 2a) R <sub>4</sub> = H, R <sub>5</sub> = OH, R <sub>6</sub> = H   |
| 1b) R <sub>1</sub> = OH, R <sub>2</sub> = OH, R <sub>3</sub> = H                               | 2b) R <sub>4</sub> =OCH <sub>3</sub> , R <sub>5</sub> =OH, R <sub>6</sub> =H                                  |
| 1c) R <sub>1</sub> = OCH <sub>3</sub> , R <sub>2</sub> = OH, R <sub>3</sub> = H                | 2c) R <sub>4</sub> = OH, R <sub>5</sub> = OH, R <sub>6</sub> = OH   |
| 1d) R <sub>1</sub> = OCH <sub>3</sub> , R <sub>2</sub> = OH, R <sub>3</sub> = OCH <sub>3</sub> | 2d) R <sub>4</sub> = OCH <sub>3</sub> , R <sub>5</sub> = OCH <sub>3</sub> , R <sub>6</sub> = OCH <sub>3</sub> |
| 1e) R <sub>1</sub> = H, R <sub>2</sub> = OH, R <sub>3</sub> = H                                |   |

A series of novel phenolipids were synthesized by coupling of methyl 12-amino octadec-9-enoate with different substituted aromatic phenolic acids. The substrate methyl 12-amino octadec-9-enoate was synthesized from methyl ricinoleate employing a 4-step procedure. The synthesized phenolipids were thoroughly characterized by FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectral studies. *In vitro* antioxidant activities of the synthesized compounds were evaluated for 2,

diphenyl-1-picrylhydrazyl (DPPH.), super oxide free radical scavenging activity and lipid peroxidation inhibitory activity. The antioxidant activities were compared with the commercial antioxidants butylated hydroxy toluene (BHT) and  $\alpha$ -tocopherol as reference compounds. The antioxidant screening data of all the synthesized compounds revealed that DPPH free radical scavenging activity, superoxide and lipid peroxidation inhibitory activity of caffeic and gallic acid substituted phenolipids showed excellent activity which was better than the commercial antioxidants BHT and  $\alpha$ -tocopherol.

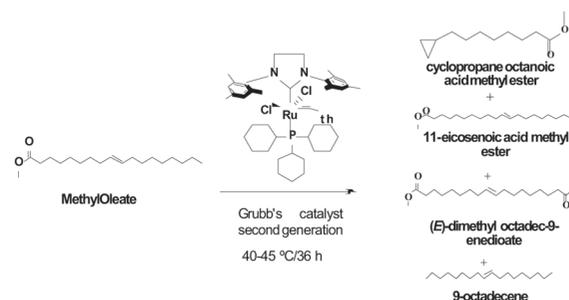
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POSTER IV/12

**Influence of Concentration of Grubbs Catalyst on Self-metathesis of 9-Octadecenoic Acid Methyl Ester**

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**Self Metathesis of 9-Octadecenoic Acid Methyl Ester**

With the increase in environmental concern, the demand for renewable sources and modern, greener synthetic routes is growing day-by-day. A number of self and cross-metathesis reactions were carried out on oleic acid, different vegetable oils, to develop a range of organic intermediates useful for lubricants, plasticizers, cosmetics, and grease applications employing Grubbs II generation catalyst. The present study involved self-metathesis of 9-octadecenoic acid methyl ester varying the concentration of Grubbs' II generation catalyst from 0.03 mmol to 0.18 mmol at 40-45 °C for 36 h to understand the influence of catalyst concentration on olefin metathesis. Only two products (9-octadecene 30%, and dimethyl-9-octadec-

dienoate 23%) resulted when 0.06 mmol of catalyst was employed, while at other concentrations four products. It was observed that 9-Octadecene generated at 0.03, 0.06 and 0.12 mmol completely disappeared and dimethyl-9-octadec-dienoate (64%) was observed in major amounts at 0.18 mmol concentration. The study also reveals the possible mechanism followed for the generation of the above products.

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#### POSTER IV/13

### A Green Recyclable $\text{SO}_3\text{H}$ -carbon Catalyst Derived from Glycerol for the Production of Biodiesel from FFA-containing Karanja (*Pongamia glabra*) Oil in a Single Step

**T. Vijai Kumar Reddy, B.L.A. Prabhavathi Devi \*, K. Vijaya Lakshmi and R.B.N. Prasad**

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Simultaneous esterification and transesterification method is employed for the preparation of biodiesel from 7.5% free fatty acid (FFA) containing karanja (*Pongamia glabra*) oil using water resistant and reusable carbon-based solid acid catalyst derived from glycerol in a single step. The optimum reaction parameters for obtaining biodiesel in >99% yield by simultaneous esterification and transesterification are: methanol (1:45 mole ratio of oil), catalyst 20 wt.% of oil, temperature 160 °C and reaction time of 4 h. Employing the optimum reaction conditions, karanja oil biodiesel was also prepared in 150 g scale using 600 ml pressure reactor maintaining at 8-10 kg pressure for the determination of biodiesel properties. After the reaction, the catalyst was easily recovered by filtration and reused for five times without any deactivation under optimized conditions. This single-step process could be a potential route for biodiesel production from high FFA containing oils by simplifying the procedure and reducing costs and effluent generation. The biodiesel obtained from karanja oil meet the biodiesel standard specifications of ASTM D6751 and EN 14103 methods.

**Scheme:** Carbon Acid Catalyzed Biodiesel Production from Karanja Oil

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#### POSTER IV/14

### ECO-FRIENDLY COATINGS FROM PLANT OILS

**Madhu Bajaji\* and Firdous Habib**

The last two decades have witnessed an exponential growth in the interest for using bioderived products, which has been driven by the need for replacing petroleum based materials reducing the fuel consumption and, equally important, for producing materials with lower environmental impact. Vegetable oils constitute a rich source for many different polymers and polymer precursors and they are being considered for the production of "greener" coatings. Plant oils offer many advantages apart from their renewability. Their worldwide availability and relatively low prices make them industrially attractive and feasible, as daily demonstrated with industrial oleochemistry. Furthermore, diverse chemistry can be applied on them, leading to a large variety of monomers and polymers. The main constituents of plant oils are triacylglycerols (triglycerides) which are the product of esterification of glycerol with three fatty acids. Fatty acids account for 95% of the total weight of triglycerides and their content is characteristic for each plant oil. Triglycerides are highly functionalized molecules, and, therefore, have been used in the synthesis of cross-linked polymers via two main approaches. The first one takes advantage of the naturally occurring functional groups present in triglycerides, such as internal double bonds, alcohols, or epoxides, which can be polymerized using different methods. The second strategy depends on chemical modifications prior to polymerization. This approach solves the draw back of the low reactivity of natural triglycerides (which usually only contain double bonds) by introducing easily polymerizable functional groups, and thus widens the synthetic possibilities.

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#### POSTER IV/15

### Synthetic Base Stock based on Non-Traditional Oils

**C. S. Waykole, Mihir Moghe and A. P. Pratap\***

Department of Oils, Oleochemicals and Surfactants Technology, Institute of Chemical Technology, (University under Section 3 of UGC Act 1956; Maharashtra Government's "Elite Status and Center

of Excellence" Formerly UDCT/UICT, University of Mumbai) Nathalal Parekh Road, Matunga (East), Mumbai - 400 019, INDIA

**Nature and scope of study:**

Plant oils as biolubricants are preferred because as they are biodegradable and non-toxic, unlike conventional mineral based oils. Non edible vegetable oils are the most significant to use as a fuel compared to edible vegetable oils. However edible oil has a tremendous demand for using as a food and also the high expense for production, hence non-edible oil is best suited for this, for effective economic purpose. Therefore many researchers are experimenting on non edible vegetable oils (Neem, Karanja and Jatropha etc.). However, vegetable oil based lubricants have a lower oxidative stability and poor cold flow properties at low temperatures. These drawbacks can be successfully improvised by chemically modifying the plant oils to produce stable biolubricant components.

**Findings:**

One potential way to improve oxidation and low temperature properties is to attach some functional groups, at their sites of unsaturation, through chemical modifications. Esterification, Epoxidation, Ring opening reaction or a combination of chemistries, have all been used in order to synthesize an improved product.

**Conclusion:**

Biolubricants have very low volatility due to the high molecular weight of the triacylglycerol molecule and have higher viscosity indices, superior anticorrosion properties, high flash and fire points, good boundary lubrication properties. In addition, plant oils have high solubilising power for polar contaminants and additive molecules. All these significant attributes present them as an ideal alternative to be used in lubricant formulations.

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**POSTER IV/16**

**Synthetic base stocks based on Estolides from Castor oil**

**Yogesh Chaudhari, Amit P. Pratap\***

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**Nature and scope of study:**

Due to the environmental concerns and the highly variable price of crude petroleum the use of vegetable oil-based lubricants to replace those based on petroleum has received significant attention. The development of biolubricants based estolide derivatives from various vegetable oils like castor, lesquerella, and meadow foam oils through chemical modifications. Estolide based on these sources have shown great promise for use in a wide variety of products with varying physical properties Cooling fluids, Cosmetics, Hydraulic fluids, Inks.

**Findings:**

Due to the presence of hydroxy functionality of ricinoleic acid (hydroxyl fatty acid - 12-hydroxy 9 cis-octadecenoic acid) upto 85-90% provides a useful site to conduct esterification reaction where the resulting product is estolide. The hydroxy moiety being a relative reactive species can be easily esterified on the intact triglyceride without degradation of glyceride molecule.

**Conclusion:**

Estolide are novel derivatives of fats that have good physical properties. Estolide ester from castor oil (ricinoleic acid) and saturated fatty acid have good physicochemical properties. Estolide from hydroxy and unsaturated fatty acids show real promise as base stock for a wide range of lubricant application.

\*\*\*

**POSTER IV/17**

**UTILIZING RENEWABLE RESOURCES IN SYNTHESIZING POLYMERS**

**Firdous Habib\*\*\* Anuradha Varshney\*\* and Madhu Bajapi\***

Already for a long time, plant oils and their derivatives have been used by polymer chemists due to their renewable nature, world wide availability, relatively low price, and their rich application possibilities. Several arguments can be found to believe in the great potential of plant oils as an alternative resource for the production of polymeric materials. The necessity of releasing the polymer industry from its dependence on depleting resources represents a major concern, pushing the search for industrially applicable renewable alternatives. In this context, plant oils offer many advantages apart

from their renewability. The chemical structure of biopolymers opens up possibilities to their reactive modification. Cashew is widely cultivated in the tropics and cashew nut is very proteinaceous tasty product popular throughout the world. CNSL is a by product of the cashew nut processing industry. It is cheap, renewable and under utilized. The phenolic nature of the constituents of CNSL along with varying degrees of unsaturation in the side chain makes it a highly polymerizable substance amenable to a variety of polymerization reactions. Of overriding importance, however, is the condensation reaction with active methylenes that formaldehyde exemplifies. This leads to a phenol- formaldehyde type condensation. Cardanol based surface coatings possess excellent gloss and surface finish with optimum levels of toughness and elasticity. Because of the dark color its uses are restricted to anticorrosion primers, black enamels, paints to prevent marine growth on structures, etc.

\*\*\*

**POSTER IV/18****An Emulsion Fuel- A New Generation Fuel****Swapnil Manea, Jyotsna Waghmare\***

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The emulsification of water in fuel is an effective technique to reduce exhaust emissions like NO<sub>x</sub>, SO<sub>x</sub>, carbon monoxide, smoke and particulate matter. Water-in-diesel (W/D) can be used as an alternative fuel for diesel engines, boilers and incinerators to improve fuel economy and reduce pollution.

The aim of the project is to formulate high stabilized water in diesel (W/D) fuel emulsion that can be utilised for oil fire boilers. The fuel emulsion is formulated using diesel, non-ionic surfactants like SPAN80, TWEEN80, Triethanolamine (TEA), various percentage of water (5%,7%, 14%) and additive like Aerosil-200. The emulsions were prepared using high energy and high pressure homogenizer (HPH) at room temperature. It was investigated that the HLB values 9.11 and 9.65 are effective for developing highly stable fuel emulsion. The emulsions were stable for 3 weeks. Further, it was observed that the emulsion transparency increases with decreasing water concentration.

**Keywords:** Emulsion fuel, Emulsifier, Hydrophilic-liphophilic balance, Synergistic effect of surfactants.

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**POSTER IV/19****Bioactive constituents as a potential agent in sesame for functional and nutritional application****Karnika Prakash and S.N Naik\***

\*Super critical fluid extraction laboratory, Faculty of Centre for rural development and technology, Indian Institute of Technology, Delhi, India.

Sesame seeds have been grown since ages in tropical regions in almost all parts of the world. Sesame is a flowering plant which is cultivated throughout the countries. Sesame is and foremost important and one of the oldest oilseed known to man. This wonder oilseed is a baggage of nutritive value as well medicinal properties. In ancient India, sesame oil was frequently used as a chief ingredient in various ayurvedic preparations and remedies. In countries like Japan and China sesame oil was also used for providing energy, soothing mind, preventing aging and for good health.

Sesame contains approximately 50% of oil which is highly resistant to oxidative stability. Along with oil sesame is an affluent source of nutritious protein, carbohydrate, dietary fibre, zinc, magnesium and many other minerals. Sesame seeds both directly and in roasted in various traditional confections in India in the form of laddus, chikki, tilgulpoli etc. Sesame seed is also used as a major ingredient in many international cuisines like Tahini, Daqqa, Gyntholik etc.

Sesame is a good source of bioactive constituents. These bioactive constituents include lignans, tocopherol and phytosterols. Lignans are the oxidative coupling products of  $\beta$ -hydroxy phenyl propane. Sesame contains both oil- dispersed lignans and glycosylated lignans. Many of these glycosylated lignans are inter convertible and have tremendous medicinal properties. Sesame seeds majorly contain sesamin, sesamol, sesaminol, sesamol and many other lignans. These lignans have many pharmacological properties e.g. antioxidant activity, anti proliferative activity, enhancing antioxidant act of Vitamin-E in lipid peroxidation systems, lowering cholesterol, neuro protective effects, reducing the incidences of breast and prostate cancer etc.

The tocopherols, the major vitamin E play a crucial role in prevention of human. Tocopherol vitamers are free radical scavengers that work as lipid soluble antioxidants. Sesame contains both  $\alpha$  and  $\gamma$ -tocopherols. The collaborative synergy of  $\gamma$ -tocopherol with sesaminol or of sesame seeds makes it to comparable to that of  $\gamma$ -tocopherol. Sesame seeds contain nearly 400-413 mg/100g of the phytosterols. These phytosterols reduces blood cholesterol levels by resembling to cholesterol in humans. They are also capable in anti-cancer, anti-atherosclerotic, anti-inflammatory and anti-oxidative effects.

\*\*\*

**POSTER IV/20**

**OPTIMIZATION OF ALGAE GROWTH FOR THE PRODUCTION OF BIODIESEL**

**Deepak Kumar Sharma, VinayaTiwari, R. K. Trivedi**

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Biofuel production from renewable sources is widely considered to be one of the most sustainable alternatives to petroleum sourced fuels and a viable means for environmental and economic sustainability. Microalgae are presently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, CO<sub>2</sub> fixation ability and high production capacity of lipids; they also do not compete with food or feed crops, and can be produced on non-arable land. Microalgae have broad bioenergy potential as they can be used to produce liquid transportation and heating fuels, such as biodiesel and bioethanol. Nowadays, increasing oil prices and climate change concerns, biodiesel has gained attention as an alternative energy source. One of the most important decisions in obtaining oil from microalgae is the choice of algal species to use. Our study investigated the influence of different media composition, pH levels, light intensities and temperature regimes on *Spirulina sp.* NCIM – 5421 growth under laboratory conditions. We found that the effect of culture media composition showed that *Spirulina* medium (1.34 O.D.) and Synthetic medium (1.24 O.D.) are the best suited media for the growth of

this species. The optimization of the physiological growth rates of this algal strain was maintained at moderate alkalinity of 7, 8 and 9 pH, (0.95, 1.48, and 1.92 O.D.), light intensities of 1500-2500 lux (1.36-2.54 O.D.), and temperature regimes of between 28°C and 32°C (0.94-1.68 O.D.). The present study signifies that the growth of microalgae not only depends on the temperature, light, pH and nutrient availability, but is also highly affected by the culture media composition. The effect of culture media composition showed that *Spirulina* medium and Synthetic medium are the best suited media for the growth of this species. The optimal growth rates of this *Spirulina* strain were obtained at pH levels of between 8-9, temperature regimes of 28-32°C and light intensities of 1500-2500 lux. Fatty acid methyl esters in biodiesel prepared from *spirulina sp.* was analyzed by FT-IR and GC-MS. The fatty acid compositions of the studied species were mainly palmitic acid, Linoleic acid, Oleic acid, Stearic acid.

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**POSTER IV/21**

**Green process for the production of Biolubricants from Castor Oil**

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During recent years the interest in environment friendly and readily biodegradable lubricant based on vegetable oil has increased. The use of environmentally acceptable vegetable oil-based product as lubricants has many advantages as they are nontoxic, biodegradable, derived from renewable resources, and have a reasonable cost when compared to other synthetic fluids. We have made an attempt to develop a green process for the production of biolubricants. In this process the transesterification reaction of castor oil extracted from wild castor seeds, was carried out in sub critical and super critical carbon dioxide with higher chain alcohols such as propanol, butanol, and amyl alcohol etc. The reaction is catalyzed by immobilized lipase in solvent free process as well as in

sub and super critical CO<sub>2</sub> medium, which led to fatty acid alkyl esters (FAAE). The reaction parameters investigated were the reaction time, pressure, temperature and molar ratio (alcohol to triglycerides), and their effect on the alkyl esters formation. The use of FAAE as additives in diesel fuel possesses good anti wear and lubricity properties studied on HFRR. FAAE have shown the improvement in low temperature fluidity as well as an effective antifriction and anti wear additive in terms of lubrication. Thus due to its high viscosity and enhanced cold flow properties; it can be used as potential bio lubricants.

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**POSTER/IV/22**

**Super absorbent Hydrogels for Sustainable Development Based on Poly (Acrylic acid-Acrylamide-Sodium Humate) for Metal ion removal from Industrial waste waters**

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In the last decades, a remarkable increase of heavy metal and dyes contaminant volume has posed many serious environmental problems due to their toxicity to many life forms. They are classified as persistent environmental toxic substances because they cannot be rendered harmless by chemical or biological remediation processes. Numerous research efforts are being done to develop methods to remove heavy metal ion and dyes, these techniques are filtration, chemical precipitation, neutralization, chelating ion exchange and adsorption.

The synthetic poly acrylates derived from acrylic acid has emerged as an important absorbent because of its superior price to efficiency as well as it has carboxylic group so it is beneficial for metal ion/cationic dye removal as the gels containing acid groups have been made to bind ions including some heavy metals for use in removal of metals and dyes from aqueous media. Humic acid is a natural product derived from green vegetation, and its principal component is humic substances. It is composed of multifunctional aliphatic components and aromatic

constituents and contains large no of functional hydrophilic groups.

This paper reports the synthesis of a series of multifunctional super absorbent hydrogel composed of Acrylic acid-Acrylamide and Sodium Humate with varying concentrations by solution polymerization ;and the effect of SH content variation on detailed swelling and diffusion characteristics its effect on Heavy metal ions/dye molecule removal was studied in detail.

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**POSTER IV/23**

**SYNTHESIS OF SUGAR ESTER USING SUGAR AND FATTY ACID**

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The Surfactant industry has in the last decades increasingly turned its attention to natural raw materials to replace petrochemical products. This interest has been clearly carried out by the development for production of few oleochemicals. Sugar based surfactant esters are produced from renewable and inexpensive substances are completely biodegradable under aerobic and anaerobic conditions, and non-toxic, non skin irritants, odour less and tasteless. Fatty acid sugar esters are non-ionic surfactants with high emulsifying, stabilizing and cleaning effect, which are widely applicable in the food, cosmetic, detergent and pharmaceutical industry.

This present study has been done for the synthesis of sugar ester using glucose and erucic, Myristic and palmitic acid individually with the azeotrop (ethanol and water) as solvent. The experiment had been carried out at different molar ratios at different temperatures with the decrement of acid value each time showing the formation of ester. The optimum condition obtained in this experiment was with molar ratio 1:2 (fatty acid: dextrose). Erucic acid ester had shown the best result among the three with acid value of 1.75 due to better solubility in desired azotrop. Effect of different parameters on the sugar acid synthesis had been studied as temperature, molar ratio as well as reaction

time. FTIR and NMR techniques had been applied to study the present functional groups and the their orientations in the formation of sugar ester.

**Keywords:** Sugar ester; Emulsifier; biodegradable; renewable; Azotrop

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**POSTER IV/24**

**PRODUCTION AND  
MICROENCAPSULATION OF FISH OIL**

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This research presents a study on the extraction of Mackerel Fish oil of Indian origin and to investigate the extent of oxidation of mackerel fish oil with added antioxidants. Production and characterization of

Mackerel fish oil was carried out using soxhlet apparatus and n-Hexane as the solvent for extraction. The extraction was carried out at the boiling point of the solvent. Antioxidant property was simulated by storing the fish oil at 4°C and 25°C. Four different antioxidants were used to evaluate oxidation during storage. The stability of fish oil was evaluated by measuring the peroxide value and the anisidine value. TBHQ and BHA gave good antioxidants effect for fish oil stored at 4°C and 25°C. They were effective to reduce the formation of peroxides in Fish oil. There was very little secondary oxidation of fish oil and no significant effects of all four antioxidants on changes of anisidine values during the storage period. Fish oil contains considerable amount of PUFAs which are very susceptible to oxidation and other possible side reactions and cause deterioration of oil. This problem would possibly remedied by using microencapsulated technology as it involves shielding of the core material by polymeric shell which inhibits the interaction of the core material with surrounding atmosphere.

**Keywords:** Antioxidants, PUFA, TBHQ, BHA, Oxidation.



## OTAI ANNUAL MERIT AWARDS

1. Dr. S Hussain Zaheer Memorial Award (Single Person Award for Basic Research): Annual Cash Award of Rs. 5,000/- was instituted with the support of Zaheer Science Foundation, New Delhi. The award is for excellence in research contribution in Oil Chemistry and Technology, Surface Coatings and Allied Subjects, through research papers, which include applicant's name among the authors and which appeared during the previous three calendar years.
2. Dr. S D Tirumala Rao Memorial Award (Single Person Award for Applied Research): Annual Cash Award of Rs. 5,000/- was instituted with the support of Anantapur Chapter of OTAI (SZ). The award is for excellence in research contributions in relevant subject "Wealth from Waste" or "Value-added Products from the Waste generated in Vegetable Oil Industry" through research papers, which include applicant's name among the authors and which appeared during the previous three calendar years.
3. RBGV Swaika Memorial Award (Team Award for Applied Research): Annual Cash Award of Rs. 5,000/- was instituted with support of Shri B K Swaika and Shri N K Swaika of M/s Swaika Vanaspati Products, Kolkatta. The award is for excellence in Specific Process or Product Development or Innovation or Improvement in the Oils, Oilseeds, Surface Coating and Allied Field over three calendar years.
4. Dr. Santinath Ghosh Memorial Research Award: Annual Cash Award of Rs.10,000/- and citation was instituted by OTAI (EZ) with corpus fund donated by Dr. Pubali Ghosh Dhar in memory of Dr. Santinath Ghosh for the Young Researcher (age below 35 years as on 1st January of the particular year). The award is for excellence in the field of Oil Technology and Allied Sciences with Best Social / Industrial Implication through patent / research paper, which include applicant's name among the authors which appeared during the previous calendar year.
5. S R Bhatnagar (SARBI) Memorial Research Award: Annual Cash Award of Rs 15,000/- and citation was instituted by OTAI (WZ) with the Corpus fund of donated by Mrs. Cherry Churi, Director, Ms Sarbi Petroleum & Chemicals Pvt. Ltd. in memory of Late Mr. S R Bhatnagar for the post graduate students. The award is for excellence in the research in the field of Tribology / Lubricant and allied fields for the research papers published which include applicant's name among the authors which appeared during the previous or current calendar year.
6. O P Narula – OTAI (SZ) Technology Award: Annual Cash Award of Rs. 7,500/- was instituted with the support of Shri O P Narula, New Delhi and OTAI (SZ). The award is for the best project report prepared for a specific topic identified by OTAI (SZ). The applicant has to submit a 10 to 15 page report (5 copies) on the above topic to the Secretary, OTAI (SZ).
7. O P Narula – OTAI (SZ) Young Scientist Award: Annual Cash Award of Rs. 5000/- was instituted with the support of Shri O P Narula, New Delhi and OTAI (SZ). This award is for a researcher who is engaged in Oils & Allied Products and should not have completed 35 years of age as on 1st January of the particular year. The award is for Publications/Patents which include applicant's name among the authors.

For further details and prescribed proforma for Award Nos. 1, 2 & 3, the applicants may contact Shri R K Srivastava, Hony. General Secretary, Oil Technologists' Association of India, C/o. HBTI, Kanpur – 208 002. For Award No. 4, the applicants may contact Dr. Mahua Ghosh, Hony. Secretary (EZ), C/o. Dept. of Chemical Technology, University of Calcutta, 92, A.P.C. Road. Kolkata 700 009, West Bengal. For Award No. 5, the applicants may contact Dr Rajeev Churi, C/o Oils, Surfactants & Oleochemicals Div., ICT, Matunga, Mumbai-19. For Award No. 6 and 7, the applicants may contact Dr. B V S K Rao, Hony. Secretary, OTAI (SZ), C/o CSIR-IICT, Hyderabad – 500 007. Any member of the OTAI engaged in an Academic or Industrial Research Organization or in industry is eligible for all the awards. The same award may be given second or more times to the same person, but only after the lapse of three years.

8. Prof. R K Khanna Memorial Award: Annual Cash Award of Rs. 5,000/- was instituted with the support of OTAI (Central Zone) in memory of Prof. R K Khanna. This team award is for the best research paper published in all issues of the Journal of Lipid Science and Technology, which appeared during previous calendar year. No application is required for this award.



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<b>Full page (Black &amp; White)</b>	Rs.5,000/- per insertion (Rs.16,000/- per four insertions)
<b>Half page (Black &amp; White)</b>	Rs.2,500/- per insertion (Rs. 8,000/- per four insertions)

#### MECHANICAL DATA

Frequency of Publication	: Quarterly
Overall size	: 28 cms x 21.5 cms
Print area	: 23 cms x 17.5 cms
No. of columns	: Two
Paper	: Art paper

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- Payment is to be made in advance by crossed cheque or demand draft in favour of "**Publication Committee, Oil Technologists' Association of India**" Payable at Hyderabad. Outstation cheques are not accepted. Please send your cheque / DD along with the advertisement material to The Editor at the above mentioned address.

This Quarterly periodical is published by Mr. K. Saravanan, on behalf of Oil Technologists' Association of India, C/o Lipid Science & Technology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad-500 007, Telephone No. 91-40-27193370 and Printed by him at Sri Balaji Graphics (Designers & Printers), # 1-8-1/B/26, Cellar & III Floor, Housefed Bhavan, Baghlingampally, Hyderabad - 500 044. Ph. 9347478010. Editor: Dr. Pradosh Prasad Chakrabarti.  
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