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# Journal of Lipid Science and Technology

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## *From Editor's Desk*

Good news for the foodies!! The US Departments of Agriculture (USDA) has removed the limit for cholesterol from 2015 Dietary Guidelines. In every five years, USDA and Health and Human Services (HHS) panel meet to identify foods and beverages that help in maintaining healthy weight and prevent diseases besides promoting health. According to their recommendations, diet programmes for school children and elderly people are designed. This panel, in their recently held meeting concluded that there is no direct link between uptake of dietary cholesterol and the risk of cardiac problems and removed the warning about this. According to the panellists' recommendations, "cholesterol is not a nutrient of concern for overconsumption." Till now, it was recommended to not exceed more than 300 mg of dietary cholesterol per day. It means not more than two eggs per day. Elimination of this warning about cholesterol was cheerfully welcomed by the foodies!! Though, according to a school of thought it was overdue since many years, another set of doctors are advising to have a cautious approach. The panel, however, still insists limiting both trans fat and saturated fat to less than 10 per cent of daily calories. Some researchers have recommended high-fat, low carbohydrate containing foods for weight loss provided one eats right kinds of fat. According to them, low fat recommendations may do more harm rather than doing good. This debate is still on and people are looking forward to more research findings before coming to any conclusion. It is beyond doubt that simplifying nutritional guideline to focus on whole foods can automatically remove unhealthy fats and added sugars from one's diet. We have to choose our sweeteners and fat intake very judiciously for our healthy life. For the time being, let us celebrate for the good news!!

Happy reading!!

**(PRADOSH PRASAD CHAKRABARTI)**

## INDEX TO ADVERTISEMENTS / ANNOUNCEMENTS




*The views and opinions expressed in this journal are those of the contributors; Oil Technologists' Association of India does not necessarily concur with the same. All correspondence should be addressed to the Honorary Editor, Journal of Lipid Science and Technology, C/o Lipid Science & Technology Division, CSIR-IICT, Hyderabad –500 007; e-mail: LST.JOURNAL@gmail.com.* ***Annual Subscription: Rs.500 (India) / US$ 60 (Overseas).***

# Wastewater Treatment for Chicken Fat Extraction Industry

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## ABSTRACT

Some processors are collecting wastes from hatcheries and chicken selling outlets and extracting fats for selling to animal poultry feed manufacturers. 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 contains protein, fat, blood, feathers, bone particles, flesh and small amount of dust etc. That contaminated water is having bad odour, dark colour, high TDS, COD and BOD. The present work describes a process for the treatment of this wastewater for possible reuse. The wastewater was initially filtered through ordinary filters to remove all suspended solids. It was then subjected to coagulant treatment. Treatment with Alum as a coagulant resulted the significant reduction of COD. The 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). Colour and the odour were reduced significantly with charcoal treatment. This PAC treated water was then passed through a microfiltration (MF) membrane having a pore size of 0.45 µm using a dead end type membrane test cell. The permeate of this filtration was then passed through a reverse osmosis membrane (BW30). Based on the results obtained, pilot scale trials were performed. PAC treated water was passed through a ceramic microfiltration membrane having 0.45 µm pore size and then the permeate treated water was passed through TFC reverse osmosis membrane of surface area 2.5 $m^2$.The permeate water samples were found to have very good characteristics of 99.6 % of COD and 92.7% of TDS were removed. The permeate water characteristics were meeting drinking water quality prescribed by Indian Standards. This treated water can be reused for various purposes.

KEYWORDS: Chicken Fat Extraction, Wastewater, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), PAC, Microfiltration (MF), Reverse Osmosis (RO).

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## INTRODUCTION

Water scarcity has forced all the environmental protection agencies to have a strict vigil on water usage and water pollution. Industries, however small its usage, have to maintain the strict discharge norms and the cost of wastewater treatment to maintain these norms has become a burden to industries. Those industries who use huge amount of water is looking for reuse of the wastewater after treatment. Chicken fat processing industry also consumes high amount of process water and produces wastewater with higher levels of different contaminants with unbearable mal odour. It was, therefore, decided to treat this wastewater to get a quality of water that can be reused or recycled.

Chicken fat extraction industries collect chicken wastes from hatcheries, hotels, marriage function halls, chicken outlets etc and collects all these remains from the whole area and then process after repeated washings. The fat extracted with hexane as solvent has its own market in feed industries and also in various industrial applications. Wastewater samples were collected from a local chicken fat extraction industry prior to any treatment in their effluent treatment plant. The samples were found to have blood, fat, oils and some other solid inorganic matters including feathers and bone particles. As the wastewater was having various decomposed organisms, it had stinking smell. The wastewater samples collected were analyzed for different characteristics like total suspended solids (TSS), total dissolved solids (TDS), conductivity, FOG, BOD, COD etc. The colour of the wastewater samples was also found to be very dark.

The primary focus of this study was to develop a process for the treatment of wastewater so that treated water can be recycled or reused. It was decided to use membrane separation science to get the desired quality of water. Application of membrane separation technique is well known in wastewater treatment and this laboratory also has already tried this process for other types of industrial wastewater like dairy wastewater, perfumery chemicals wastewater and oil refinery wastewater[1-3]. The same technique was applied to treat wastewater coming out of chicken fat extraction industry.

Membrane processing requires elaborate pre-treatments to safe-guard the membrane life. After adjusting the pH of the wastewater to 7(neutral pH), the wastewater was subjected to coagulation treatment. This is an important step in which the particle size of some contaminants are increased from sub microscopic microfloc to visible suspended particles due to the addition of suitable coagulant and are removed[4-8]. Contaminants like FOG's that can inhibit oxygen and other gas transport necessary for plants and aquatic animals are also to be removed[9]. Alum is the most common coagulant used for wastewater treatment[10-12]. In this study, alum was used as coagulant and the dosages were optimised by performing laboratory scale experiments.

In the next step of processing the wastewater, powdered activated charcoal (PAC) was used as adsorbent following reported literature[13-16]. This pretreatment step was performed to remove the colour and the odour of the water sample. The dosages of PAC were also optimized for maximum efficiency. The pH of the resultant water sample was further adjusted to 7. This water was then passed through a microfiltration (MF) membrane having 0.45 micron pore size. The permeate water from the MF membrane system was then passed through BW 30 reverse osmosis (RO) membrane.

The permeate coming out from reverse osmosis membrane unit was then analyzed for all parameters. It was observed that all the parameters were within the range of the drinking water standard as per IS10500 specification. This water was found to be suitable for reuse and recycling back into the process. This will definitely result in significant reduction in environmental pollution and cause substantial savings in process water.

## MATERIALS AND METHODS

### Materials

*Wastewater from chicken fat extraction unit*: wastewater samples were collected from a local chicken fat extraction industry is located in the outskirts of Hyderabad. The wastewater generated from various operations was collected in tanks and then aerated in a tank. The aerated wastewater samples were collected and filtered. The filtered water samples were then evaluated for complete analysis. Three different samples were collected and all the parameters were evaluated as per standard procedure [17].

*Membranes*: A hydrophilic PVDF flat sheet membrane having 0.45 µm pore size (HLVP09050) procured from Millipore Corporation, MA, USA was used for laboratory scale studies. The reverse osmosis membrane BW30 of 99% salt rejection was procured of M/S.Film Tec. Corporation, USA. For pilot scale microfiltration, a ceramic microfiltration membrane having 0.45 µm pore size was procured from Orelis, France. It is having a tubular configuration with 19 channels, having 800 mm of length and 0.167 $m^2$ surface area. For RO studies in pilot scale, TFC reverse osmosis membrane of surface area 2.5 $m^2$ , module length 40 inches and diameter 2.4 inches was used and was procured from M/S. Permionics, Baroda, India. It was specified for seawater desalination with 99.4% salt rejection.

*Membrane Equipment*: For laboratory scale membrane study, the micro filtration and RO experiments were performed in a stainless steel test cell of a dead-end type having maximum pressure limit of 60 bar. The test cell was supplied by Snowtech Pvt. Ltd., Mumbai, India. The test cell was fitted with magnetic stirrer. The Trans Membrane Pressure (TMP) was generated by nitrogen gas. For pilot scale studies, a 50 L capacity stainless steel cross flow membrane unit which have recirculation arrangement was used and this was supplied by M/S Nishotech Pvt Ltd, Mumbai, India.

*Chemicals and reagents*: Alum and Powdered activated charcoal (LR grade) were procured from S.D. Fine Chem. Ltd., Mumbai, India. Commonly used chemicals like NaOH (LR grade) and $H_2SO_4$ (LR grade) were procured from S.D. Fine Chem. Ltd., Mumbai, India and Ranbaxy Fine Chemicals Ltd, New Delhi, India respectively. Potassium dihydrogen orthophosphate, potassium dichromate($K_2Cr_2O_7$), Potassium chromate, Silver Nitrate($Ag_2NO_3$), Ferroin solution ($C_{36}H_{24}FeN_6O_4S$), Barium chloride(Extra Pure), were also procured from SD Fine Chem. Ltd. Ammonium Ferrous sulphate hexahydrate $(NH_4)_2SO_4.FeSO_4.6H_2O$, Sodium acetate trihydrate (extra pure) $CH_3COONa.3H_2O$, Solochrome black-T (Eriochrome black-T) (extra pure) $C_{20}H_{12}N_3NaO_7S$ were purchased from Finar Chemicals Limited Ahmadabad, India. EDTA( Ethylene Diamine Tetra Acetic Acid $CH_2N(CH_2COOH)CH_2.COONa_2\ 2H_2O$, Potassium nitrate KNO3, Magnesium chloride $MgCl_2.6H_2O$ were procured from Qualigens Fine Chemicals, Mumbai. Silver sulphate ($Ag_2SO_4$) procured from Spectrochem Pvt Ltd, Mumbai. Magnesium sulphate (extra pure) (Heptahydrate) $MgSO_4.7H_2O$ was procured from Molychem, Thane, India. Sodium sulphate (Anhydrous) $Na_2SO_3$, Ranboxy Laboratories Limited, Chemical Division, S.A.S Nagar, India. Di Potassium Hydrogen Phosphate is procured from M.S Life Sciences, Hyderabad.

*Analytical methods*: Wastewater samples and various samples after each treatment step were analysed for TSS, TDS, Hardness, Chloride, FOG, COD, BOD etc according to the standard methods for analysis of water

and wastewater described by APHA[17]. The value of pH were measured with the help of digital pH meter(model; DI707) supplied by Digisun Electronics, Hyderabad and the values of conductivity were measured with the help of digital conductivity meter (DCM 900) supplied by Global Electronics, Hyderabad, India. For COD estimation the digestion of the sample was done in a COD reactor supplied by HACH, Colorado, USA followed by titration with standard ferrous ammonium sulphate. For BOD test, 5 days 20°C BOD values were estimated using YSI500 dissolved oxygen meter, supplied by YSI Incorporated, Ohio, USA.

**Methods**

*Coagulant treatment*: The coagulant dosage was optimised by increasing the alum dosage from 0.2% to 0.3% and monitoring COD values. At 0.25 % dosage COD reduction was found to be maximum.

*Powdered activated charcoal (PAC) treatment*: Adsorption studies were performed using powdered activated charcoal giving the required dosage under stirring for 30 minutes. The water was then filtered.

*Laboratory scale studies on membrane processing*: The PAC treated water was then passed through a micron filter in dead end cell membrane equipment. It is having the capacity of 200 ml and 60 bar pressure withstanding capacity and stirring bead is fixed inside. 150 ml of PAC treated water was taken inside and closed. The commercial nitrogen cylinder was connected to this closed system for generating pressure and permeate was collected at the bottom and quality of water samples were evaluated for all parameters. The microfiltered water was then passed through the reverse osmosis membrane (BW 30) with the characteristic rejection of NaCl (98-99%) in same cell. The permeate was analysed to evaluate the quality characteristics.

*Pilot scale membrane studies*: Pilot scale membrane separation studies were conducted in a cross flow membrane unit having spiral wound module with 2.5 $m^2$ effective area. The TMP is maintained by a high pressure pump.

**RESULTS AND DISCUSSION**

The primary objective of this investigation was to develop a membrane separation technique based process for treatment of wastewater coming out of a chicken fat extraction unit. Accordingly, raw wastewater samples were collected from a local chicken fat processing industry. The characteristics of wastewater samples collected from the industry were evaluated and presented in Table 1.

In the initial stages of study, laboratory scale experiments were performed to optimize the dosages of alum and PAC for further experimentation in Pilot scale studies. Initially, ordinary filtration was performed and the filtered water was subjected to coagulant treatment with alum. In this study COD was taken as the major parameter. It was observed that addition of 0.25% of alum gave maximum reduction of COD and further increase in alum dosage did not result any further decrease of COD. Hence, a dosage of 0.25% of alum

**TABLE 1**
**Characteristics of Wastewater Samples**

| Parameter | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| pH | 7.67 | 7.96 | 7.63 |
| Conductivity, µs/cm | 1830 | 1750 | 1550 |
| TDS, mg/L | 2420 | 2130 | 1842 |
| TSS, mg/L | 145 | 188 | 173 |
| Hardness, mg/L | 320 | 285 | 250 |
| Chloride, mg/L | 137 | 163 | 145 |
| Sulphate, mg/L | 34.4 | 36.6 | 45.6 |
| COD, mg/L | 3600 | 3200 | 2600 |
| BOD, mg/L | 780 | 700 | 660 |
| FOG, mg/L | 1200 | 950 | 1000 |
| Odour | high | high | high |
| Colour | greenish brown | greenish brown | greenish brown |

was selected for pilot scale experiments (Fig.1). The wastewater sample was then filtered for the removal of coagulant particles. This was then subjected to powdered activated charcoal (PAC) treatment. PAC is a natural adsorbent that are generally used for removal of colour and odour. Since the chicken fat extraction unit wastewater was having very dark colour and irritating smell, it was decided to have PAC treatment. The pH of the coagulant treated and filtered water was again adjusted to 7. It was observed that with 0.2% PAC dosage and stirring time of 30 minutes, the extent of colour removal was maximum. The odour also was significantly reduced. The treated wastewater sample was then filtered. This was then passed through microfiltration membrane to remove any FOG, odoriferous compound and also to safeguard the reverse osmosis membrane.

For laboratory scale studies a dead-end type test cell was used for both microfiltration and reverse osmosis. The MF permeate water showed around 60-63 % reduction of COD and RO permeate water showed around 99.5 % reduction in COD. The wastewater sample showed some increase in conductivity and TDS after coagulant treatment. It may be due to addition of alum as coagulant. Based on these finding, the work was extended to pilot scale. 50 litres of freshly generated wastewater sample was collected from the same industry. The quality of the wastewater sample was presented Table 2. This water was then subjected to coagulant treatment with 0.25% dosage of alum and kept under stirring a mechanical

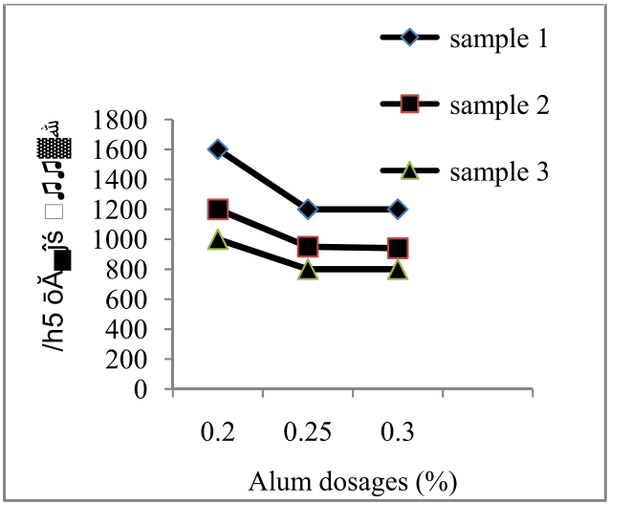


**Fig.1 : Effect of COD for Alum dosages.**

stirrer for 15 minutes and then allowed to settle for 30 minutes. The agglomerates were filtered using ordinary filter paper and the filtrate was subjected to adsorption by powdered activated charcoal. 0.2% (w/v) PAC was used for study and stirred for 30 minutes. It was again filtered using ordinary filtration. The pH of the treated water was then adjusted to 7. The resultant water sample was then passed through a tubular ceramic microfiltration membrane having 0.45µm of pore size. The quality of water obtained after microfiltration is also shown in Table 2 . At this stage, the colour and odour of the water were completely removed. The

**TABLE 2**

**Wastewater Characteristics and Characteristics of Treated Water at Various Stages.**

| Parameter | Raw wastewater | Micro filtered water | RO treated water | Drinking water IS 10500 |
|---|---|---|---|---|
| pH | 7.99 | 7.83 | 7.0 | 6.5-8.5 |
| Conductivity, µs/cm | 1700 | 3120 | 140 | 250 |
| TDS, mg/L | 1332 | 1640 | 98 | 500 |
| TSS, mg/L | 155 | nil | nil | nil |
| Hardness, mg/L | 225 | 220 | 50 | 300 |
| Chloride, mg/L | 137.45 | 87.47 | 8.74 | 250 |
| Sulphate, mg/L | 44.43 | 64 | 23.3 | 200 |
| COD, mg/L | 2400 | 800 | 8 | <10 |
| BOD, mg/L | 650 | 150 | nil | nil |
| FOG, mg/L | 896 | 26 | nil | nil |
| Odour | high | odour less | odour less | odour less |
| Colour | greenish brown | colour less | colour less | colour less |

permeate was then passed through a spiral wound reverse osmosis membrane module where a thin film composite membrane having 2.5 $m^2$ surface area and 99.4% salt rejection characteristics was used. The water was found to have parameters like TDS, COD, Conductivity, BOD, Chloride, Sulphate etc. well within the limit of drinking water specification as per IS 10500.

## CONCLUSION

In the present investigation wastewater samples from chicken fat extraction unit was collected and a process was developed at pilot scale (50 litre wastewater) for the treatment of this particular wastewater. The treated water obtained had the quality parameters within the range of drinking water standards prescribed in India. The treated water, thus obtained can be reused for different purposes.


## ACKNOWLEDGEMENT

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V

# Antioxidant Properties and Stability Study of γ-oryzanol Separated from Rice Bran Oil Distillate

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## ABSTRACT

In present study, γ-oryzanol separated from rice bran oil distillate (Industrial waste) was utilized to stabilize sunflower oil. Stability study experiments were carried out in rancimat 743 by varying temperature (110-130oC) and concentration of γ-oryzanol (100-300 ppm). Obtained results were also compared with synthetic antioxidants (BHA, BHT and TBHQ). Antioxidant potential of γ-oryzanol and crude rice bran oil (CRBO) were evaluated by using DPPH and ABTS antioxidant assays and results were reported in terms of % inhibition activity and IC50 values. Values of total phenol (TPC) and total flavonoid content (TFC) of γ-oryzanol were observed to be 2.7±0.18 mg GAE/g and 1.28±00.06 mg QE/g respectively. Above 80% inhibition activity of γ-oryzanol in both DPPH and ABTS assays were observed at 500 and 600 µg/mL concentration. Antioxidant capacities of CRBO and γ-oryzanol in terms of IC50 values were also calculated. In comparison to ABTS assay, DPPH assay give highest antioxidant activity at lower values of IC50 for both γ-oryzanol and CRBO. Sunflower oil was found to be benefitted with the addition of γ-oryzanol. Results of antioxidant index for γ-oryzanol was also found to be comparable with BHA and BHT.

KEYWORDS: γ-oryzanol, phenols, flavonoids, antioxidant activity, rancimat, sunflower oil.

## INTRODUCTION

The increasing demand for food products increases load on food processing industries and raw material producer. In view of this, food processing industries work continuously to bridge gap between demand and production. As the production increases, production of industrial waste (by-products and co-products) also increases. Wastage of food is also one of the reason of producing waste. Food industries are on second position after household food waste which produces approximately 39% industrial wastes during production[1]. Recycling and utilization of such kind of industrial waste is necessary in order to recover functional food ingredients in terms of value addition.

In the case of oil industries, wastes generated through different physical and chemical refining process. These wastes are nothing but the soap-stock produced after neutralization process, deodorized distillate produced during deodorization process and oil seed cake remained after oilseed crushing. During refining several nutrient lost through waste. Therefore recycling of these waste is necessary in recovery and value addition point of view. Some researcher extracted functional food ingredients from wastes/by-products of turmeric, waste water of olive oil mill, palm and rice bran oil refining industries[2-4]. In previous study, we separate γ-oryzanol from rice bran oil distillate and utilize it to stabilize linseed and peanut oil[5].

Keeping above broad aspects, present research has been aimed to stabilize sunflower oil by adding γ-oryzanol. In addition to this, comparable antioxidant study experiments of γ-oryzanol and crude rice bran oil (CRBO) were also performed to measured antioxidant activities in terms of phenol, flavonoid, DPPH and ABTS assays.

## MATERIAL AND METHODS

### Materials

Dilution solvents like methanol, ethanol and standards of synthetic antioxidants (BHA, BHT) were purchased from Merck (Darmstadt, Germany). TBHQ was from Acros organics (New Jersey, USA). Reagents, Folin–Ciocalteu phenol reagent, 1, 1-diphenyl-2-picryhydrazyl radical (DPPH), 2, 2-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) was from Sigma-Aldrich (St. Lous. MO). Anhydrous sodium carbonate ($Na_2CO_3$), anhydrous sodium sulphate ($Na_2SO_4$), sodium acetate ($CH_3COONa$), aluminium chloride ($AlCl_3$) and potassium per sulphate were purchased from CDH fine chemicals (New Delhi, India). Milli-Q water was used for all the testing procedures.

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## Methods

### *Gas chromatography (GC) analysis*

Fatty acid methyl esters of sunflower oil was prepared as per the method of Hammond[6]. Nucon 5765 GC (Nucon Engineers, New Delhi, India) equipped with flame ionization detector (FID), fused silica capillary column BPX-70 (60X0.25 mm) with 0.25 µm particle size, GC panel, Nuchrom software was used. Air, hydrogen and nitrogen was used as carrier gas with the flow rate of 30 mL/minute, 300 mL/minute and 45 mL/minute respectively. The column temperature was set between 240 to 280°C with gradual increase 4°C/minute. Detector and injector temperature was set at 280 and 240°C respectively. 1 µL sample was injected in injector with a split ratio of 60 mL/minute.

### *Total phenol content (TPC)*

Total phenol content was determined by a well-known Folin-Ciocalteu method with slight modification in sample preparations[7]. 200 µL extract/standard was transferred into a test tube containing, 1 mL of freshly diluted (10 fold) Folin-Ciolateu reagent. The mixture was allowed to stand at room temperature. After 8 min, 3 mL of 7.5% (w/v) sodium carbonate was added to the mixture and shaken manually. Then the mixture was incubated at room temperature for 60 min. The absorbance was recorded at 765 nm using UV – visible spectrophotometer (Shimadzu, UV-2600 Kyoto, Japan). Acidified methanol was used as blank. The calibration curve was plotted against gallic acid (GA) and expressed in terms of milligram GA equivalents per gram dry weight basis (mg GAE/g).

Total flavonoid content (TFC)

Total flavonoid content (TFC) of extracts was determined by the method of Hosu[8] with slight modification in the standard (Quercetin) used. A mixture of 400 µL of $AlCl_3$ (25g/L), 500 µL of sodium acetate (100 g/L) and 4 mL distilled water was added in 500 µL of extracts/standards. After 15 min, the absorbance of the mixture was measured in spectrophotometer at 415 nm. Quercetin standard concentration curve for flavonoids was obtained in the range of 0-100 µg/mL. Results are expressed in milligram Quercetin equivalent (QE) per gram extract (mg QE/g).

### *DPPH method*

DPPH radical scavenging activity of extracts was measured by using the method of Patel[9]. A reaction mixture of 100 µL extracts and 3900 µL DPPH solution (0.004%) was prepared and incubate it in dark for 60 minutes. Readings were recorded on spectrophotometer at 515 nm against blank methanol. All the readings was taken in triplicates and percent DPPH inhibition was expressed in mean±standard deviation. $IC_{50}$ values was also calculated in order to determine 50% inhibitory activity.

### *ABTS method*

ABTS radical scavenging activity was measured as the method described by Jaiswal[10]. ABTS stock solution was prepared by adding 7 mM ABTS and 2.45 mM potassium persulphate in equal quantities and allowed this reaction mixture for 16 hours incubation in dark. Working stock solution of 0.700±0.005 absorbance at 734 nm was prepared by diluting reaction mixture with 80% ethanol. 3900 µL ABTS working stock solution was added in test tube containing 100 µL extracts and mixture was allowed to incubate for 5 min to measure absorbance. ABTS radical scavenging activity results of extracts was measured in terms of percent inhibition (Inhibition %). IC50 values was also calculated in order to determine 50% inhibitory activity. All the reported values of percent inhibition as well as 50% inhibitory activity are the mean of three measured readings.

### *Rancimat study*

Stability test for sunflower oil after addition of ORC was performed in rancimat-743 (Model Metrohm-743, Herisau, Switzerland). Operating parameters like temperature (110-130oC), γ-oryzanol concentration (100-300 ppm) and air flow rate (20 L/hour) were arrange as described by Jaiswal[11]. All the experiments were performed thrice and results were reported in terms Mean ±SD. Antioxidant index of oil was also measured by calculating ratio of induction period of oil with antioxidant and without antioxidant.

## RESULT AND DISCUSSION

### *GC analysis*

Before stability study of sunflower oil, fatty acid composition has been studied in order to understand composition of unsaturated fatty acids (oleic, linoleic and linolenic acids). Figure 1 shows the chromatograms for analysed fatty acid methyl esters (FAME) of sunflower oil. Oleic acid (C18:1) and linoleic acid (C18:2) were found to be predominant fatty acids with 36.26 and 52.06 % of total fatty acid composition. Linolenic acid (C18:3) was found to be absent. Other fatty acids namely palmitic (C16:0), stearic (C18:0) and archidic (C20:0) were found to be 5.90, 3.29 and 0.44%. Results of this study indicate that percentage of unsaturated fatty acids (88.32%) is more than saturated fatty acids (9.63%) present in sunflower oil. Results of GC analysis was found to be comparable with the previous findings[12]. Presence of these higher percentage of unsaturated fatty acids in sunflower increases the possibilities of oxidation.

Therefore sunflower oil has been chosen for present investigation to stabilize it with γ-oryzanol.

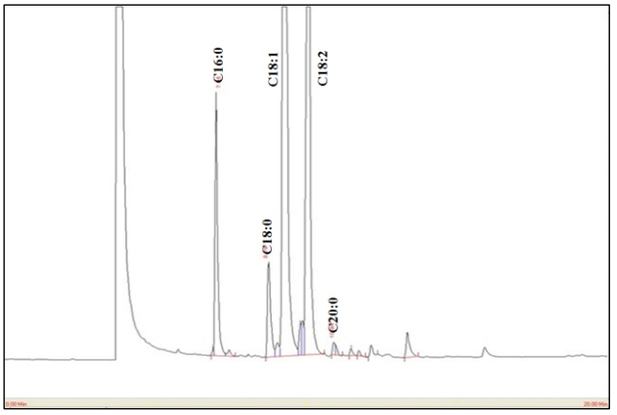


**Fig. 1 : GC-FID chromatogram for sunflower oil methyl ester**

TPC and TFC analysis

Total phenol (TPC) and flavonoid (TFC) content of RBO and γ-oryzanol were measured by using gallic acid and quercetin standards respectively (Table 1). Samples were prepared in methanol and raw readings repeated thrice in order to report the value of mean and standard deviation. TPC values for crude rice bran oil and purified fraction of oryzanol rich concentrate was found to be 4.18±0.16 and 2.7±0.18 mg GAE/g respectively. Besides TFC values for crude rice bran oil and oryzanol rich concentrate was found to be 3.68±0.08 and 1.28±00.06 mg QE/g. Overall, TPC values for both CRBO and γ-oryzanol was found to be higher than the TFC values obtained. Moreover TPC and TFC values for CRBO was observed to be higher than γ-oryzanol. This higher value of TPC and TFC of CRBO is may be due to presence of other antioxidant compounds (tocopherols, tocotrienols and other phytosterols). During refining process this bioactive constituents lost in every step of refining operation. Due to this, there is a possibility of higher value of TPC and TFC for CRBO. There are few reports available on TPC and TFC of rice bran extracts or oil which are as follows.

TPC and TFC values of methanolic extracts of four Indian variety rice bran studied by Rao[13] were found in the range of 3.2-12.4 mg GAE/g and 1.65-8.5 mg QE/g. Our results for crude rice bran oil of TPC and TFC was also found in the range of this reported results.

In another study, Chotimarkorn14 studied five Thai variety long grained rice bran for TPC and TFC. Extraction was done by using methanol as extraction solvent. Results of this study revealed that TPC values were found in the range of 2.2-3.2 mg GAE/g and TFC were in 0.03-0.10 mg catechin equivalent/g (et al., 2008). TFC values were observed to be lower than that of our reported result. This may be due to changing standard used for flavonoid study. Catechin is a very strong standard for flavonoid study than quercetin. Therefore measurement of TFC against Catechin standard gives lower values than quercetin standard. Lee15 evaluated seventy one rice varieties for antioxidant activities. Extraction of all varieties were done in methanol solvent. Range of total phenols were obtained in the range of 0.5-2.01 mg GAE/g.

From the above results it is clear that rice bran oil is a potent source of phenols and flavonoids rather than oryzanol. This phenol and flavonoids play an important role in antioxidant activity. On the basis of obtained results of lower phenol and flavonoid content for γ-oryzanol, it is cleared that refining process loses antioxidant compounds and lowers antioxidant activities in a substrate.

***Antioxidant activity by DPPH and ABTS method***

On the basis of obtained phenol and flavonoid content of CRBO and γ-oryzanol, DPPH and ABTS antioxidant study has been carried in spectrophotometer

**TABLE 1**

**Total Phenol Content (TPC), Total Flavonoid Content (TFC) and Antioxidant Capacities of γ-oryzanol and CRBO**

| Sample | TPC* | TFC# | Antioxidant capacity ($IC_{50}$)A | |
|---|---|---|---|---|
| | | | DPPH | ABTS |
| γ-oryzanol | 2.7 ± 0.18 | 1.28 ± 00.06 | 0.071 | 1.14 |
| CRBO | 4.18 ± 0.16 | 3.68 ± 0.08 | 0.090 | 2.34 |

Values are the means of triplicate determinations.

A = mg/mL, * = mg GAE/g, # = mg QE/g

as per the standard method described in methodology section. Antioxidant activities of CRBO and γ-oryzanol were measured in terms of percent inhibition (%) activity and values of antioxidant capacities (IC50). Figure 2 describe the effect of changing concentration of antioxidants on % inhibition activity.

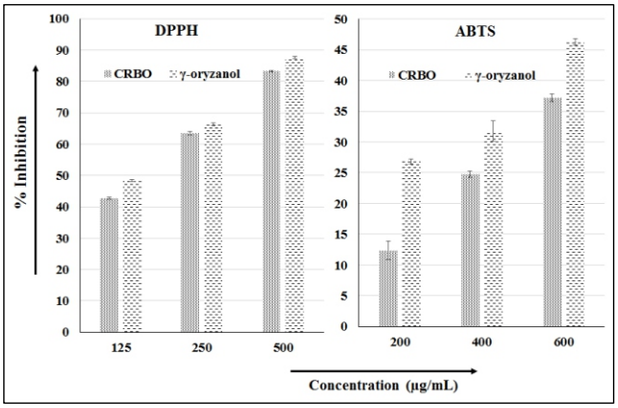


**Fig. 2 : DPPH and ABTS % inhibition activity of CRBO and γ-oryzanol**

All the results of both DPPH and ABTS antioxidant activity was taken in triplicates. DPPH % inhibition activities for CRBO and γ-oryzanol were measured at three different concentrations starting from 125, 250 and 500 µg/mL. Values of DPPH % inhibition for CRBO at three different concentrations was obtained in the range of 42.71±0.34% to 83.27±0.15% while γ-oryzanol was observed in the range of 48.41±0.35% to 87.67±0.25%. Result of γ-oryzanol for DPPH % inhibition was found to be slightly higher than CRBO. Therefore, γ-oryzanol gives more radical scavenging activity than crude rice bran oil. In the case of ABTS % inhibition activity, % inhibition activity of CRBO at three different concentrations 200, 400 and 600 µg/mL was observed in the range of 12.39±1.48% to 37.18±0.59% while for γ-oryzanol was found in the range of 26.83±0.41% to 46.26±0.54%. Again, result of ABTS % inhibition activity of γ-oryzanol was found to be higher than CRBO. In comparison to both DPPH and ABTS % inhibition activity, DPPH method give better result of % inhibition while ABTS method performs very poor activity by giving values of % inhibition less than 50%. For both γ-oryzanol and CRBO, DPPH and ABTS method gives increasing trend of % inhibition activity with increasing concentration.

Rather than % inhibition activity, antioxidant apacities of CRBO and γ-oryzanol were also measured in terms of calculating IC50 values. In DPPH, 0.071 and 0.090 mg/mL concentration of γ-oryzanol and CRBO give 50% inhibition activity. In the case of ABTS antioxidant capacity, 1.14 and 2.34 mg/mL concentration of γ-oryzanol and CRBO is required for 50% inhibition activity. From the IC50 results, it is cleared that DPPH method gives better antioxidant capacity results at lower concentration while ABTS method require more antioxidant concentration for 50% inhibition. Due to presence of ferulate compounds in γ-oryzanol, both the antioxidant method gives more antioxidant activity results than CRBO. Our IC50 values for DPPH antioxidant capacity of rice bran oil was lye in the range of previously reported results by Rao13 and Lee15.

Stability study of γ-oryzanol in rancimat

Two types of study has been carried out in the case of sunflower oil as shown in Figure 3 and 4. Result of changing concentration and temperature on induction period of sunflower oil shows that as the concentration of antioxidant in oil increased from 100-300 ppm, induction period also found to be increased while temperature shows negative effect on induction period of oil. Induction period of control sample (without antioxidant) of sunflower oil at 110°C was recorded to be 5.53±0.20. After addition of antioxidants (γ-oryzanol, BHA, BHT and TBHQ) in 100 ppm concentration, induction period of control sample at 110°C for different antioxidants were found to be 5.64±0.05 (γ-oryzanol), 5.58±0.06 (BHA), 6.63±0.12 (BHT) and 9.54±0.09 (TBHQ). This indicates that all the antioxidants work effectively in stabilizing sunflower oil. TBHQ was found to be more effective among all while γ-oryzanol observed to be comparable with BHA and BHT.

Another important namely antioxidant index (AI) has been studied in order to understand effectiveness of antioxidants in sunflower oil preservation. In Table 2, AI data of changing concentration of antioxidants and temperature has been summarized. All the values reported are the means of triplicate determinations. Coefficient of variation (%RSD) was also calculated. From Figure 4, it is cleared that TBHQ has strong

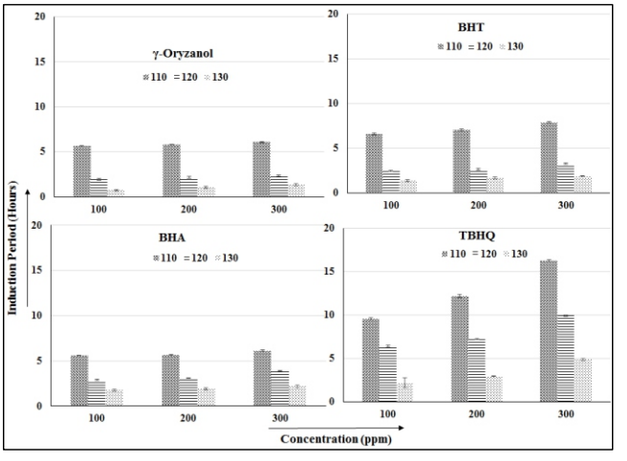


**Fig. 3 : Comparative stability study of γ-oryzanol with synthetic antioxidants in sunflower oil**

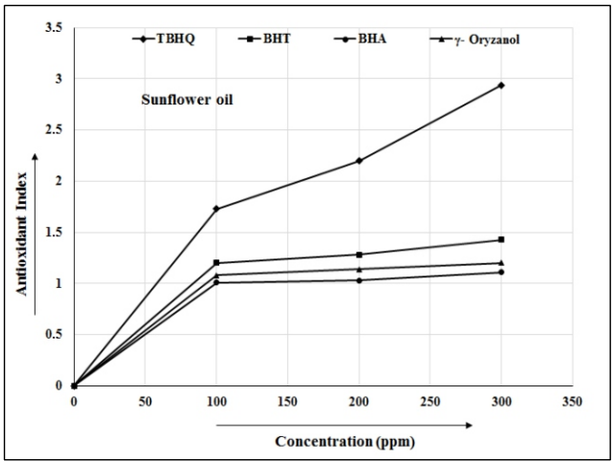


**Fig. 4 : Effect of changing concentration of antioxidants on Antioxidant Index (AI)**

antioxidant index while γ-oryzanol gives comparable AI values between BHT and BHA.

## CONCLUSION

On the basis of obtained results of separated γ-oryzanol for antioxidant activity and stability study, it can be concluded that antioxidant activity in terms of IC50 values for DPPH and ABTS assay gives highest activity at lower concentration. In terms of % inhibition activity, DPPH assay for both CRBO and γ-oryzanol gives above 80% activity at the concentration of 500 µg/mL while ABTS activity gives poor performance with lower percent inhibition activity (below 50%) at 600 µg/mL concentration. TPC and TFC results for γ-oryzanol was found to 2.7±0.18 mg GAE/g and 1.28±00.06 mg QE/g respectively. Result of stability study of γ-oryzanol in sunflower oil was found to be positive and comparable with BHA and BHT. Therefore, future study should be needed to study the effect of γ-oryzanol at higher concentration in oil. Moreover γ-oryzanol could be used synergistically with other natural antioxidants to stabilize oils and other lipid based food products.

**TABLE 2**

**Effect of temperature and antioxidant concentrations on antioxidant index of sunflower oil**

| Oil | Anti-oxidants | 110 ºC | | | 120 ºC | | | 130 ºC | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100* | 200* | 300* | 100* | 200* | 300* | 100* | 200* | 300* |
| Sun-flower | γ-oryzanol | 1.02± 0.04 (4.40) | 1.04± 0.05 (5.00) | 1.10± 0.05 (4.69) | 0.90± 0.08 (8.55) | 0.99± 0.11 (10.96) | 1.09± 0.09 (8.39) | 0.76± 0.09 (12.13) | 1.08± 0.11 (9.91) | 1.37± 0.18 (13.39) |
| | BHA | 1.01± 0.04 (4.39) | 1.03± 0.05 (4.72) | 1.11± 0.06 (5.06) | 1.34± 0.09 (6.66) | 1.43± 0.11 (7.97) | 1.01± 0.11 (11.28) | 1.76± 0.14 (7.99) | 1.97± 0.14 (6.93) | 2.22± 0.23 (10.42) |
| | BHT | 1.20± 0.02 (2.06) | 1.28± 0.06 (4.49) | 1.43± 0.06 (4.11) | 1.14± 0.07 (5.78) | 1.22± 0.05 (4.13) | 1.50± 0.11 (7.12) | 1.39± 0.09 (6.77) | 1.67± 0.13 (7.82) | 1.92± 0.14 (7.28) |
| | TBHQ | 1.73± 0.05 (2.97) | 2.20± 0.06 (2.54) | 2.94± 0.01 (0.24) | 2.95± 0.14 (4.76) | 3.34± 0.17 (5.13) | 4.58± 0.28 (6.12) | 2.19± 0.12 (5.66) | 2.99± 0.11 (3.85) | 4.98± 0.19 (3.74) |

All values are expressed as antioxidant index ± standard deviation,
(n = 3) and coefficient of variation (% RSD);
* = concentration in ppm.

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# Kinetic Study of Lipase Catalyzed Synthesis of Decyloleate

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## ABSTRACT

Decyl oleate is an important skin conditioning agent derived from the esterification of oleic acid and decanol. It is widely used in many cosmetic products as it has a composition similar to that of natural skin lipids. This study presents the kinetics of wax ester synthesis from oleic acid and decanol in the presence of Novozym 435 enzyme. The effects of various parameters such as reaction time, enzyme concentration, reaction temperature, and oleic acid-decanol mole ratio were investigated. The optimal conditions were found to be 1:1 oleic acid-decanol mole ratio, 2.5% enzyme concentration, 45°C temperature and 60 min reaction time. A Michaleis–Menten type kinetic model has been used to predict the esterification kinetics and the kinetic parameters were evaluated.

KEYWORDS: Wax ester, Esterification, Novozym 435, Kinetics

## INTRODUCTION

Wax esters are long chain esters which belong to an important class of fine chemicals that are widely used as base materials in pharmaceuticals, cosmetics, lubricants, paints, wood coatings and perfumery products [1-3]. Wax esters are biodegradable, non-toxic and are prepared from renewable sources such as vegetable oils which make them industrially important chemicals. Speciality liquid wax esters such as jojoba and sperm whale oil have wide industrial applications as premium lubricants, parting agents, anti foaming agents and in cosmetics.

The product of interest in this study, i.e. decyl oleate, is synthesized from decanol and oleic acid, a naturally occurring fatty acid. Decyl oleate has good lubrication properties and possesses low viscosity. It is widely used in moisturizers, anti-aging treatments, sunscreens, eye shadows and hand, foot and eye creams. It is also recommended for make up and lipsticks without the draw back of unsaturation[4]. It has a composition similar to that of natural skin lipids and has special properties of re-fatting and good solvency for lipophilic active ingredients[5].

Several researchers worked with different types of enzymes for the synthesis of various wax esters. Garcia *et al.*[6] optimized the conditions of enzymatic synthesis of myristyl myristate ester by factorial design and analysis of experiments using a fungal lipase from *Candida antarctica.* Parameter estimation for kinetic model was carried out by Garcia *et al.* [7,8] by numerical calculation algorithms. The validity of the proposed models was quantified by ANOVA. Kinetic models for the lipase-catalyzed esterification in a biphasic organic–aqueous system has been proposed by Oliveira *et al.*[9] for ethyl oleate, and Shintre *et al.*[10] and Radzi *et al.* [11] for oleyl oleate.

Hadzir *et al.*[12] screened five immobilized lipases for the alcoholysis reaction of triolein and oleyl alcohol. Similar product was synthesized by Kapucu *et al.* [13] using Novozym 435 and optimized by adopting four variable central composite rotatable design. The maximum oleyl oleate concentration predicted by the model (737 g/L) agreed well with the experimental value (734 g/L). *Candida rugosa* lipase (CRL) was used in a solvent less esterification reaction to yield twelve wax esters by Guncheva and Zhiryakova[14]. Within 10 h, they reported complete conversion at 50°C when immobilized $PEG_{2000}$-activated *Candida rugosa* lipase was added to the reaction mixture.

Woodcock *et al.*[15] synthesized nine alkyl esters employing Novozym 435 in a pressure driven, packed-bed, miniaturized, continuous flow reactor. Gunawan and Suhendra[16] used lipozim for palm kernel oil and oleyl alcohol. Gunawan *et al.*[17] carried out alcoholysis of palm oil and oleyl alcohol using *Rhizomucor meihei* (Lipozyme IM). Cetyl oleate was synthesized using immobilized lipase from *Candida* sp. 99-125[18] and the optimum conditions reported for 98% conversion were 40°C, 1:0.9 acid-alcohol molar ratio, lipase dosage of 10% of oleic acid and reaction time of 8 h.

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Kuo *et al.* [19] employed response surface methodology (RSM) and 5-level-4-factor central composite rotatable design (CCRD) to evaluate the effect of reaction parameters on the biocatalytic preparation of cetyl octanoate using two commercial immobilized lipases, i.e. Lipozyme RMIM (*Rhizomucor miehei*) and Novozym 435 (*Candida antarctica*). Similar methodology was adopted by Sellami *et al.* [20] for the preparation of different wax esters.

In the present study, enzymatic synthesis of decyl oleate was carried out for the first time. The optimization of process parameters for the preparation of wax esters from oleic acid and decanol and Michaleis–Menten kinetic model was used to evaluate the kinetic parameters of the reaction.

## MATERIALS AND METHODS

### Materials

Analytical grade decanol (99.5%) was procured from Sd. Fine Chem. Ltd., Mumbai. Oleic acid used in the study was 99% pure and was prepared in the laboratory from enriched high oleic sunflower oil methyl esters. Hexane used was of HPLC grade obtained from Merck (Darmstadt, Germany) and was used as such. Novozym 435 was obtained from Novozymes A/S (Bagsvaerd, Denmark) and consisted of *Candida antarctica* lipase B immobilized on macroporous acrylic resin.

### Experimental Procedure

Oleic acid and decanol were taken in 1:1 mole ratio (i.e. 0.01773 moles) in a 100 ml stirred vessel. 10 ml hexane along with 2.5% enzyme and 10% molecular sieves (3Å) were added to the reactor. The reaction mixture was maintained at constant temperature of 45°C and kept under continuous stirring for 60 min. This study was carried out by comparing two sets of reactions, i.e. reaction with enzyme and without enzyme (taking without enzyme reaction as control reaction). The samples were drawn at various time intervals for each set of reactions, and were filtered, desolventized, dried and analyzed by titration for the evaluation of unreacted oleic acid in the reaction mixture using automatic titrator. Conversion of ester was calculated as

$$Conversion\ of\ wax\ ester = \frac{\left[\begin{matrix}Volume\ of\ NaOH\ used\ (without\ enzyme) - \\ Volume\ of\ NaOH\ used\ (with\ enzyme)\end{matrix}\right]}{Volume\ of\ NaOH\ used\ (without\ enzyme)} \quad \ldots (1)$$

Different sets of experiments were carried out for the generation of data under different operating conditions to arrive at optimum process conditions. The first set of experiments was carried out at different enzyme concentrations ranging from 0 to 10% based on total weight by keeping fixed molar ratio of oleic acid-decanol at 1:1, at a temperature of 45°C. The second set of experiments was carried out at different molar ratios of oleic acid-decanol of 1:0.5, 1:1, 1:1.5 and 1:2 at 45°C temperature and 2.5% enzyme concentration. The third set of experiments was carried out at different temperatures ranging from 35°C to 50°C with oleic acid-decanol mole ratio of 1:1 using 2.5% enzyme concentration.

### Analytical methods

The fatty acid composition of the final decyl oleate was analyzed using Gas Chromatograph Agilent 6890 series equipped with flame ionization detector. The stationary phase used was a capillary column, DB-225 MS (i.d. 0.25 mm, length 30 m). The oven temperature was programmed as follows: 160°C for 2 minutes and rised to 230°C at 5°C per minute and held at this temperature for 20 min. The carrier gas used was nitrogen with a flow rate of 1 mL/min. The injector and detector temperatures were maintained at 230ºC and 270ºC respectively. The area percentage was recorded using HP Chem Station Data System.

## RESULTS AND DISCUSSION

The experimental data was used to study the effect of different parameters on the rate of reaction and the same is discussed in this section.

### Effect of reaction time

The extent of esterification reaction depends on the time of reaction. The time of reaction was optimized for 2.5% enzyme concentration and 1:1 oleic acid-decanol mole ratio at 45°C reaction temperature. It was found that the conversion increased with respect to time and reached a maximum value of 96.5% within 60 min. A further increase in the reaction time did not produce any change in conversion. This may be due to the fact that the equilibrium is being achieved within 60 min reaction time.

### Effect of enzyme concentration

One of the parameters which affects the rate of reaction is enzyme concentration. This effect was studied by varying the weight percent of enzyme used from 1 to 10% based on total reaction mass at constant 1:1 oleic acid-decanol mole ratio and 45°C temperature, and is given in Fig. 1. It can be inferred from Fig. 1 that there was increase in the conversion from 71.8% to 96.5% with the increase in amount of enzyme from 1% to 2.5%, and the conversion increased only to 96.9% with further increase in enzyme to 5%. So, all the other experiments were conducted with 2.5% enzyme concentration.

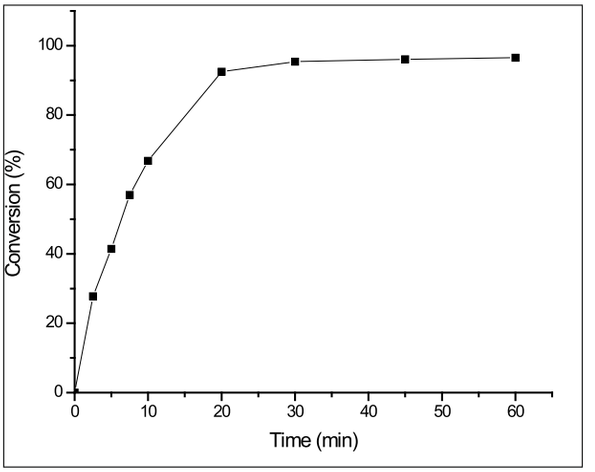


**Fig. 1 : Effect of time of reaction on esterification of oleic acid and decanol**

(Reaction temperature 45°C, enzyme concentration 2.5%, oleic acid-decanol mole ratio 1:1)

### Effect of oleic acid-decanol mole ratio

The mole ratio of oleic acid-decanol is one of the most important variables affecting the conversion. Esterification is an equilibrium limited reaction. Therefore, excess alcohol will shift the equilibrium towards the production of more ester and also increase the rate of esterification. The effect of mole ratio of oleic acid-decanol on conversion was studied at various mole ratios ranging from 1:0.5 to 1:2, and is shown in Fig. 2. It is clearly observed from the Fig. 2 that the conversion increased from 47.7% to 96.5% as the mole ratio increased from 1:0.5 to 1:1, and further increase in mole ratio to 1:2 increased the conversion marginally to 97.1%. So, 1:1 oleic acid-decanol mole ratio was used in all the other experiments as it is the optimum ratio.

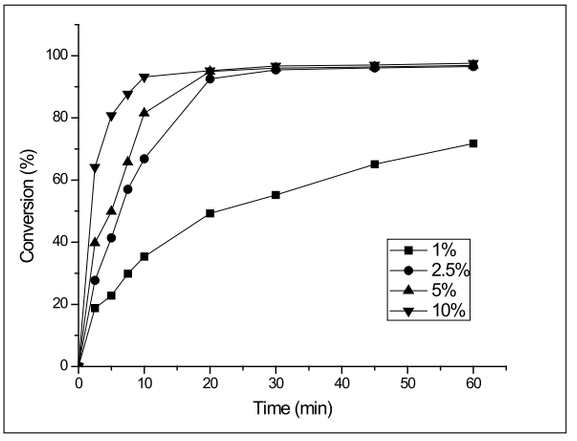


**Fig. 2 : Effect of enzyme concentration on esterification of oleic acid and decanol**

(Reaction temperature 45°C, oleic acid-decanol mole ratio 1:1)

### Effect of reaction temperature

The reaction temperature affects the activity and stability of the enzymes and thus the reaction rate. The effect of temperature was determined at different temperatures ranging from 35°C to 50°C, by keeping other reaction parameters constant, i.e. 2.5% enzyme and 1:1 oleic acid-decanol mole ratio, and is shown in Fig. 3. It was found that rate of reaction increased rapidly from 75.7% to 96.5% with increase in temperature from 35°C to 45°C. This is because increasing temperature allows the molecules to move quickly with greater energy causing more collisions and therefore increasing the rate of reaction. But increasing the temperature further to 50°C decreased the conversion. This may be due to thermal denaturation of the lipase and thus making the lipase inactivate.

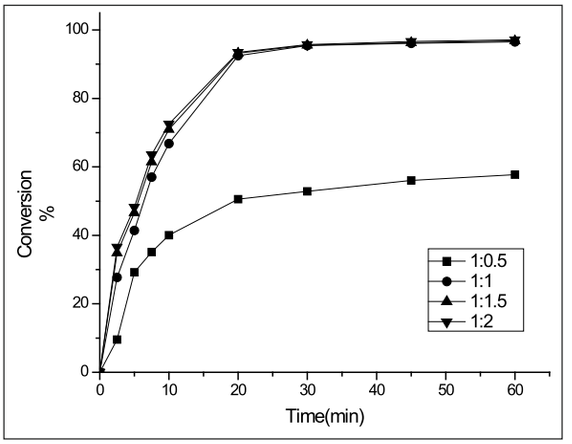


**Fig. 3 : Effect of oleic acid-decanol mole ratio on esterification of oleic acid and decanol**

(Reaction temperature 45°C, enzyme concentration 2.5%)

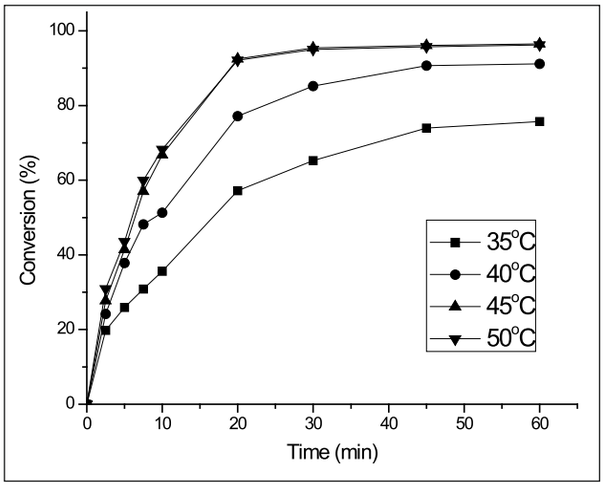


**Fig. 4. Effect of reaction temperature on esterification of oleic acid and decanol**

(Enzyme concentration 2.5%, oleic acid-decanol mole ratio 1:1)

**Validation of kinetic model**

It is assumed that the enzymatic synthesis of decyl oleate follows a Michaelis-Menten mechanism.

$$E + S \int \quad E \quad \text{ç} \quad E + P \qquad \text{... (2)}$$

According to the Michaelis-Menten model, reaction velocity can be determined for enzyme catalysed reactions by following equation

$$v = v_{\max} \frac{[S]}{K_m + [S]} \qquad \text{... (3)}$$

Where $v$ denotes reaction velocity, $K_m$ represents the Michaelis-Menten constant (mol/cm$^3$), [S] the substrate concentration (mol/cm$^3$) and $v_{max}$ is the maximum reaction rate (µmol/cm$^3$.min)

The kinetic parameters $K_m$ and $v_m$ for the reaction systems were estimated using the Lineweaver-Burk transformation of the Michaelis-Menten equation:

$$\frac{1}{v} = \frac{1}{v_{\max}} + \frac{K_m}{v_{\max}} \cdot \frac{1}{[S]} \qquad \text{... (4)}$$

The values of $K_m$ and $v_{max}$ are obtained by plotting a curve for eq. (4) i.e., between 1/*v vs.* 1/[*S*] as shown in Fig. 5, the slope of this curve provides $K_m/v_{max}$ and the intercept provides $1/v_{max}$.

**TABLE 1**

**Michaelis-Menten Kinetic Data for Synthesis of Decyloleate**

| S.No | [*S*] | *v* | 1/[*S*] | 1/ *v* |
|---|---|---|---|---|
| 1 | 0.008865 | 1.46E-04 | 112.8032 | 6868.132 |
| 2 | 0.01773 | 2.97E-04 | 56.40158 | 3364.738 |
| 3 | 0.026595 | 3.52E-04 | 37.60105 | 2843.332 |
| 4 | 0.03546 | 4.06E-04 | 28.20079 | 2461.236 |

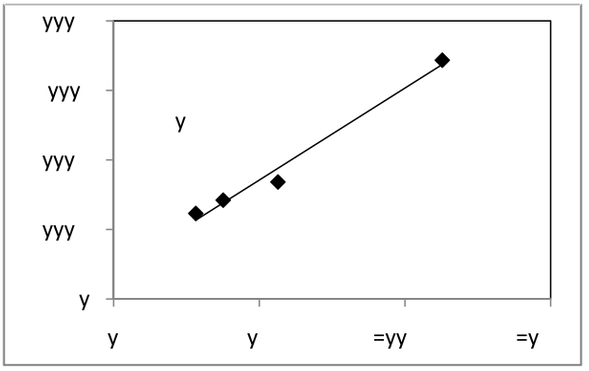


**Fig. 5 : Lineweaver-Burk plot for esterification of oleic acid and decanol**

For enzymatic synthesis of decyl oleate, from oleic acid and decanol with Novozym 435, the Michaelis-Menten constant and maximum reaction velocity $v_{max}$ were obtained as 0.0683 mol/cm$^3$and 0.00129 µmol/cm$^3$.min.

**CONCLUSIONS**

The synthesis of wax ester, decyl oleate, from oleic acid and decanol with Novozym 435 in hexane medium was studied at different oleic acid-decanol molar ratios (1:0.5-1:2), enzyme concentrations (1%-10%) and temperatures (35-50°C). Under optimal conditions of 2.5% enzyme concentration, 1:1 oleic acid-decanol mole ratio and 45°C temperature, a conversion of 96.53% was reached within a reaction time of 60 min. It can be concluded that the enzymatic synthesis of decyl oleate follows Michaelis-Menten kinetics.

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V

# RESEARCH ROUNDUP

## Detection of virgin coconut oil adulteration with animal fats using quantitative cholesterol by GC × GC–TOF/MS analysis

A new method based on the cholesterol level was developed by **Baocheng Xu *et al*** to detect the presence of animal fats in virgin coconut oil (VCO). In this study, the sterols in VCO and animal fats was separated using conventional one-dimensional gas chromatography (1D GC) and comprehensive two-dimensional gas chromatography (GC × GC). Compared with 1D GC, the GC × GC system could obtain a complete baseline separation of the sterol trimethylsilyl ethers derived from cholesterol and cholestanol, so that the cholesterol content in pure VCO and false VCO adulterated with animal fats could be accurately determined **[Food Chemistry, 178** , pp128-135, (2015)]. Cholesterol, a main sterol found in animal fats, represented less than 5 mg/kg of VCO. The study demonstrated that the determination of the cholesterol level in VCO could be used for reliable detection of the presence of lard, chicken fat, mutton tallow, beef tallow, or their mixture in VCO at a level as little as 0.25%

## Optimal extraction and fingerprinting of carotenoids by accelerated solvent extraction and liquid chromatography with tandem mass spectrometry

Accelerated solvent extraction (ASE) is applied for the extraction of carotenoids from orange carrot and the extraction parameters were optimized by **Supradip Saha *et al*** . Two carotenoids, lutein and β-carotene, are selected as the validation process. Hildebrand solubility parameters and dielectric constant of solvents were taken into consideration in selecting solvent mixture **[Food Chemistry, 177**, pp 369-375, (2015)]. The effects of various experimental parameters, such as temperature, static time, drying agent etc., on the ASE extraction efficiency are investigated systematically. Interactions among the variables were also studied. Furthermore, two carotenoids were analyzed and characterized by LC–ESI MS. The study concluded that Hildebrand solubility parameter approach may be applicable for less polar bioactive molecules like carotenoids. The properties of solvent and extraction temperature are found to be the most important parameters affecting the ASE extraction efficiency of thermolabile natural compounds.

## Enzyme-assisted process for DAG synthesis in edible oils

This study by **Daniela von der Haar *et al*** deals with the enzymatic synthesis of diacylglycerols in rapeseed oil by the esterification of free fatty acids and monoacylglycerols **[Food Chemistry, 176**,pp 263-270, (2015)]. As enzymatic reactions are influenced by many factors, a statistical design of experiments was conducted to investigate the enrichment of diacylglycerols, systematically. Simultaneously, the investigated method contributes to the refining process, as the amount of free fatty acids could be reduced significantly from 2% to 0.3%. Utilizing an immobilized lipase from *Rhizomucor miehei*, a maximum diacylglycerol content of 23% was obtained, after optimization. By washing wit iso-propanol and hexane the immobilised lipase could be reused in 14 consecutive batches. In addition, glycerol was proven to be an alternative to MAG as acyl-group acceptor. However, the diacylglycerol enrichment was not accomplished in the same yields as for monoacylglycerols. Summarizing, the present study revealed the potential of an enzymatic diacylglycerol synthesis in edible oils as a suitable alternative to conventional processes also enabling the reduction of free fatty acids in crude oils.

## Characterisation and oxidation stability of monoacylglycerols from partially hydrogenated corn oil

***Zhen Zhang et al*** reported the characterisation of some types of monoacylglycerols (MAGs) obtained by the glycerolysis of different partially hydrogenated corn oils (PHCOs) catalysed by $Al_2O_3$ loaded with $K_2CO_3$ ($K_2CO_3/Al_2O_3$) under the previous selected conditions**[Food Chemistry, 173**, pp 70-79, (2015)]. A two-stage molecular distillation method of purifying the MAGs was introduced, and the obtained MAG products were more than 90.0 wt.% pure. The fatty acid composition of corn oil significantly changed after hydrogenation sequentially catalysed by Pricat™ Ni catalysts (9908 Ni/kieselguhr and 9920 Ni/$Al_2O_3$). The PHCO samples generated typical structures with β′-form crystals. Moreover, the melting regions of all hydrogenated samples and their MAGs shifted to higher temperatures. The oxidation stability of MAGs has been significantly increased using hydrogenation to change the fatty acid composition.

## Development of rice bran oil blends for quality improvement

Six rice bran oil (RBO) blends were prepared by **Monika Choudhary *et al*** in two ratios i.e., 80:20 and 70:30 and analysed for physicochemical properties, and antioxidants and fatty acid composition. Among all the RBO blends, rice bran oil + groundnut oil (70:30) had the highest smoke point (204 °C) and rice bran oil + olive oil (70:30) was the most stable blend in terms of chemical parameters **[Food Chemistry, 173,** pp 770-777, (2015)]. The highest value of total antioxidants was observed in rice bran oil + sunflower oil (70:30) (2568.7 mg/kg). Fatty acid composition (SFA:MUFA:PUFA) (1:1.5:2) of rice bran oil + palm oil (80:20), and products prepared using this RBO blend, were close to the recommended intake. Boiling with sautéing was a better cooking method in terms of maintaining fatty acid ratios

## Use of various vegetable oils in designing photoprotective nanostructured formulations for UV protection and antioxidant activity

Vegetable oils are well-known for their beneficial health effects, mainly due to their antioxidant activity, which is maintained or enhanced when they are encapsulated at nanoscale. This study aims by **Gabriela Badea *et al*** to design new nanostructured lipid carriers (NLCs) containing various vegetable oils and their combinations in order to obtain efficient formulations with UV protection performance and antioxidant activity **[Industrial Crops and Products, 67,** pp 18-24, (2015)]. Pomegranate seed oil, wheat germ oil, blackcurrant seed oil, sesame seed oil, carrot root oil, raspberry seed oil and rice bran oil were used for obtaining NLCs, as carriers for a photoprotective agent that absorbs the UVA radiation: diethylamino hydroxybenzoyl hexyl benzoate. The NLCs were characterized by mean particle size, physical stability over time, and entrapment efficiency of the UVA filter. The UV protection performance was assessed using both *in vitro* sun protection factor (SPF) and the UVA protection factor (UVAPF). The antioxidant activity was determined by chemiluminescence analysis. All vegetable oils used led to the development of appropriate NLCs, having mean particle sizes ranging between 100 nm and 145 nm and good physical stability with zeta potential values less negative than −35 mV. The UV protection factors were evaluated on the individual vegetable oils and on cream formulations based on NLCs. Out of the seven cream formulations, the best UV protection was assured by the pomegranate seed oil based cream resulting in a SPF of 4.1 and an UVAPF of 7.8. Knowing that a synergistic interaction can occur among the active compounds of different oils, new NLCs were developed by using a mixture of pomegranate seed oil and one of the other oils. The NLCs based on pomegranate seed oil combined with wheat germ oil have shown the best entrapment efficiency (70%) and photoprotection (SPF of 5.1 and a UVAPF of 9.5). All vegetable oils showed a good antioxidant activity, which was improved by their incorporation into NLCs. The results have shown that vegetable oils and especially their combinations can be used as renewable raw materials in designing effective and eco-friendly photoprotective nanostructured formulations.

## Lipase-catalyzed process for biodiesel production: Enzyme immobilization, process simulation and optimization

Transesterification of oil feedstocks using immobilized lipase (IL) is a promising process for biodiesel production. However, the running cost of this process is still higher than that of conversional chemical-catalyzed approaches. To address this challenge, both upstream and downstream processes have to be optimized. This review by ***Xuebing Zhao et al*** provides an overview of recent progresses in improving IL-catalyzed biodiesel production, focusing on mid- and down-stream processing such as immobilization of lipase, bioreactors development, process optimization, simulation and techno-economic evaluation **[Renewable and Sustainable Energy Reviews, 44**, pp 182-197, 2015]. The immobilization of lipase is a costly process. Most of the commercial ILs are prepared by adsorption of free lipase on polymeric materials. However, to further reduce cost, works should be focused on developing cheap carriers and strengthening the interaction between enzyme and carrier but without significant loss of lipase activity. Running cost of lipase also can be reduced by improving its lifetime during transesterification. To achieve this goal, solvents can be used to prevent lipase leaching and eliminate the inhibitive effects of alcohol (usually methanol) and glycerol. Downstream processing includes important units to purify biodiesel products. In this part, works should be focused on minimizing energy consumption and waste effluents. A global process integration and optimization with economic evaluation also should be figured out to improve the economic feasibility of Il-catalyzed production of biodiesel.

## Characterizing the epoxidation process conditions of canola oil for reactor scale-up

Novel epoxidized vegetable oils are of current interest for a variety of applications in coatings and polymeric materials. Samples are needed at quantities beyond gram scale to test such applications. However,

scale-up of this very exothermic reaction requires an increased understanding of actual reaction temperature ($T_r$), which may be much higher than the water bath temperature ($T_b$). Canola oil (300 g) was epoxidized by **Ewumbua M. Monono *et al*** at three $H_2O_2$ addition rates (4.6, 2.3, and 1.2 g $min^{-1}$ $mol^{-1}$ unsaturation); at three $T_b$ (55, 65, and 75 °C); and at three reaction times (2.5, 4, and 5.5 h) **[Industrial Crops and Products, 67,** pp 364-372, (2015). The addition rates were obtained by adding 180 g of $H_2O_2$ over 0.5, 1, and 2 h. Maximum $\Delta T$ ($T_r$–$T_b$) was attained within 30–40 min of reaction time; thereafter, reactor temperatures gradually declined even though $H_2O_2$ addition continued. The maximum $\Delta T$ (8.7–15.8 °C) increased as the $T_b$ and $H_2O_2$ addition rate increased; but, no clear trend was observed in the time maximum $\Delta T$ values were attained. The $\Delta T$ vs time profile was a useful indicator of the extent of epoxidation and can be used as a criteria to stop epoxidation reaction. The greatest source of variation in oxirane content was from $T_b$ and reaction time. Therefore, $T_r$ must be maintained within the optimal range during the high exothermic periods, if high resin quality and minimal process time is to be achieved during scale-up.

## The synthesis of bio-lubricant based oil by hydrolysis and non-catalytic of palm oil mill effluent (POME) using lipase

Synthesis of bio-lubricant from palm oil mill effluent (POME) using enzymatic hydrolysis and non-catalytic esterification has been investigated in this article by **M.T.S Syaima *et al***. The effects of essential parameters, which are temperature, pH, agitation speed, enzyme loading, ratio of oil to fatty acid and alcohol to fatty acid, on the reaction rate were examined **[Renewable and Sustainable Energy Reviews, 44**, pp 669-675,(2015)]. The optimum hydrolysis rate (0.1639 mg/sec.L) was achieved at 40 °C, pH 7.0, 650 rpm, 20 U/mL of enzyme loading and 50% (v/v) of POME. As for non-catalytic esterification, the highest reaction rate attained was 0.0018 mg/sec.L at the operating conditions of 75 °C, 950 rpm, and alcohol to fatty acid ratio of 3:1. Viscosity and density of the produced bio-lubricant were also evaluated.

## Performance of whole-cells lipase derived from *Mucor circinelloides* as a catalyst in the ethanolysis of non-edible vegetable oils under batch and continuous run conditions

The catalytic performance of whole *Mucor circinelloides* URM 4182 cells immobilized in polyurethane foam particles was assessed by **Ana K.F. Carvalho *et al*** for the ethanolysis of different vegetable oils, including andiroba (*Carapa guianensis*), coconut (*Cocos nucifera*), jatropha (*Jatropha curcas*), macaw palm (*Acronomia aculeata* ), and palm tree (*Elaeis guineensis*) **[Industrial Crops and Products, 67,** pp 287-294,(2015)]. In a typical batch run, the immobilized cells were added at a vegetable oil-to-ethanol molar ratio of 1:8 using *tert*-butanol as the solvent. Under these conditions, the biocatalyst showed consistent selectivity by producing the corresponding ester from each fatty acid. The highest yield was achieved in the ethanolysis of coconut (97%) and macaw palm (95%) oils whose fatty acid profiles showed predominant concentrations of lauric acid. These results suggested high specificity of intracellular lipases to convert saturated fatty acids into their respective ethyl esters. The ethanolysis activity of the immobilized cells was also assessed at different space times (60–80 h) in a continuous packed-bed reactor using coconut oil as the feedstock. Better reactor performance was found at space time of 80 h. In this condition, 92.7 ± 1.5% of the fatty acids present in the coconut oil were converted into the corresponding ethyl esters. The average volumetric productivity was 3.5 ± 0.7 $mg_{ester}$ $g^{-1}_{biocatalyst}$ $h^{-1}$ with no significant reduction in the reactor efficiency during 25 days.

## Comparative study of diesel and biodiesel on CI engine with emphasis to emissions—A review

Biodiesel is a renewable, nontoxic, eco-friendly and sustainable alternative fuel for compression ignition engines. In spite of having some application problems, biodiesel, in recent times, being considered as one of the most promising alternative fuels in internal combustion engine. It has been proven that the pollutants in the vehicular emissions have significant impacts on the ecological systems and on the health of human beings. Thus there is an increasing demand on tightening the emission standards of motor vehicles, as well as an ever increasing need for developing means of reducing emissions from in service motor vehicles. Biofuel, such as alcohol and biodiesel, could partly replace petroleum fuel, reduce toxic emissions and more importantly restrain the life-cycle emission of CO. In the present paper, earlier studies have been collected and analyzed. The aim of the present study by **V.K. Shahir *et al*** is to evaluate the feasibility of biodiesel in automobiles, with special emphasis on emission aspects **[Renewable and Sustainable Energy Reviews, 45**, pp 686-697, (2015)]. Few aspects on durability and performance are also included. Evaluation is done on both conventional and CRDI engines, though literature on the latter are few and insufficient when compared to the former. Biodiesel use, when compared to diesel as a fuel, in conventional diesel engines with little or no modification

leads to the substantial reduction in particulate matter (PM), hydro carbon (HC) and carbon monoxide (CO) emissions. This is accompanied by a light power loss, increase in fuel consumption and an increase in nitrogen oxide ($NO_x$) emission.

## A systematic study substituting polyether polyol with palm kernel oil based polyester polyol in rigid polyurethane foam

The future depletion of petroleum resources is driving development of sustainable alternatives based on biomaterials. This study by **Athanasia A. Septevani *et al*** is aimed at developing rigid polyurethane foam using high bio-based polyester polyol content without sacrificing the mechanical or thermal insulation performance associated with traditional polyether polyol based rigid polyurethane foam **[Industrial Crops and Products, 66,** pp 16-26, (2015)]. In this paper, we quantify the properties of a model rigid polyurethane foam formulation based on a commercially available polyether polyol and then systematically substitute the polyether polyol with a commercially available palm kernel oil based polyester polyol. The influence of the palm kernel oil based polyester polyol content on reaction kinetics, structure, morphology and mechanical properties of rigid polyurethane foam were evaluated by cup test, Fourier transform infra-red spectroscopy, optical microscopy, and compression testing. Reaction rate was increased by the substation of polyether polyol with palm kernel based polyester polyol. Rigid polyurethane foams were successfully prepared by blending up to 50% of palm kernel oil based polyol with polyether polyol. Mechanical and thermal properties, as well as dimensional stability of rigid polyurethane foam with up to 30% palm kernel oil based polyester polyol gave comparable or better properties to the 100% polyether polyol based foams. Improved compressive strength without compromising thermal insulation was achieved at around 20% palm kernel oil based polyester polyol. This can be due to the formation of hard block segments of rigid urethane linkages composed of palm-based-polyols, into the discrete domains. In terms of the thermal conductivity, the improved thermal insulation properties were achieved at a composition of around 10% palm kernel oil based polyester polyol. Upon substitution of palm based polyols, while the onset degradation temperature was slightly reduced, stability (50% weight loss) above 350 °C was improved.

## Recent trends of biodiesel production from animal fat wastes and associated production techniques

Non-edible feedstocks such as animal fat wastes (AFWs) have recently increased in popularity as alternatives to vegetable oils in the production of biodiesel. They are low cost, mitigate environmental damage and increase the quality of the resultant biodiesel fuel (low $NO_x$ emissions, high Cetane number and oxidative stability). Therefore, AFWs are an excellent feedstock for biodiesel production. Here **Peter Adewale *et al*** provide a comprehensive review trends and techniques in biodiesel production from AFWs **[Renewable and Sustainable Energy Reviews, 45**, pp 574-588,(2015)]. A critical overview of homogeneous and heterogeneous (one- or two-step) catalytic transesterification of AFWs is presented. Similarly, enzyme-catalyzed transesterification and the application of supercritical fluids conversion techniques in the production of biodiesel from AFWs are thoroughly assessed. Finally, cutting edge advances in assisted transesterification processes for biodiesel production are critically reviewed.

## Thermal processing of soybean oil to obtain bio-based polymers and bio-oil

**Vinicius M. Mello *et al*** evaluated the use of triacylglycerides to produce bio-based resins and bio-oils suitable to be used, respectively, as a binder in printing inks (offset) and as diesel-like fuel. Soybean oil was kept under nitrogen atmosphere at temperatures ranging from 260 °C to 370 °C up to 12 h in the presence or absence of a nickel complex as a catalyst precursor **[Industrial Crops and Products, 66**, pp 255-261,(2015)]. It was observed that the reaction occurs in two steps. In the first one, occurs the consumption of the double bonds via Diels–Alder to form a polymer increasing the viscosity of the material. In a second step, the pyrolysis of ester groups and the alkyd chains takes place, reducing a viscosity of the polymers. Besides, using Nickel complex as a catalyst precursor it was observed a high activity to produce polymers with higher viscosity in a shorter time than when comparing with reactions without catalyst. It were also analyzed the bio-oil formed during the reaction. It was observed that without catalyst the pyrolysis leads to the formation of high amounts of carboxylic acids with short chain. However, the presence of Nickel complex increased the formation of hydrocarbons and reduced the amount of formed carboxylic acids, strongly indicating its activity in the deoxygenation.

## Extraction of oil from rubber seeds for biodiesel application: Optimization of parameters

Rubber seed comprises of 40–48% shell and 52–60% kernel by weight of seed was subjected for oil extraction study. The effects of process parameters such as extraction time (3–12 h), kernel size range (0.5–3 mm), ratio of kernel to solvent (0.03–0.09 g/ml), and variety of solvents (polar and non-polar) on

Soxhlet extraction process were studied. Design of experiment (DOE) schemes was considered to prepare an experimental matrix using central composite design (CCD) approach by **Ali Shemsedin Reshad *et al***. Response surface methodology (RSM) was applied to optimize the process parameters to achieve maximum oil yield **[Fuel ,150**, pp 636-644, (2015)]. The maximum oil recovery, 49.36 wt% was obtained during the experiment conducted with hexane as solvent, 0.08 g/ml solute to solvent ratio, average kernel size of 1 mm and 8 h extraction time. Physico-chemical properties of oil obtained from the rubber seed were estimated to measure its suitability for biodiesel production. Proton nuclear magnetic resonance ($^1$H NMR) spectra of the obtained rubber seed oil (RSO) revealed 13.17% linolenic, 39.86% linoleic, 27.06% oleic and 19.91% saturated fatty acid in its composition. These compositional data were qualitatively confirmed with Fourier transform infrared (FT-IR), thermal gravimetric (TG) and differential scanning calorimeter (DSC) analyses of extracted oil samples.

## Process development for scum to biodiesel conversion

A novel process was developed by **Chong-hao Bi *et al*** for converting scum, a waste material from wastewater treatment facilities, to biodiesel. Scum is an oily waste that was skimmed from the surface of primary and secondary settling tanks in wastewater treatment plants. Currently scum is treated either by anaerobic digestion or landfilling which raised several environmental issues **[Bioresource Technology, 185,** pp 185-193, (2015)]. The newly developed process used a six-step method to convert scum to biodiesel, a higher value product. A combination of acid washing and acid catalyzed esterification was developed to remove soap and impurities while converting free fatty acids to methyl esters. A glycerol washing was used to facilitate the separation of biodiesel and glycerin after base catalyzed transesterification. As a result, 70% of dried and filtered scum was converted to biodiesel which is equivalent to about 134,000 gallon biodiesel per year for the Saint Paul waste water treatment plant in Minnesota.

## Palm oil: Processing, characterization and utilization in the food industry – A review

The oil palm tree is an ancient tropical plant that originated from West Africa. Palm oil has centuries' long use as food and medicine. This review covers the recent significant materials found in the literature on palm oil processing, refining, and use in frying especially in blends with other vegetable oils. Crude palm oil (CPO) is obtained from the fruit of the oil palm tree (*Elaeis guineensis*). The oil is rich in palmitic acid, β-carotene and vitamin E. CPO has been fractionated mainly into liquid palm olein and solid palm stearin in order to diversify its food applications. Palm oil is highly stable during frying especially due to the synergistic activity of β-carotene and tocotrienol. In recent years there has been a shift from the use of animal fats and hydrogenated vegetable oils in frying and other food applications. The use of naturally stable oils such as palm oil and composite oils like blends of palm oil and other fats and oils is practiced to ensure that maximum benefits are derived from the oils. Blending offers functional, nutritional and technical advantages, such as tailoring the oil to suit frying applications. The objective of this review by **Ogan I. Mba *et al*** is to combine and condense the body of research on the processing, characterization and use of palm oil especially in frying as well as suggest areas that need further research **[Food Bioscience, 10** , pp 26-41,(2015)].

## In situ transesterification of *Cynara cardunculus* L. seed oil via direct ultrasonication for the production of biodiesel

Alkaline transesterification of *Cynara cardunculus* L. seed oil with methanol for biodiesel production is investigated. Both in situ transesterification and conventional transesterification were studied by **A.A. Koutsouki *et al*** using ultrasonication (24 kHz, without external heating) and mechanical stirring (600 rpm, 60 °C). For in situ transesterification, the use of ultrasonication and mechanical stirring led to similar high % FAME content (96.0 and 93.0% respectively) after 20 min. However the % yield of the extracted methylesters using mechanical stirring was lower compared to ultrasonication (50.4 and 85.1% respectively) **[Fuel Processing Technology, 134**, pp 122-129,2015]. For in situ transesterification via ultrasonication the optimum conditions were: 9.5% NaOH w/w of oil and a 550:1 methanol to oil molar ratio. In conventional transesterification, using ultrasonication, a high % FAME content of methylesters (97.0%) was obtained after 20 min. Respective % FAME content for mechanical stirring was 95.8% after 1 h. In both cases of conventional transesterification 1% w/w of oil NaOH as catalyst and a 7:1 methanol to oil ratio were used. The kinetics used for methanolysis reaction using sonication or mechanical stirring involved the irreversible second order reaction followed by the reversible second

order reaction close to equilibrium. *Cynara* biodiesel properties determined, comply with the specifications of the European Standard EN 14214.

### Flaxseed (*Linum usitatissimum* L.) oil processing and selected products

Flaxseed may be consumed directly as milled seed or crushed to produce familiar products including flaxseed oil and defatted flaxseed meal. However, processes that generate these fractions may play an important role in their biological activity if processes denature, enrich or deplete active ingredients present. A traditional crushing process is used in China while "cold pressing" is commonly used for production of food products. Most flaxseed products arise from these processes. In this review, **Youn Young Shim *et al*** reported on common and newly introduced processes for crushing and fractionating flaxseed and information on the impact of processing on bioactive ingredients **[Trends in Food Science & Technology, 43**, pp 162-177,(2015)]. In addition, we describe some of the diversity of flaxseed containing industrial food and cosmetic product offerings found in several marketplaces.

### Stabilisation of phytosterols by natural and synthetic antioxidants in high temperature Conditions

The aim of the study by **Dominik Kmiecik *et al*** was to assess the potential applicability of natural antioxidants in the stabilisation of phytosterols. A mixture of β-sitosterol and campesterol was incorporated into triacylglycerols (TAGs **[Food Chemistry,173,** pp 966-971( 2015)]. The following antioxidants were added to the prepared matrix: green tea extract, rosemary extract, a mix of tocopherols from rapeseed oil, a mix of synthetic tocopherols, phenolic compounds extracted from rapeseed meal, sinapic acid and butylated hydroxytoluene (BHT). Samples were heated at a temperature of 180 °C for 4 h. After the completion of heating, the losses of phytosterols were analysed, as well as the contents of β-sitosterol and campesterol oxidation products. The total content of phytosterol oxidation products in samples ranged from 96.69 to 268.35 μg/g of oil. The effectiveness of antioxidants decreased in the following order: phenolic compounds from rapeseed meal > rosemary extract > mix of tocopherols from rapeseed oil > mix of synthetic tocopherols > green tea extract > sinapic acid > BHT.

### Sonochemistry Approach to Reducing Biodiesel Reaction Time From *Jatropha Curcas* Oil by Clamp on Tubular Reactor [Energy Procedia,68, pp 480-489, (2015)]

Biodiesel is a form of diesel fuel manufactured from vegetable oils, animal fats, or waste cooking oil. It is safe, biodegradable, and produces less air pollutants than petroleum-based diesel. **Achmad Praptijanto *et al*** present the sonochemistry approach to achieve short biodiesel reaction time from *jatropha curcas* oil by clamp on tubular reactor. The effect of 21 mm, 60 mm diameter of clamp on tubular reactor and conventional stirring method to removing free fatty acid (FFA) and optimizing time of reaction at the acid catalyzed esterification were observed. Transesterification of *jatropha curcas* oil with methanol, in the sodium hydroxide as a catalyst was investigated using clamp on tubular reactor. The effect of different operating parameter such as methanol to oil molar ratio, catalyst concentration to reach optimum condition was studied. The influence of output power on water to oil concentration and time of reaction in the purification were investigated. In the esterification process, the high FFA concentration was removed lower 1% by tube of diameter 60 mm at 5minutes time of reaction. The optimum condition of the transesterification process was achieved at molar ratios of methanol to oil of 7:1, catalyst concentration of 1%, time of reaction of 5 minutes. On the other hand the optimal condition of the purification process at water concentration of 10% (v/v), power output of 240 w, time of reaction of 5 minutes was obtained. Compared with conventional stirring method, the time of esterification reaction by clamp on tubular reactor reduces until 96%, while for the transesterification reaction reduces until 83%.

### Extraction of Spent Bleaching Earth in the Production of Renewable Diesel

The extraction of oils based on animal fat and vegetable oil from two types of spent bleaching earths, namely from the acidic sepiolite and the nonacidic palygorskite, was investigated by the Soxhlet method with hexane as a solvent by ***Vilppu Kuuluvainen et al***. The yields of oil were independent of the feedstock, whereas a much lower oil yield was obtained with palygorskite exhibiting also a smaller surface area as compared to sepiolite which provided a higher yield. The glyceride compositions were very similar in bleached and extracted oils, while slightly lower melting and crystallization energies were determined by

differential scanning calorimetry for the extracted oils bleached with acidic clay indicating minor hydrolysis of triglycerides [Chemical Engineering & Technology, 38, pp 769-776, (2016)]

## Continuous enzymatic interesterification of milkfat with soybean oil produces a highly spreadable product rich in polyunsaturated fatty acids

**Ariela V. Paula *et al*** studied the physical properties of milkfat by enzymatic interesterification with soybean oil in a continuous fluidized bed reactor (FBR) to obtain healthy interesterified fat blends having suitable texture properties for the food industry. The immobilized commercial non-regioselective *Candida antarctica* lipase (Novozym®435) and *sn*1,3-regioselective *Rhizopus oryzae* lipase, immobilized in an organic-inorganic hybrid matrix of polysiloxane-polyvinyl alcohol, were used as biocatalysts in a FBR **[European Journal of Lipid Science and Technology, 117,** pp 608–619,(2015)**].** The minimum value of the ascendant flow of the medium for allowing fluidization in the system was 3.13 $mL \cdot min^{-1}$. The reaction was evaluated in terms of the interesterification yield (IY), consistency values and solid fat content (SFC). The IY values of 10.50 ± 1.64% and of 5.70 ± 1.46% were attained for Novozym®435 and for immobilized *R. oryzae* lipase, respectively. The consistency of the initial 65:35 milkfat/soybean oil mixture (1000 gf/$cm^2$) decreased to 732.35 ± 75.30 gf/$cm^2$ and to 478.02 ± 71.80 gf/$cm^2$ in interesterified blends catalyzed by Novozym®435 or by *R. oryzae* lipase, respectively. SFC was considered an inadequate parameter for following the interesterification of this blend formulation because the values were similar for initial and for interesterified blends. Free fatty acid levels of 1.5%, the non-notable deactivation of Novozym®435 and a half-life of 190 h for *R. oryzae* lipase were observed during the operation time.

## Green Diesel from Hydrotreated Vegetable Oil Process Design Study

A systematic approach was applied by **Tim J. Hilbers *et al*** to study the process of hydrotreating vegetable oils. During the three phases of conceptual, detailed, and final design, unit operations were designed and sized. Modeling of the process was performed with UniSim Design®. Producing green diesel and jet fuel from vegetable oils was found to be technically possible via a flexible process of hydrotreatmen t[Chemical Engineering & Technology [**Special Issue: CHISA: 21st International Congress of Chemical and Process Engineering, 38,** pp 651–657, (2015)] . The resulting mass and energy balances indicated high carbon atom and energy yield. An economic evaluation proved that the operational expenses mainly depend on the cost of raw materials. Currently, the margin between crude palm oil and the retail diesel price is too low to operate an economically viable process. However, production and utilization of biofuels is required by international regulations.

## Improving the Olive Oil Yield and Quality Through Enzyme-Assisted Mechanical Extraction, Antioxidants and Packaging

**Rakesh Sharma *et al*** investigate the use of different enzymes viz. pectinase, cellulase and pectinase CCM alone or in combinations to enhance olive oil recovery and quality. The enzymatic treatment significantly ($P < 0.05$) increased the oil yield and clarity of oil without adversely affecting the quality parameters **[Journal of Food Processing and Preservation, 39,** pp 157–166, (2015)**].** The combination of pectinase + cellulase (1:1) at 0.05% gave maximum oil recovery (11.0%), clarity (optical density = 0.242) and total phenols (165 mg/kg) compared with untreated samples. The oils showed increase in free fatty acid contents (0.82–2.95%), peroxide value (7.30–16.50 meq/kg), $K_{232}$ (1.2–2.2), $K_{270}$ (0.08–0.20) while decrease in iodine value (80.0–78.0) and total phenols (165–139 mg/kg) during 6 months of storage. The overall acceptability scores of oils packed in colored glass bottles with the addition of antioxidant tert-butyl hydroquinone were above 7.50 and placed in the category of "*liked very much* ".

## Modeling vapor pressure of fatty acid and fatty acid methyl esters using cubic equations of state

Biodiesel is one of the most promising alternates to fossil fuels. Production process of biodiesel requires separation of fatty acids (FAs) and their esters from the other products and reactants. This requires the phase equilibrium studies of the FA and their esters. For phase equilibrium study through equation of state (EOS), accurate prediction of vapor pressure is necessary. It was shown that most of the predictive methods fail to estimate acceptable values of vapor pressure. Peng–Robinson EOS was used in the present study by **M. H. Joshipura *et al*** to predict the vapor pressure. Proper values of critical properties are required for accurate prediction of vapor pressure

through cubic EOS. Obtaining experimental critical properties of FAs and their ester are difficult **[Asia-Pacific Journal of Chemical Engineering,10,** pp 170–177, (2015)**].** Ten methods for the prediction of critical properties were compared, and suitable methods for FA, FAME, and their mixtures were proposed. For accurate prediction of vapor pressure using cubic EOS, one requires suitable cohesion factor model. Predictive cohesion factor models did not perform well; hence, four compound-specific cohesion factor models were compared. Constants for all the four models for the compound considered were presented, and the models were compared for the accurate prediction of vapor pressure. It was observed that modified Trebble–Bishnoi type of cohesion factor model outperformed other models. Boiling points for pure FA and FAME were obtained with these four models. Prediction of boiling points for real-world biodiesel was also carried out. Modified Trebble–Bishnoi type model was found to be suitable for predictions of boiling points as well.

**[ Contributed by KN Prasanna Rani ]**

## FORTHCOMING EVENTS

1. World Conference on Fabric and Homecare, at Montreux Music and Convention Centre, Montreux, Switzerland during October 6-9, 2014. This meeting is organized by American Oil Chemists' Society. For details, contact: AOCS Meetings Department, Phone no. +1 217-693-4821; Fax: +1 217-693-4865; e-mail: meetings@aocs.org; website:http:// aocs.org/ meetings.

2. Oils and Fats International Congress 2014 (OFIC 2014) at Kualalampur Convention Centre, Kualalampur, Malaysia during November 5-7, 2014. For details, Contact: Ms. Michelle Lim, OFIC Secretariat, MOSTA, Selangor, Malaysia. E-mail: mosta.secretariat@gmail.com.

3. 69th Annual Convention of Oil Technologists' Association of India and International Conference on Sustainable Technologies and Futuristic Trends: Oilseeds, Oil Processing and Surfactants & Expo 2014 at Hotel Radisson Blue, Agra, India during November 14-16, 2014. For details, contact: Prof R K Trivedi, President, OTAI Central Zone. Phone: +91-9415024771. E-mail: otai2014@otaicentralzone.org; website:www.otaicentralzone.org.

4. Fundamentals of Oilseed and Edible Oil Processing and Refining at Hotel Crowne Plaza, Shanghai Pudong, Shanghai, China during November17-18, 2014. For details, contact: meetings@aocs.org.

5. 4th International Conference on Soaps, Detergents & Cosmetics at Hotel Marriott, Panjim, Goa, India during December 7-9, 2014. For details, contact: Indian Home and Personal Care Industry Association. Tel. +91 2228771857; Fax.: +912228733619. E-mail: ihpcia@ihpcia.org.

6. Refresher Course on Processing and Analytical Methodologies of Oils & Fats at Centre for Lipid Research, CSIR-Indian Institute of Chemical Technology, Hyderabad, during February 25-27, 2015. The Members of SEA, India may avail concession for this course by mentioning their membership numbers. For further details, contact: Dr P P Chakrabarti, Convener, Refresher Course. Tel. +914027193179, +914027191851, Telefax: +914027193370. E-mail: pradosh@iict.res.in.

## NEW BOOKS PUBLISHED

1. Molecules That Amaze Us by Paul May and Simon Cotton, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN : 9781466589605, $53.96, 2014.

2. Chemistry of Sustainable Energy by Nancy E Carpenter, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN : 9781466575325, $71.96, 2014.

3. Industrial Biocatalysis by Peter Grunwald, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN : 9789814463881, $359.96, 2014.

4. Surface Chemistry Essentials by K S Birdi, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN : 9781439871782,$129.95, 2013.

5. Refining Used Lubricating Oils, by James Speight and Douglas I Exall, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN: 9781466551497, $161.96, 2014.

6. Differential Scanning Calorimetry: Applications in Fat and Oil Technology, by Emma Chiavaro, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN: 9781466591523, $152.96, 2014.

7. Lipids: Nutrition and Health, by Claudia Leray, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN: 9781482242317, $89.96, 2014. .

8. Lipids: Nutrition and Health, by Claudia Leray, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN: 9781482242317, $89.96, 2014.

9. Improving Food Quality with Novel Food Processing Technologies by Ozlem Tokusoglu and Barry G Swanson, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN: 9781466507241, $152.96, 2014.

10. Methods in Food Analysis by Rui M S Cruz, Igor Khmelinsku and Margarida Vieira, CRC Press, Cheriton House, North Way, Andover, Hants, SP10 5BE, UK. ISBN: 9781482231953, $116.96, 2014.

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# 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 applicants 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 applicants 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 applicants 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 applicants 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 applicants 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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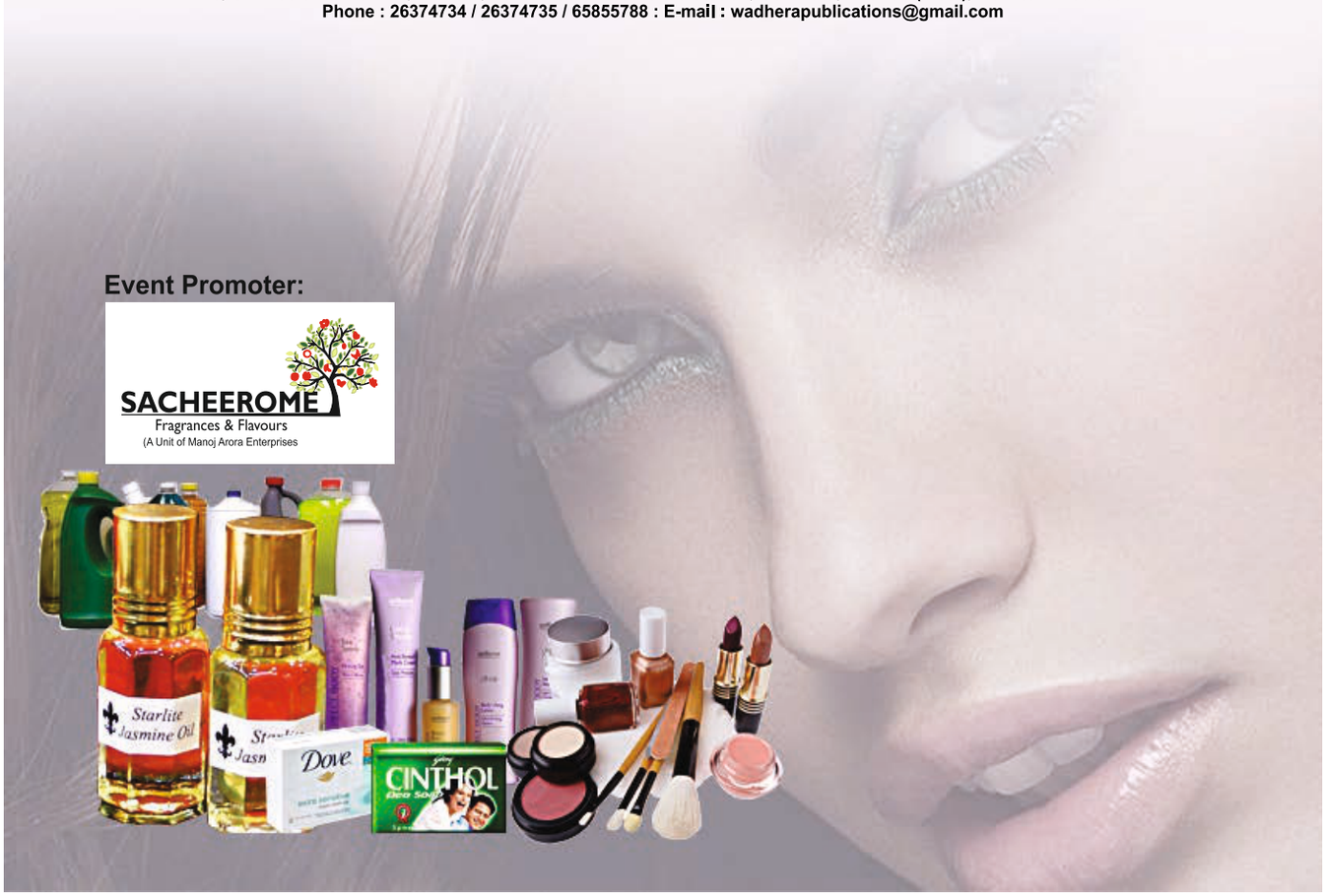

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