ISSN 0973 - 6379

JOURNAL OF LIPID SCIENCE AND TECHNOLOGY

Volume 48 No. 4 Oct - Dec 2016

Published by
Oil Technologists' Association of India

FARELABS

FOOD ANALYSIS & RESEARCH LABORATORY

A commitment for a better tomorrow

Research and Development

Excellence

Creative industry oriented solutions

Analytical Testing

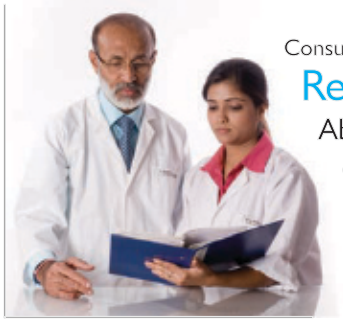
Accuracy

Attention to detail

Credentials

Trustworthiness

Successful business relationships



Consultancy and Training

Reliability

Abundant & diverse experience

Founded in 1999, FARE Labs Private Limited is a leading NABL accredited, FSSAI authorised & MOEF recognised laboratory institution of India. It is based in Gurgaon, and is promoted by Dwijendra Mathur and Chandra Shekar Joshi, a team of chemical engineers with a combined industry work experience of over 51 years.

FARE Labs possesses world-class competencies in Analytical Testing and Research & Development. In addition, it also has a strong Consultancy and Training division to provide holistic solutions to its clients.

Water, Fuels & bio-fuels
Food & Agriculture
Fats, Oils & Allied products
Pollution & Environment
Essential Oils, Herbs & Cosmetics
Soaps, Detergents & Toiletries
Packing material, Petrolium & Lubricants
Industrial & Fine Chemicals
Wood Testing & Soil Strength

FARE LABS Private Limited
L-17/3, DLF Phase-II, IFFCO Chowk, M. G. Road, Gurgaon-122002, Haryana, INDIA.
Phone : +91-124-4223207-08, 4034205, Fax : +91-124-4036038, Cell : +91-93126 64533
Email : farelabs@farelabs.com, Website : www.farelabs.com

Journal of Lipid Science and Technology

Vol. 48 | No. 4 | Oct - Dec 2016

CONTENTS

EDITOR

R.P. Singh

E-mail : journalotai@gmail.com, editorinchief@otai.org, otaihbtu@gmail.com

ASSOCIATE EDITORS

A Madhavan | **PKS Yadav** | **KN Prasanna Rani**

PUBLICATION COMMITTEE

Ashok Mahindru
President

D Mathur
Vice President

CS Joshi
Secretary

RK Srivastava
Hon. Gen. Secretary, OTAI

DN Tewari
Hon. Gen. Treasurer, OTAI

SN Jhunjhunwala
President (CZ)

JP Singh
President (EZ)

RK Singh
President (NZ)

Rajeev Churi
President (WZ)

KVSA Rao
President (SZ)

EDITORIAL ADVISORY BOARD

DK Bhattacharyya | **DN Bhowmick** | **BR Gaikwad**

NB Godrej | **OP Goenka** | **V Mannari**

BVSK Rao | **E Krishnarajan**

AGMARK
C.A.No. A 006186

BL Agro
Pure Perfection

प्रकृति से पकवानों तक

बैल कोल्हू®

• कच्ची घानी सरसों का तेल • रिफाइंड ऑयल्स
• चना बेसन

लाजवाब स्वाद, बेजोड़ शुद्धता

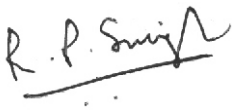
B.L. AGRO OILS LTD., Head Office: B-31, Road No.2, Parsakhera Industrial Area, Bareilly (U.P.)
T: 0581-2518300-399 F: 0581-2518371 | EPBAX: 9927092630 enquiry@blagro.org | www.blagro.org

From Editors Desk

Friends, the Indian farmers are losing interest in cultivating the oil seeds. Consequently, the production has become stagnated to around 28-30 MT in last two decades. The yields are also extremely low and are around 30% of the world average. Due to this reason, the oilseed crushing industry has been suffering from the paucity of oil seeds. Whereas, the oil import in India has risen to 70% of our total need. Even with such high import, the Indian oil refining industry continues to suffer due to import of refined palmolein to the tune of 40%. This is due to the reason that the crude oils fetch 7.5% import duty vis-à-vis 15% duty on refined oils. Therefore, this gives enough justification that we should be importing oilseeds not refined oils into our country. This will enable the crushing industry also to be happier, at the same time the refiners will also get crude oil and will be able to utilize the processing capacity adequately. Therefore, Indian Govt. should impose the higher duty on refined palm to promote the import of crude oil.

The average production of oil per hectare in our country is ~500 kg, whereas palm oil production per hectare is ~4000 kg. Keeping in mind the scarcity of oil/ oilseed availability for our need, we should promote the cultivation of palm plantation with advanced technological interventions. This will help in improving the earnings of farmers as well as it will provide adequate oils to refiners for processing. Beside this, it will also be effectively reducing the burden of FOREX and enhancing the GDP of our country.

We are happy to note that GST is going to be implemented soon. In GST, four tier structure is being proposed by the GST Council, keeping the lowest slab for essential food items and for other food items, it is higher. From the food security point of view, the oil, oilseed and oil cake/ meal are considered as essential food items, therefore GST council should place the minimum slab of GST for these too.



(R.P. SINGH)

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, 7/105-E, Ratan Kunj, Villa-3, Swaroop Nagar, Kanpur - 208 002, e-mail : rpshbti@rediffmail.com, journalotai@gmail.com, otaihbtu@gmail.com, editorinchief@otai.org.
Annual Subscription : Rs 500 (India)/ US $ 60 (Overseas).


घड़ी
डिटर्जेन्ट
भारत को
स्वच्छ रखना है,
कपड़े तो गंदे
होंगे ही
बेफ़िक्र करो देश की सफ़ाई,
कपड़ों की चिंता हम पर छोड़ो
पहले इस्तेमाल करें
फिर विश्वास करें
New Improved
घड़ी
डिटर्जेन्ट पाउडर
पहले इस्तेमाल करें
फिर विश्वास करें
महा शक्तिशाली
घड़ी
डिटर्जेन्ट केक
पहले इस्तेमाल करें, फिर विश्वास करें
RSPL
EVOLUTION IS CONSTANT
For any suggestion or query, contact: 0512-2223606 | Toll Free No. 1800 120 2800

Kinetic Study of Lipase Catalyzed Synthesis of Decyloleate

K. N. Prasanna Rani[1]*, T. S. V. R. Neeharika[1], Ch. Sailu[3], B. Satyavathi [2], T.Prathap Kumar[2]

[1]Centre for Lipid Research, [2] Chemical Engineering Division, CSIR-Indian Institute of Chemical Technology, Hyderabad - 500007

[3]University College of Technology, Osmania University Hyderabad - 500007

**Corresponding author:* Mrs. K.N. Prasanna Rani, E-mail: knpr@iict.res.in

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.

1. 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.

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.

2. Materials and Methods

2.1. 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.

2.2. 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

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

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.

2.3. 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.

3. 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.

3.1. 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.

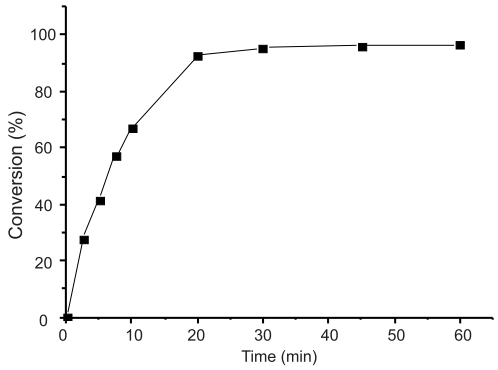


Fig. 1. Effect of time of reaction on esterification of oleic acid and decanol
(Reaction temperature 45°C, enzyme concentration 2.5%, oleic acid-decanol mole ratio 1:1)

3.2. 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.

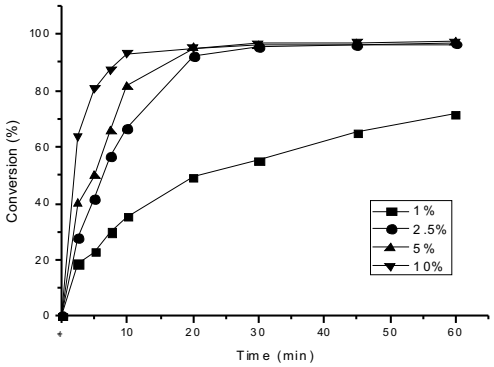


Fig. 2. Effect of enzyme concentration on esterification of oleic acid and decanol
(Reaction temperature 45°C, oleic acid-decanol mole ratio 1:1)

3.3. 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.

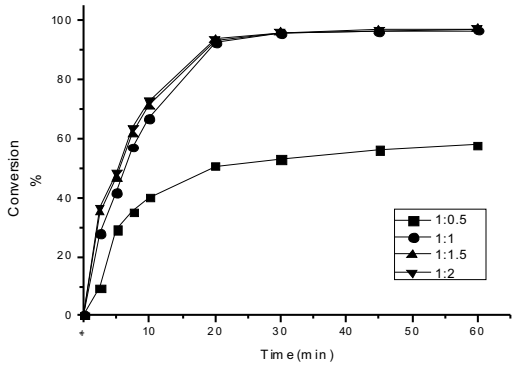


Fig. 3. Effect of oleic acid-decanol mole ratio on esterification of oleic acid and decanol
(Reaction temperature 45°C, enzyme concentration 2.5%)

3.4. 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.

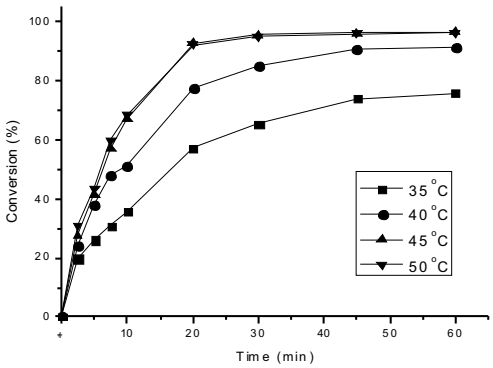


Fig. 4. Effect of reaction temperature on esterification of oleic acid and decanol
(Enzyme concentration 2.5%, oleic acid-decanol mole ratio 1:1) *4.5. Validation of kinetic model*

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

$$\mathcal{E} + \mathcal{S} \; <=> \mathcal{ES} \; --> \mathcal{E} + \mathcal{P} \qquad (2)$$

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

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

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:

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

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

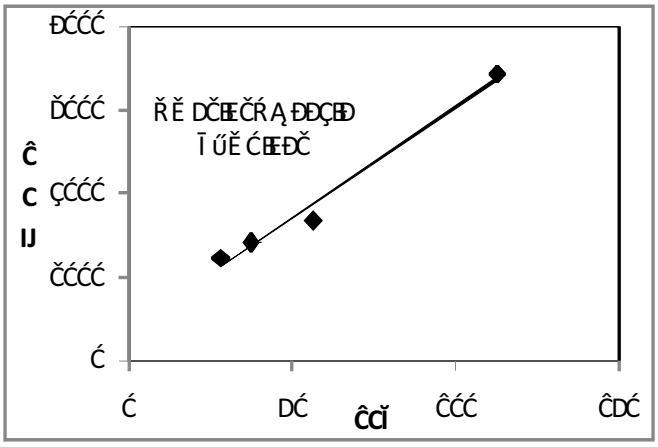


Fig. 5. Lineweaver-Burk plot for esterification of oleic acid and decanol

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.

5. 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.

References

[1] Mukerjee K D and Kiewitt I, **J. Agric. Food Chem. 36,** 1333 (1988).

[2] Doncescu N G and Legoy M D, **J. Am. Oil Chem. Soc. 74,** 1137 (1997).

[3] De B K, Bhattacharya D K and Bandhu C, **J. Am. Oil Chem. Soc. 76,** 451(1999).

[4] Flick E W, **Cosmetics additives: an industrial guide,** Noyes Publications New Jersey, (1991).

[5] Hills G, **Eur. J. Lipid Sci. Technol. 105,** 601 (2003). Garcia

[6] T, Martinez M and Aracil J, **Biocatal. Biotranf. 14,** 67 (1996).

[7] Garcia T, Coteron A, Martinez M and Aracil J, **Chem. Eng. Sci. 51,** 2841(1996).

[8] Garcia T, Coteron A, Martinez M and Aracil J, **Chem. Eng. Sci. 55,** 1411(2000).

[9] Oliveira A C, Rosa M F, Aires-Barros M R and Cabral J M S, **J. Mol. Cat. B Enzy. 11,** 999 (2001).

[10] Shintre M S, Ghadge R S and Sawant S B, **J. Chem. Technol. Biotechnol. 77,** 1114 (2002).

[11] Radzi S M, Mohamad R, Basri M, Salleh A B, Ariff A and Rahman M B, **J. Oleo Sci. 59,** 127 (2010).

[12] Hadzir N M, Basri M, Rahman M B A, Razak C N A, Rahman R N Z and Salleh A B, **Malaysian J. Anal. Sci. 7,** 213 (2001). Kapucu

[13] N, Guvenc F, MehmetoGLU U, Calimli A and Kapucu H, **Chem. Eng. Comm. 190,** 779 (2003). Guncheva

[14] M H and Zhiryakova D, Biotechnol. Lett**.** 30**,** 509 (2008). Woodcock L L, Wiles

[15] C, Greenway G M, Watts P, WellsA and Eyley S, **Inform 26,** 466 (2008).

[16] Gunawan E R and Suhendra D, **Jurnal Matematika Sains 13,** 76 (2008).

[17] Gunawan E R, Basri M and Suhendra D, **Jurnal Natur. Indonesia 14,** 37 (2011).

[18] Deng L, Wang X, Nie K, Wang F, Liu J and Wang P, **Chin. J. Chem. Eng. 19,** 978 (2011).

[19] Kuo C H, Chen H H, Chen J H, Liu Y C and Shieh C J, **Int. J. Mol. Sci. 13,** 11694 (2012).

[20] **Sellami M, Aissa I, Frikha F, Gargouri Y** and **Miled N, BMC Biotechnol. 11,** 68 (2011).

Detection of adulteration in *ghee* using season variation in iodine value studied before and after the application of dry fractionation technique

Anil Kumar[1*], Neelam Upadhyay[2], Kamal Gandhi[3], Satya Narayan Naik[4]
*Corresponding Author: kumaranil_16@rediffmail.com
Centre for Rural Development & Technology, Indian Institute of Technology, Delhi[1&4]
Division of Dairy Chemistry, National Dairy Research Institute, Karnal[2&3]

Abstract

Being a costlier product, adulteration of milk fat (*ghee*) is **a** more common malpractice in India and also abroad because it will fetch more profit to the traders and also result in increased supply. Common adulterants used in *ghee* are cheaper vegetable oils/fats, animal body fat, mineral oil and *vanaspati* etc. Although, in order to ensure a genuine product to the consumer, the Government of India has prescribed the compositional standards for the milk fat (*ghee*) under FSSAI, Regulations (2011) and Agmark Rules (1983) but they have their own limitations because the composition of milk fat **is** greatly affected by season and other factors and also the analytical characteristics of milk fat vary over a wide range, permitting fairly high degree of adulteration while still keeping the constants within the normal limits. In the present study, iodine value was used to establish the authenticity of milk fat (cow and buffalo) before and after addition of soybean oil and buffalo body fat with respect to seasons. The results also revealed that the iodine value showed a wide range (33.58 to 41.03 and 30.28 to 36.21 for cow and buffalo milk fat, respectively) and were found lowest in winter and highest in summer for both type of fats. In addition, dry fractionation technique was coupled with iodine value to increase the sensitivity of the test which enabled the partitioning of the adulterants on the basis of their melting points in different fractions.

Introduction

Milk fat plays an important role in the economics, nutrition and sensory properties of milk and milk products. *Ghee* (anhydrous milk fat) is one of the costlier dairy product and is almost 4 times more than the price of edible vegetable oils/fats. The supply of *ghee* is also far short of its demand. These gaps between price and availability lead**s** to several malpractices. Adulteration of *ghee* is more common malpractice in India and also in abroad because it will fetch more profit to the traders and also result into increased supply. The main types of adulterants are used in *ghee* are cheaper vegetable oils/fats, animal body fat, mineral oil and *vanaspati* etc. The problem of adulteration is further complicated by the fact that the composition of milk fat varies with the species, season and the diet given to the animals (Kelsey *et al*, 2003; Janu *et al*, 2007; Kalac *et al*, 2010) and also the analytical characteristics of milk fat vary over a wide range, permitting fairly high degree of adulteration while still keeping the constants within the normal limits.

We still depend on the traditional methods i.e. Butyro-refractometer reading (BR), Reichert Meissl (RM) value, Polenske value, Iodine value, saponification value etc. (BIS, 1981) to detect adulteration in *ghee* because of the lack of modern techniques (which demand high cost) at reception dock of dairy, where milk is tested for quality before its acceptance or rejection. Now it is realized that these physico-chemical constants have their own limitations in detecting the type and level of all the adulterants particularly in view of multiple component adulteration malpractices. Although, in order to ensure a genuine product to the consumer, the Government of India has prescribed the compositional standards for the milk, butter and milk fat (*ghee*) under FSSAI, Rules (2011) and Agmark Rules (1983).

Earlier, several workers also recommended fractionation technique coupled with different parameters including complete liquification time (Upadhyay *et al*, 2016) iodine value (Gandhi *et al*, 2015), Reichert-Meissl value (Gandhi *et al*, 2014), butyro-refractometer reading (Kumar *et al*, 2013), apparent solidification time (Kumar *et al*, 2010), fatty acid profile using gas chromatography (Frag *et al*, 1983, Kumar *et al*, 2015) etc for the authentication of milk fat to increase the sensitivity of the method. Similarly, present investigation is based on the partitioning of the adulterants in milk fat (*ghee*) on the basis of their melting point using dry fractionation process of ghee to detect adulteration using iodine value.

Methodology

Preparation of pure milk fats (*ghee*):

Cow and buffalo milk fat samples were prepared in laboratory from their respective milks, collected after every two consecutive months up to complete one year (in November, January, March, May, July and September) from the cattle yard of the National Dairy Research Institute, Karnal using creamery butter method (De, 2012) followed by filtration using ordinary filter paper and finally with Whatman No. 4 using vacuum filter assembly.

Collection and preparation of adulterant oil and fat

One vegetable oil (soybean oil) and one body fat (buffalo body fat) were used as adulterant oil/fat in the present investigation.

Vegetable oil:

Refined soybean oil of different popular brands was procured from super market of Karnal (Haryana), India.

Animal depot fat:

Buffalo depot fat was prepared from the respective adipose tissue collected from MDA Exports, Industrial Area, Lawrence Road, New Delhi, India. Adipose tissues were first washed thoroughly with running water and then heated at about 150°C over a direct flame till a transparent liquefied depot fat is obtained. The liquid fat was then filtered through 6 to 8 folds of muslin cloth followed by further filtration by Whatman No. 4 filter paper using vacuum filter assembly.

Preparation of Adulterated Milk fat Samples

For the preparation of adulterated milk fat samples, pure milk fat samples prepared from Institute herd milks were heated to 60-65°C for 10 min before adding and mixing of adulterants.

Adulterants (vegetable oil and depot fat) were added, individually as well as in their combinations, directly to milk fat and mixed thoroughly. At individual levels, the adulterants were added separately @ 5, 10 & 15 percent levels. In case of their combined proportions, milk fat was added with vegetable oil (5, 10 & 15 percent) and depot fat (5, 10 & 15 percent) separately in equal amount thus representing adulteration of total 10, 20 & 30 percent levels.

Since the iodine values of adulterated milk fat samples, as a result of addition of mixture of adulterants (animal depot fat and vegetable oil) were likely to fall within the range of values that would be obtained from pure milk fats, the fractionation approach, as delineated below (Figure I), was adopted so as to get the clear cut separation of added adulterants in to different fractions i.e. solid and liquid fractions of milk fat. This process was done in such a way that most of the depot fats got concentrated in solid fraction, whereas vegetable oil got concentrated in liquid fraction.

Fractionation of pure milk fats and milk fat added with combination of adulterants

For the enrichment of animal depot fat into the solid fraction of milk fat, and of vegetable oil in the liquid fraction, milk fat samples containing combinations of vegetable oil and depot fat were fractionated using dry fractionation (also called melt crystallization or solvent-less fractionation). Fractionation was planned to be studied at two different temperatures for the better partitioning of the particular adulterants into particular fractions of milk fat, depending upon the physico-chemical makeup of adulterant fats/oils. For getting the fractions in a minimum possible time, two temperatures, viz., 15±0.1°C and 10±0.1°C, were selected through trial and error method for fractionation of milk fat samples in successive order so as to give finally a total of 3 fractions (2 solids and 1 liquid) in approximately 20, 60 and 20 percent proportions (Figure I). For this, 200 ml of liquid milk fat samples were taken in 250 ml glass beaker, equilibrated for 5 min at around 60°C in a water bath and then kept in a Biological Oxygen Demand (BOD) incubator maintained at 15±0.1°C. After about 1 Hr 10 min to 1 Hr 20 min of incubation at 15±0.1°C, approximately 20 percent of the whole fat got solidified. This first solid fraction (S_{15}) obtained at 15±0.1°C was

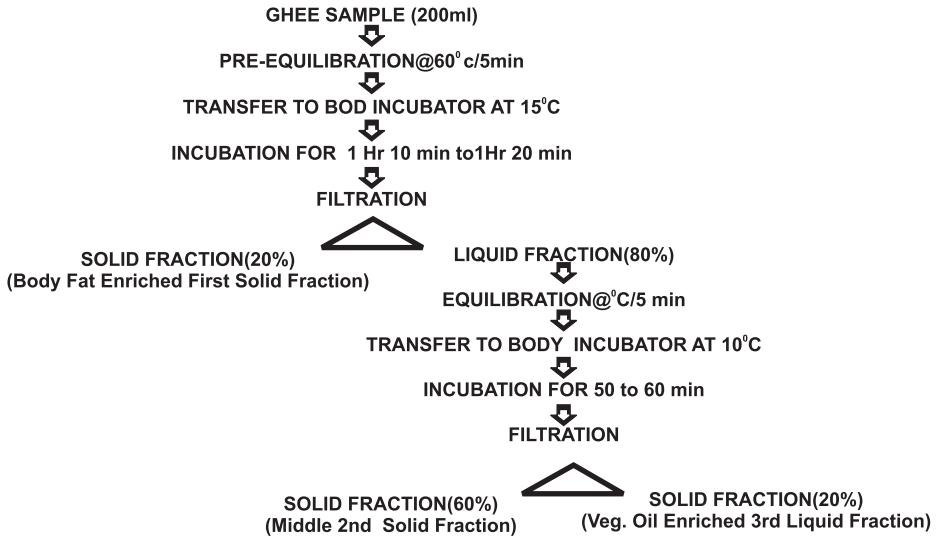


Figure I: Flow diagram showing the dry fractionation technique used for the partitioning of adulterants.

separated from the remaining liquid portion of the whole fat by filtration through single layer of muslin cloth by applying slight pressure, inside the BOD incubator itself so as to avoid the influence of temperature on the yield of the fractions during filtration. The remaining liquid portion obtained above after filtration was again equilibrated at 60 °C for 5 min to remove the opaqueness of the sample and then further fractionated by keeping it in BOD incubator maintained at 10±0.1°C. After about 55 to 60 min of incubation at 10±0.1°C, this liquid portion which further got partitioned into two fractions, one solid (S_{10}) and one liquid (L_{10}) which are middle and last fractions in approximately 60 and 20 percent proportions, were also separated by filtration through single layer of muslin cloth inside the BOD incubator itself. First solid fraction (S_{15}) and last liquid fraction (L_{10}) thus obtained were analyzed so as to characterize these fractions for the presence of depot fat and vegetable oil in milk fat. Middle solid fraction was not analyzed based on the assumption that the enrichment of depot fat and vegetable oil has occurred in first and last fractions.

The iodine value of all the pure milk fat (cow and buffalo) samples, adulterants (soybean oil and buffalo depot fat), milk fat samples added individually with adulterants and combination thereof and the first solid (S_{15}) and last liquid (L_{10}) fractions of pure milk fats and milk fat added with combination of adulterants were determined as per the standard procedure described in IS: 3508 (ISI, 1966).

Results & Discussion

Iodine value is a measure of the degree of unsaturation of a fat or fatty acid and expressed as the number of grams of iodine capable of reacting with 100 g of the sample, is usually determined indirectly by measuring the amount of an iodine-containing reagent, e.g. iodine monochloride, that remains after excess has been added and reaction is complete.

It can be seen from the Tables I and III that the Iodine value for pure cow ghee ranged from 33.58 to 41.03 with an average of 37.50, whereas that for pure buffalo ghee ranged from 30.28 to 36.21 with an average of 33.42. When the values of pure cow and buffalo ghee were combined together, the Iodine values ranged from 30.28 to 41.03 with an average of 35.46 (Table III). The Iodine value for **soybean oil** ranged from 122.58 to 127.82 with an average of 125.25, while buffalo body fat it ranged from 45.17 to 51.53 with an average of 47.70 (Tables I).

It was observed from the results that the Iodine value for pure cow ghee was lowest (33.58) in January and highest (41.03) in May. For pure buffalo ghee also, the lowest Iodine value (30.28) was observed to be in January and highest (36.21) was noticed to be in May, as observed for pure cow ghee (Table I).

Analysis of variance of data (Table II) for Iodine value revealed that pure ghee (cow and buffalo) and adulterant oil and fat differed significantly ($P<0.05$) from each other. Comparison among all the pure samples of ghee, and adulterant fat and oil revealed that pure cow and pure buffalo ghee, soybean oil and buffalo body fat differed significantly from each other ($P<0.05$). Adulterants (soybean oil and buffalo body fat) showed the higher Iodine values as compared to pure ghee samples (Table II). Of the two pure milk fats, the average Iodine value was observed to be higher for cow ghee. Soybean oil showed very high Iodine value, almost 3.5 times to that of pure cow and buffalo ghee samples. Similarly, buffalo body fat also showed higher Iodine value, about 1.35 times to that of pure cow and buffalo

Table I : Iodine value of pure cow and buffalo ghee and adulterants fat/ oil in different months of the year

Parameter	Months							Range*	Average ± SE
	Type of sample	January	March	May	July	September	November		
Iodine Value	PCG	33.58	39.92	41.03	38.53	36.68	35.28	33.58-41.03	37.50+/-1.16^{b}
	PBG	30.28	35.12	36.21	34.36	32.62	31.95	30.28-36.21	33.42+/-0.90^{a}
	SO	122.58	126.36	127.82	125.73	124.64	124.38	122.58-127.82	125.25+/-0.74^{d}
	BBF	48.28	48.13	51.53	46.72	46.36	45.17	45.17-51.53	47.70+/-0.90^{c}
CD Value ($P\leq0.05$)									1.953

PCG- Pure cow ghee PBG- Pure buffalo ghee SO- Soybean oil BBF- Buffalo body fat

*** Data represents mean + SE of six determinations.**
Values bearing different superscripts in each column differ significantly.

ghee samples.

Adulteration of cow as well as buffalo ghee with vegetable oil (soybean oil) and animal body fat (buffalo body fat) individually at 5, 10 and 15 percent levels and in their combinations at 5+5 (10), 10+10 (20) and 15+15 (30) % levels increased the Iodine value and this increase was dependent upon the amount of adulterants added to the pure cow and buffalo ghee. Higher the level of adulterant added, greater was the increase in the Iodine value of ghee samples. It was also observed that addition of vegetable oil (soybean oil) caused higher increase in the Iodine value of ghee.

By keeping in view of the overall range (30.28 to 41.03) for Iodine value of both cow and buffalo pure ghee samples, the results obtained on the basis of comparison of average values showed that the adulteration of both cow and buffalo ghee with vegetable oil individually could easily be detected at all the levels, except 5 percent level in case of buffalo ghee. Whereas, animal body fat added individually in cow and buffalo ghee samples could not be detected at all the levels studied.

When the results on the ghee samples added with mixture of adulterants at 5+5 (10), 10+10 (20) and 15+15 (30) were compared, it was noticed from the results of the average values (Table III) that vegetable oil in the presence of animal body fat studied at all the levels could easily be detected in case of cow ghee samples. On the other hand, in case of buffalo ghee samples, the lower level i.e. 5+5 (10) percent of both the adulterants remained undetectable, while higher levels i.e. 10+10 (20) and 15+15 (30) percent could be detected easily.

When the data of pure and adulterated cow and buffalo ghee samples was combined together, the results revealed that the soybean oil added individually could not be detected at 5 percent level while, higher levels (10 and 15 percent) were detectable. Whereas, buffalo body fat was found to be not detectable at any of the level. Likewise, mixture of soybean oil and buffalo body fat was not detectable at 5+5 (10) percent level, while higher levels i.e. 10+10 (20) and 15+15 (30) percent could easily be detected.

On comparison of the data of samples collected after every two months interval for a complete one year, it was observed from the results of ghee samples added with individual adulterants that the vegetable oil added individually to cow ghee at 5 percent level was detectable in all the months except January and November, while at 10 and 15 percent levels it could easily be detected in all the months. Whereas, addition of animal body fat to cow ghee at 5 and 10 percent levels could be detected only in the month of May, while 15 percent level was detectable in the months of March and May. In case of buffalo ghee samples, vegetable oil added individually was undetectable at 5 percent level throughout the year, while 10 and 15 percent levels could easily be detected in all the months except for 10 percent level in the month of January. Whereas, animal body fat added to buffalo ghee could not be detected at all the levels in any of the month studied (Table IV).

On the other hand, it was observed from the results of Iodine values of a complete one year that the mixture of adulterants in equal ratios at all the levels (5+5, 10+10, 15+15 percent) added to cow ghee could easily be detected in all the months of a year except 5+5 (10) percent level of each adulterant in the months of January and November. Whereas in case of buffalo ghee samples, addition of the mixture of vegetable oil and animal body fat, could be detected at 5+5 (10) percent level in the month of May only, while for remaining month, it remained undetectable *possibly because of the highest iodine value was obtain in this month which crossed the overall range*. However, the higher levels i.e. 10+10 (20) and 15+15 (30) percent of mixture of adulterants could easily be detected in all the months studied (Table IV).

When the data on Iodine values of pure and

Table II : ANOVA on Iodine value for pure cow and buffalo ghee, soybean oil and buffalo body fat in different months throughout the year.

Type of interaction	Sum of Squares	df	Mean Square	F Value
Between fat samples	33706.639	3	11235.546	2135.308*
Within months	105.236	20	5.262	
Total	33811.874	23		

*** Significant at 5% level (p<0.05)**

adulterated (individually as well as in combination) cow and buffalo ghee samples collected after every two months of interval for a complete one year was combined together and compared, it was observed that soybean oil added individually at 5 percent level was detectable in the months of March and May only, while the higher levels (10 and 15 percent) could easily be detected in all the months except 10 percent level in the month of January, whereas the adulteration of buffalo body fat in ghee at all the levels could not be detected in any of the month studied. On the other hand, in case of mixture of adulterants, 5+5 (10) percent level could be detected in the months of March, May and July, while the higher levels i.e. 10+10 (20) and 15+15 (30) percent could easily be detected in all the months throughout the year (Table IV).

The pure ghee samples and the samples adulterated with mixture of adulterants, as explained earlier, were subjected to fractionation at 15°C and 10°C for enrichment of animal body fat in first solid fraction and vegetable oil in last liquid fraction. The first and last fractions were also analyzed for Iodine value so as to know whether fractionation increases the sensitivity of detection of adulteration. The results obtained on Iodine value of first solid (S_{15}) and last liquid (L_{10}) fractions along with their respective combined values are shown in Table V.

For the first solid (S_{15}) fraction of pure cow ghee, the Iodine value ranged from 33.21 to 40.64 with an average of 37.09, whereas the similar values for S_{15} fraction of pure buffalo ghee ranged from 30.02 to 36.02 with an average of 33.25. The Iodine value of the last liquid (L_{10}) fraction of pure cow ghee ranged from 35.64 to 41.68 with an average of 38.58, while that of L_{10} of pure buffalo ghee ranged from 32.87 to 37.15 with an average of 34.92 (Table V). It was observed from the results that the Iodine value of the first solid (S_{15}) fractions of both cow and buffalo pure ghee were lower and that of last liquid (L_{10}) fractions were found to be higher than the corresponding (unfractionated) pure cow and buffalo ghee samples from which the fractions were obtained. Of the two fractions (S_{15} and L_{10}), last liquid (L_{10}) fractions obtained from pure cow and buffalo ghee samples and samples added with combinations of adulterants showed the higher Iodine value as compared to corresponding first solid (S_{15}) fractions. Further, it was also observed that the Iodine value of the first solid (S_{15}) and last liquid (L_{10}) fractions of ghee samples adulterated with mixture of vegetable oil and animal body fat in equal proportion (5, 10 and 15 percent each) increased with the level of adulteration which was supposed to be mainly because of vegetable oil as it possessed very high Iodine value as compared to pure ghee samples.

The low Iodine values for S_{15} and high Iodine value of L_{10} of pure ghee samples as compared to Iodine values of unfractionated pure ghee may be possibly due to the reason that the fractionation process helped in partitioning of triglycerides containing more of saturated fatty acids in to first solid (S_{15}) fraction and triglycerides containing more of unsaturated fatty acids in to last liquid (L_{10}) fraction on the basis of their melting points.

By using the overall range of Iodine value of 30.02 to 40.64 for first solid (S_{15}) fractions and 32.87 to 41.68 for last liquid (L_{10}) fractions of both pure cow and buffalo ghee samples together as the basis and then comparing it with the average Iodine values for the respective first solid (S_{15}) and last liquid (L_{10}) fraction of cow ghee samples added with mixture of soybean oil and buffalo body fat at 5, 10 and 15 percent each, totaling to 10, 20 and 30 percent levels of adulterations, it was noted that the adulteration of soybean oil and buffalo body fat in equal proportions could easily be detected at all the levels (5, 10 and 15 percent of each adulterants) studied. Similarly, when the results on the Iodine value of S_{15} and L_{10} fractions of buffalo ghee added with mixture of adulterants, were compared with the overall range of S_{15} and L_{10} fractions of both pure cow and buffalo ghee, lower level of 5+5 (10) percent could not be detected, while the higher levels i.e. 10+10 (20) and 15+15 (30) percent could easily be detected.

When the data on first solid (S_{15}) fraction of pure and adulterated cow and buffalo ghee samples was combined together and the overall range (30.02 to 40.64) of Iodine value was used as the basis for comparison of Iodine value of first solid (S_{15}) fractions of adulterated ghee samples, it was observed that the lower level of adulteration done at 5 percent of each adulterants could not be detected while, higher levels of adulteration (10 and 15 percent of each) could easily be detected. On the other hand, when the data of last liquid (L_{10}) fractions of pure and adulterated cow and buffalo ghee samples was combined together and the overall range (32.87 to 41.68) of Iodine value was used as the basis for comparison of Iodine value of last liquid (L_{10}) fraction of adulterated ghee samples, the results revealed that all the levels (5, 10 and 15 percent of each adulterants) could easily be detected. This indicated that using the Iodine value of last liquid (L_{10}) fractions, all the levels of adulteration studied for the mixture of soybean oil and buffalo body fat could easily be detected.

However, when the data on S_{15} and L_{10} fractions of ghee samples collected after every two months of interval for a complete year was examined, it was observed that for the first solid (S_{15}) fractions of cow ghee samples added with soybean oil and buffalo body fat at 5+5 (10) percent level could be detected in the months of March, May and July, while on the basis of last liquid (L_{10}) fractions, the same levels of adulterants could easily be detected in all the months. Likewise, the results of first solid (S_{15}) fractions in case of buffalo ghee samples showed that as low as 5+5 (10) percent level could be detected in all the months except for the months of

January and November. Whereas, on the basis of last liquid (L_{10}) fractions of buffalo ghee, the same level could be detected in the month of May only. On the other hand, the higher levels i.e 10+10 (20) and 15+15 (30) percent studied throughout the year, could easily be detected in all the months by using both S_{15} and L_{10} fractions in both the cases of cow and buffalo ghee samples, except the S_{15} fraction of buffalo ghee added with 10+10 (20) percent

level in the month of January (Table VI).

When the data on Iodine values of first solid (S_{15}) fractions of adulterated cow and buffalo ghee samples with mixture of adulterants collected after every two months of interval for a complete one year was combined together and compared with the combined data of cow and buffalo pure ghee samples, it was observed that 5+5 (10) percent level could be detected only in the months of March and May, while it remained undetectable in rest of the months. On the other hand, by using last liquid (L_{10}) fractions, as low as 5+5 (10) percent level of each adulterant could be detected in all the months except January and November. Whereas, the higher levels of 10+10 (20) and 15+15 (30) percent could easily be detected in all the months of a year on the basis of S_{15} and L_{10} fractions, except the S_{15} fraction of ghee sample added with 10+10 (20) percent level in the month of January (Table VI).

On comparing the results obtained on the Iodine value of fractionated ghee samples with these of unfractionated ghee samples, based on the combined data of cow and buffalo ghee together, it may be inferred that fractionation technique has offered advantage in lowering the detection limit using Iodine value because even that level of adulteration (5+5 percent each of soybean oil and buffalo body fat totaling to 10 percent) which could not be detected in case of unfractionated ghee samples, was found to be detectable on the basis of last liquid (L_{10}) fractions.

The similar trend of variation between iodine values of cow and buffalo ghee as noticed in the present study was also observed by earlier workers (Rangappa and Achaya, 1974; Bector and Narayanan, 1974; Lal and Naryanan, 1984). However, Singhal (1973) observed an opposite trend and reported higher iodine values for buffalo ghee than cow ghee. Further, the Iodine value was found to be maximum in the month of May while minimum in January for both cow and buffalo ghee. The similar seasonal variation in iodine value was also observed by earlier workers (Rangappa and Achaya, 1974; Arun Kumar, 2003; Amit Kumar, 2008; Ashvin Kumar, 2010) for both cow and buffalo milk fats, but they have reported different minimum and maximum values for different months of a year.

Table III : Iodine values of cow and buffalo ghee pure and adulterated with individual adulterants and combination thereof.

Adulterant	Level (%)	Iodine value					
		Cow ghee		Buffalo ghee		Mixed (Cow + Buffalo) ghee	
		Range*	Average ± SE	Range*	Average ± SE	Range**	Average ± SE
Control	0	33.58-41.03	37.50±1.16	30.28-36.21	33.42±0.90	30.28-41.03	35.46±0.93
SO	5	38.07-45.35	41.90±1.13	34.93-40.76	38.01±0.88	34.93-45.35	39.96±0.90
	10	42.45-49.73	46.28±1.12	39.55-45.37	42.62±0.87	39.55-49.73	44.45±0.87
	15	46.93-54.08	50.65±1.09	44.14-49.96	47.21±0.87	44.14-54.08	48.93±0.84
BBF	5	34.35-41.53	38.00±1.13	31.23-36.94	34.14±0.86	31.23-41.53	36.07±0.89
	10	35.06-42.03	38.56±1.09	32.12-37.95	34.89±0.89	32.12-42.03	36.71±0.86
	15	35.77-42.62	29.04±1.07	32.94-38.51	35.57±0.85	32.94-42.62	37.31±0.84
SO+BBF	5+5	38.74-45.92	42.42±1.11	35.76-41.56	38.74±0.87	35.76-45.92	40.57±0.87
	10+10	43.98-50.78	47.30±1.05	41.36-46.91	43.95±0.89	41.36-50.78	46.63±0.83
	15+15	49.13-55.64	52.20±1.00	46.85-52.26	49.36±0.81	46.85-55.64	50.78±0.75

SO- Soybean oil BBF- Buffalo body fat

*** Data represents mean + SE of six determinations.**

**** Data represents mean + SE of twelve determinations.**

Table IV : Iodine values of ghee (Cow and Buffalo) adulterated with individual adulterants (Soyabean oil and Buffalo body fat) and combination thereof in different months of the year.

Type of ghee	Type of adulterant fat/oil	Level of adulteration (%)	Iodine Value						Average ± SE
			January	March	May	July	September	November	
Cow Ghee	Control	0	33.58	39.92	41.03	38.53	36.68	35.28	37.50 (+/-) 1.16
	SO	5	38.07	44.26	45.35	42.92	41.09	39.76	41.90 (+/-) 1.13
	SO	10	42.45	48.57	49.73	47.26	45.51	44.21	46.28 (+/-) 1.12
	SO	15	46.93	52.83	54.08	51.52	49.88	48.65	50.65 (+/-) 1.09
	BBF	5	34.35	40.36	41.53	38.91	37.14	35.73	38.00 (+/-) 1.13
	BBF	10	35.06	40.76	42.03	39.35	37.67	36.28	38.56 (+/-) 1.09
	BBF	15	35.77	41.18	42.62	39.76	38.12	36.78	29.04 (+/-) 1.07
	SO+BBF	5+5	38.74	44.64	45.92	43.41	41.52	40.26	42.42 (+/-) 1.11
	SO+BBF	10+10	43.98	49.35	50.78	48.07	46.46	45.18	47.30 (+/-) 1.05
	SO+BBF	15+15	49.13	54.12	55.64	52.85	51.32	50.12	52.20 (+/-) 1.00
Buffalo Ghee	Control	0	30.28	35.12	36.21	34.36	32.62	31.95	33.42 (+/-) 0.90
	SO	5	34.93	39.65	40.76	38.95	37.25	36.54	38.01 (+/-) 0.88
	SO	10	39.55	44.26	45.37	43.45	41.86	41.21	42.62 (+/-) 0.87
	SO	15	44.14	48.83	49.96	48.07	46.45	45.79	47.21 (+/-) 0.87
	BBF	5	31.23	35.75	36.94	34.96	33.32	32.62	34.14 (+/-) 0.86
	BBF	10	32.12	36.46	37.95	35.62	33.96	33.28	34.89 (+/-) 0.89
	BBF	15	32.94	37.09	38.51	36.23	34.72	33.94	35.57 (+/-) 0.85
	SO+BBF	5+5	35.76	40.35	41.56	39.56	37.93	37.27	38.74 (+/-) 0.87
	SO+BBF	10+10	41.36	45.52	46.91	44.76	43.23	41.93	43.95 (+/-) 0.89
	SO+BBF	15+15	46.85	50.78	52.26	49.94	48.53	47.81	49.36 (+/-) 0.81
Mixed (Cow+Buffalo) Ghee	Control	0	31.93	37.52	38.62	36.44	34.65	33.61	35.46 (+/-) 0.93
	SO	5	36.50	41.95	43.05	40.93	39.17	38.15	39.96 (+/-) 0.90
	SO	10	41.00	46.41	47.55	45.35	43.68	42.71	44.45 (+/-) 0.87
	SO	15	45.53	50.83	52.02	49.79	48.16	47.22	48.93 (+/-) 0.84
	BBF	5	32.79	38.05	39.23	36.93	35.23	34.17	36.07 (+/-) 0.89
	BBF	10	33.59	38.61	39.99	37.48	35.81	34.78	36.71 (+/-) 0.86
	BBF	15	34.35	39.13	40.56	37.99	36.42	35.36	37.31 (+/-) 0.84
	SO+BBF	5+5	37.25	42.49	43.74	41.48	39.72	38.76	40.57 (+/-) 0.87
	SO+BBF	10+10	42.67	47.43	48.84	46.41	44.84	43.55	46.63 (+/-) 0.83
	SO+BBF	15+15	47.99	42.45	53.95	51.39	49.92	48.96	50.78 (+/-) 0.75

SO- Soyabean Oil

BBF- Buffalo body fat

Table V- Iodine value of first solid (S_{15}) and last liquid (L_{10}) fractions of cow and buffalo ghee pure and added with combination of adulterants.

Type of Ghee	Type of adulterant fat/ oil	Level of adulteration (%)	Iodine Value			
			Range		Average ± SE	
			S_{15}	L_{10}	S_{15}	L_{10}
Cow Ghee	Control	0	33.21-40.64	35.64-41.68	37.09(+/-)1.16	38.58(+/-)1.02
	SO+BBF	5+5	37.72-45.43	42.34-47.36	41.67(+/-)1.15	44.71(+/-)0.75
	SO+BBF	10+10	41.86-49.82	46.93-51.72	46.01(+/-)1.16	49.46(+/-)0.70
	SO+BBF	15+15	46.83-54.41	52.65-56.63	50.63(+/-)1.22	54.34(+/-)0.64
Buffalo Ghee	Control	0	30.02-36.02	32.87-37.15	33.25(+/-)0.89	34.92(+/-)0.70
	SO+BBF	5+5	34.43-40.67	38.26-41.86	37.58(+/-)0.96	40.17(+/-)0.65
	SO+BBF	10+10	38.64-45.28	43.84-47.73	42.30(+/-)0.94	45.63(+/-)0.64
	SO+BBF	15+15	44.04-49.32	48.92-53.52	46.57(+/-)0.90	50.99(+/-)0.71
Mixed Cow (+) Buffalo Ghee	Control	0	30.02-40.64	32.87-41.68	35.17(+/-)0.91	36.75(+/-)0.81
	SO+BBF	5+5	34.43-45.43	38.26-47.26	39.63(+/-)0.94	42.44(+/-)0.83
	SO+BBF	10+10	38.64-49.82	43.84-51.72	44.16(+/-)0.90	47.54(+/-)0.73
	SO+BBF	15+15	44.04-54.41	48.92-56.63	48.60(+/-)0.95	52.66(+/-)0.68

SO- Soyabean Oil BBF- Buffalo body fat S_{15}- First Solid Fraction L_{10}- Last Liquid Fraction

Table VI- Iodine value of first solid (S_{15}) and last liquid (L_{10}) fractions of pure ghee (cow and buffalo) samples and ghee samples adulterated with mixture of adulterants oil/ fat in different months of the year.

Type of Ghee	Type of adulterant fat/ oil	Level of adulteration (%)	Iodine Value												Average ± SE	
			January		March		May		July		September		November			
			S_{15}	L_{10}	S_{15}	L_{10}	S_{15}	L_{10}	S_{15}	L_{10}	S_{15}	L_{10}	S_{15}	L_{10}	S_{15}	L_{10}
Cow Ghee	Control	0	33.21	35.64	39.37	40.82	40.64	41.68	38.21	39.46	36.35	37.94	34.74	35.93	37.09(+/-)1.16	38.58(+/-)1.02
	SO+BBF	5+5	37.72	42.34	43.92	46.24	45.43	47.36	42.40	44.86	40.62	43.85	39.95	43.63	41.67(+/-)1.15	44.71(+/-)0.75
	SO+BBF	10+10	41.86	46.93	48.08	50.52	49.82	51.72	46.87	50.24	44.93	48.85	44.52	48.47	46.01(+/-)1.16	49.46(+/-)0.70
	SO+BBF	15+15	46.83	52.65	53.68	55.48	54.41	56.63	51.22	54.73	48.82	53.74	48.83	52.78	50.63(+/-)1.22	54.34(+/-)0.64
Buffalo Ghee	Control	0	30.02	32.87	34.92	36.12	36.02	37.15	34.14	35.84	32.43	34.32	31.97	33.21	33.25(+/-)0.89	34.92(+/-)0.70
	SO+BBF	5+5	34.43	38.26	39.45	41.38	40.67	41.86	38.42	41.38	36.76	39.79	35.76	38.36	37.58(+/-)0.96	40.17(+/-)0.65
	SO+BBF	10+10	38.64	43.84	44.15	46.71	45.28	47.73	42.36	46.27	41.76	45.36	41.62	43.84	42.30(+/-)0.94	45.63(+/-)0.64
	SO+BBF	15+15	44.04	49.24	48.63	51.95	49.32	53.52	47.53	51.64	45.23	50.64	44.67	48.92	46.57(+/-)0.90	50.99(+/-)0.71
Mixed Cow (+) Buffalo Ghee	Control	0	31.61	34.25	37.14	38.47	38.33	39.41	36.17	37.65	34.39	36.13	33.35	34.57	35.17(+/-)0.91	36.75(+/-)0.81
	SO+BBF	5+5	36.07	40.30	41.68	43.81	43.05	44.61	40.41	43.12	38.69	41.82	37.85	40.99	39.63(+/-)0.94	42.44(+/-)0.83
	SO+BBF	10+10	40.25	45.38	46.11	48.61	47.55	49.72	44.61	48.25	43.34	47.10	43.07	46.15	44.16(+/-)0.90	47.54(+/-)0.73
	SO+BBF	15+15	45.43	50.94	51.15	53.71	51.86	55.07	49.37	53.18	47.02	52.19	46.75	50.85	48.60(+/-)0.95	52.66(+/-)0.68

SO- Soyabean Oil BBF- Buffalo body fat S_{15}- First Solid Fraction L_{10}- Last Liquid Fraction

Conclusion

From the above part of the study, it may be concluded that Iodine value is an important parameter which can be used as an indicator for checking adulteration in milk fat, especially with vegetable oil. Further, using this parameter, fractionation process has helped in lowering the detection limit of adulteration on the basis of last liquid (L_{10}) fractions.

References

AGMARK (1981). Ghee Grading and Marking Rules, 1938 (as amended). Government of India, Ministry of Food and Agriculture, Department of Agriculture, New Delhi.

Amit Kumar (2008). Detection of Adulterants in ghee. Ph.D. **Thesis submitted to National Dairy Research Institute (Deemed University), Karnal, India.**

Arun Kumar (2003). Comparative studies on physico-chemical properties of various oils and fats with a view to detect adulteration in ghee. **Ph.D. Thesis submitted to National Dairy Research Institute (Deemed University), Karnal, India.**

Ashwin Kumar (2010). A study on physico-chemical qualities of market ghee. **M.Tech. Thesis submitted to National Dairy Research Institute (Deemed University), Karnal, India.**

Bector,B.S. and Narayanan, K.M. (1974). Effect of thermal oxidation on the physico chemical constants of ghee. **Indian J. Dairy Sci., 27: 90-93.**

BIS (1981). Handbook of Food analysis, SP- 18, Part XI-Dairy Products. Bureau of Indian Standards. Manak Bhavan, New Delhi.

De Sukumar (2012). **Outlines of Dairy Technology. Oxford University Press, New Delhi.**

Deffense E (1993). Milk Fat Fractionation Today: A Review. ***J Am Oil Chem Soc.* 70: 1193–1201.**

Dimick PS, Reddy SY, and Ziegler GR (1996). Chemical and thermal characteristics of milk-fat fractions isolated by a melt crystallization. ***J Am Oil Chem Soc.* 73:1647–1652.**

Farag RS, Abo-Raya, Ahmed SH, Hewedi FA and Khalifa HH (1983). Fractional crystallization and gas chromatographic analysis of fatty acids as a means of detecting butterfat adulteration. ***J Am Oil Chem Soc.* 60 (9):1665-1669.**

FSSAI Act (2006) and rules (2011). Akalank's food safety and standards Act, rules and regulation, **Akalank publication, pp-40-43.**

Gandhi K, Kumar A and Lal D (2015). Iodine value integrated with solvent fractionation technique as a tool for detecting palmolein and sheep body fat adulteration in ghee (clarified milk fat). ***Indian J Dairy Sci*. 68 (4): 347-351.**

Gandhi K, Upadhyay N, Aghav D, Sharma V and Lal D (2014). Detection of adulteration of ghee (clarified milk fat) with palmolein and sheep body fat using Reichert-Meissl (RM) value coupled with solvent fractionation technique. ***Indian J Dairy Sci*. 67 (5): 387-393.**

German JB and Dillard CJ (1998). Fractionated milk fat: Composition, structure, and functional properties. ***Food Technol*. 52: 33–38.**

Janů L, HanuŠ O, Macek a, Zajíčková I, Genčurová V, Kopecký J (2007). Fatty acids and mineral elements in bulk milk of Holstein and Czech Spotted cattle according to feeding season. ***Folia Veterinaria*, 51: 19-25.**

Kalač P, Samková E (2010). The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. ***Czech J Animal Scie*nce, 55: 521-537**

Kelsey J A, Corl B A, Collier RJ, Bauman D E (2003). The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. ***J ournal dairy Sci*, 86: 2588-2597.**

Kumar A, Lal D, Kumar A, Sharma V and Seth R (2013). Detection of adulteration in milk fat using Butyro-Refractometer (BR) reading before and after fractionation. ***Indian J Dairy Sci.* 64 (4): 290-297.**

Kumar A, Lal D, Seth R and Sharma V (2010). Detection of milk fat adulteration with admixture of foreign oils and fats using a fractionation technique and the apparent solidification time test. ***Inter J Dairy Tech.* 63 (3): 457–462.**

Kumar A, Upadhyay N, Padghan PV, Gandhi K, Lal D and Sharma V (2015). Detection of Vegetable Oil and Animal Depot Fat Adulteration in Anhydrous Milk Fat (Ghee) using Fatty Acid Composition. ***MOJ Food Process Tech*. 1 (3): 00013. DOI: 10.15406/ mojfpt./ 2015.01.00013.**

Lal, D. and Narayanan, K.M. (1984). Effect of lactation number on the PUFAs and oxidative stability of milk fat. **Ind. J. Dairy Sci., 37: 225-228.**

Rangappa, K.S. and Achaya, K.T. (1974). **Indian dairy products. Asia Publishing House, Bombay. pp 255-326.**

Singhal, O.P. (1973). Studies on ghee (clarified butterfat) and animal body fats with a view to detect adulteration. **Ph.D. Thesis. Submitted to Punjab University, Chandigarh.**

Upadhyay N, Kumar A, Goyal A and Lal D (2016). Complete liquification time test coupled with solvent fractionation technique to detect adulteration of foreign fats in ghee. ***Inter J Dairy Technol*. Vol. 68, doi: 10.1111/1471-0307.12323**.

RESEARCH ROUNDUP

Creating an Efficient Methanol-Stable Biocatalyst by Protein and Immobilization Engineering Steps towards Efficient Biosynthesis of Biodiesel

Two ternary sol–gel matrices, an octyltriethoxysilane-based aliphatic matrix and a phenyltriethoxysilane (PTEOS)-based aromatic matrix, were used by **Shalev Gihaz *et al*** to immobilize a methanol-stable variant of lipase from *Geobacillus stearothermophilus* T6 for the synthesis of biodiesel from waste oil **[Chemsuschem, 9**, 3161–3170, (2016)]. Superior thermal stability of the mutant versus the wildtype in methanol was confirmed by intrinsic protein fluorescence measurements. The influence of skim milk and soluble *E. coli* lysate proteins as bulking and stabilizing agents in conjunction with sol–gel entrapment were investigated. *E. coli* lysate proteins were better stabilizing agents of the purified lipase mutant than skim milk, as evidenced by reverse engineering of the aromatic-based system. This was also shown for commercial *Candida antarctica* lipase B (CaLB) and *Thermomyces lanuginosus* lipase (TLL). Uniform, dense, and nonaggregated particles imaged by scanning electron microscopy and a small particle size of 13 µm pertaining to the system comprising PTEOS and *E. coli* lysate proteins correlated well with high esterification activity. Combining protein and immobilization engineering resulted in a durable biocatalyst with efficient recycling ability and high biodiesel conversion rates.

Eco-friendly Pretreatment of Oil with High Free Fatty Acid Content Using a Sulfamic Acid/Ethanol System

In recent years, sulfamic acid (SA, NH_2SO_3H) has emerged as a novel green catalyst for organic synthesis because of several advantages, including its non-volatility, non-hygroscopicity, non-corrosivity, and low cost. The use of sulfamic acid as a catalyst in the pretreatment of oil with a high content of free fatty acids (10 and 20 % FFA) in ethanol or methanol was reported by **Marcelo G. Montes D'Oca *et al.*** The process (esterification reaction) used in the pretreatment of the oil was carried out under a variety of conditions, initial fatty acid content, temperature, type of alcohol, and % catalyst. The experiments using an NH_2SO_3H/ethanol system resulted in a high fatty acid ethyl ester conversion, 88.53 % (±0.95) from an initial acidity of 40 mg KOH/g (20 % FFA), using 8 % NH_2SO_3H as the catalyst [J. Amer. Oil Chem. Soc**. 93,** p. 1393–1397 (2016)]. According to the results, the methodology using sulfamic acid and ethanol demonstrated elevated potential for a environmentally-friendly means of biodiesel production.

Blending of Palm Mid-Fraction, Refined Bleached Deodorized Palm Kernel Oil or Palm Stearin for Cocoa Butter Alternative

In a detailed study **L.F. Siow *et al.*** evaluated the physicochemical properties of palm mid-fraction (PMF), refined bleached deodorized palm kernel oil (RBDPKO) and refined bleached deodorized palm stearin (RBDPS) as binary mixtures in terms of their fatty acid compositions (GC), triacylglycerols (HPLC), solid fat contents (p-NMR), melting behaviors (DSC) and polymorphisms (XRD) for cocoa butter (CB) alternative formulations [J. Amer. Oil Chem. Soc**.93**, p. 1415-1427 (2016)]. All the PMF/RBDPKO and RBDPS/RBDPKO blends showed mixtures of short/long-chain fatty acids with corresponding triacylglycerols. 10–70 % PMF in RBDPKO showed a eutectic effect between 20 and 30 °C. However, a monotectic effect was observed at 10–15 °C for 20–40 % PMF in RBDPKO and 40–80 % of RBDPS in RBDPKO. For PMF/RBDPS blends, a monotectic effect was observed at less than 30 °C. Broad endotherms at 20–38 °C were observed for 30–50 % RBDPS in RBDPKO which are closer to CB, with polymorphs of $\beta'_1 > \beta'_2 \gg \beta_2$ based on XRD analysis. 50–80 % PMF in RBDPS exhibited significantly higher contents of long-chain fatty acids with the exception of stearic and lower constituents of monounsaturated triacylglycerols compared to CB. Broad endotherms were observed at 20–38 °C for 50–80 % PMF in RBDPS which are closer to CB, with $\beta'_1 \gg \beta'_2 > \beta_2$. Therefore, 20–40 % PMF in RBDPKO, 30–50 % RBDPS in RBDPKO and 50–80 % PMF in RBDPS could be used as CB substitutes because of their comparable physicochemical behaviors.

Production and Emulsifying Effect of Polyglycerol and Fatty Acid Esters with Varying Degrees of Esterification

Esters with acyl groups can be formed by the esterification of polyglycerol. **Nadezhda V. Stolpovskaya *et al.*** prepared different fatty acid esters [hexanoic (caproic), octanoic (caprylic), decanoic (capric), dodecanoic (lauric), tetradecanoic (myristic), hexadecanoic (palmitic), octadecanoic (stearic)] of polyglycerol (average number-of degrees of polymerization of 5) with varying degrees of esterification and examine their emulsifying properties [J. Amer. Oil Chem. Soc**.93**, p. 1429-1440 (2016)]. A number of fundamental catalysts of polyglycerol acylation reactions by methyl esters of carboxylic acid were studied, and sodium methoxide was found to be the best choice. The temperature rate of transesterification increased from 180 to 220 °C with the fatty acid chain alkyl residue. Synthesized mono-, di-, tri-, tetra-, and heptaesters of various fatty acids and polyglycerol provided the highest hydroxyl values from 15 to 815 mg KOH g^{-1} and saponification values from 82 to 321 mg KOH g^{-1}. The emulsifying properties were assessed for all polyglycerol and fatty acid esters, with results showing maximum emulsifying effect for tri- and tetraesters of capric, lauric, and caprylic acids. Regardless of the hydrophilic–lipophilic balance value (HLB) of polyglycerol esters and carboxylic acid, a 4:1 ratio of sunflower oil to water formed a water-in-oil type

emulsion. When mixing oil and water in a 1:1 ratio, mono- and diesters of polyglycerol formed an oil-in-water type emulsion, heptaesters formed a water-in-oil type emulsion, and tri- and tetraesters formed both of types of emulsions, depending on the length of the acid hydrocarbon radicals.

Enhanced Structuring of Fat with Reduced Saturates Using Mixed Molecular Compounds

Molecular compound formation was characterized by **A. G. Marangoni *et al.*** between multicomponent triacylglyceride (TAG) mixtures using palm oil mid fraction enriched in *sn*-1,3-dipalmitoyl-2-oleoylglycerol (cPOP) and a commercial fraction enriched in *sn*-1,3-dioleyl-2-palmitoylglycerol containing a wide range of TAGs (cOPO). Compound formation occurred at a 1:1 (w/w) ratio of cPOP to cOPO corresponding to an equal parts ratio of two different families of TAGs. One family of TAGs consisted of a grouping of all TAGs composed of a saturated–oleic–saturated (StUSt) positioning of fatty acids. The second family consisted of a grouping of all TAGs composed of all oleic–saturated–oleic and saturated–saturated–oleic fatty acids (UStU, StStU). An increase in solid fat content, peak melting temperature, and crystalline domain size and a change in crystalline microstructure were observed at this ratio corresponding to an overall equality in the amount of cPOP to cOPO [J. Amer. Oil Chem. Soc.**93**, p. 1441-1452 (2016)]. This indicated that it is possible to achieve that same level of solid fat content and hardness as a mixture containing more saturates, simply by slightly decreasing the saturates and substituting an oleic monounsaturated fatty acid containing TAG mixture such that a molecular compound is formed. The increase in full-width at half-maximum indicated a decrease in crystalline order and/or decrease in crystal domain size. The characteristic spherulitic crystalline microstructure of OPO changed to small granular crystals upon addition of cPOP. This discovery may allow for the incorporation of a larger proportion of healthy monounsaturated fats into foods while decreasing the use of saturated and *trans* fats.

'Speed of sound in methyl caprate, methyl laurate, and methyl myristate: measurement by Brillouin light scattering and prediction by Wadas group contribution method

The speed of sound related with the isentropic bulk modulus has a significant effect on fuel injection and NO_x emissions in diesel engines. Nevertheless, the speed of sound in pure fatty acid (methyl and ethyl) esters, which were widely used and investigated as the main components of biodiesel, is scarce in the literature. Most of the experimental data are available only at atmospheric pressure or in a narrow range of temperatures. In this work, the speed of sound in three fatty acid methyl esters [FAMEs = caprate (MeC10:0), laurate (MeC12:0), myristate (MeC14:0)] was measured by **Ying Zhang Xiong *et al*** by the Brillouin light scattering method. The measurements were carried out at temperatures ranging from 288 to 498 K along four isobaric lines of 0.1, 4.0, 7.0, and 10.0 MPa **[Energy Fuels, 30**, 9502–9509 (2016)]. The relative expanded uncertainty in the speed of sound was estimated to be less than 1.0%. A rational function that correlates $1/c^2$ as a function of the pressure and temperature was used to correlate the experimental speed of sound in liquid MeC10:0, MeC12:0, and MeC14:0, respectively. In comparison of the experimental speed of sound to the correlation results in the measured range, the absolute average deviations (AADs) are 0.15% for MeC10:0, 0.10% for MeC12:0, and 0.17% for Mec14:0. Moreover, the data were also used to assess the predicted ability of Wada's model.

Formation of 3-MCPD fatty acid esters from monostearoyl glycerol and the thermal stability of 3-mcpd monoesters

Formation of 3-monochloropropanediol (3-MCPD) esters from monostearoyl glycerol (MSG) was investigated under high temperature and low moisture conditions. Different organic and inorganic chlorides, including lindane, KCl, $CaCl_2$, NaCl, $MgCl_2$, $AlCl_3$, $CuCl_2$, $MnCl_2$, $SnCl_2$, $ZnCl_2$, and $FeCl_3$, were evaluated by **Yue Zhao Yaqiong *et al*** for their potential to react with MSG to form 3-MCPD and glycidyl esters at 120 and 240 °C using a UPLC-Q-TOF MS analysis. The results indicated that different chlorine compounds differed in their capacity to react with MSG and formed different products including 3-MCPD mono- and diesters, distearoylglycerol, and glycidyl esters **[J. Agric. Food Chem, 64**, 8918–8926, (2016)]. According to electron spin resonance (ESR) and Fourier transform infrared (FT-IR) spectroscopies, free radical mediated formation mechanisms involving either five-membered or six-membered cyclic acyloxonium free radicals (CAFR) from monoacylglycerol (MAG) were proposed. Tandem quadrupole-time-of-flight (Q-TOF) MS and MS/MS analyses confirmed the free radical mechanisms. In addition, the results from the present study showed that 3-MCPD monoester could be degraded upon thermal treatment and suggested a possible catalytic role of Fe^{3+} under the experimental conditions.

Castor oil based biothiol as a highly stable and self-initiated oligomer for photoinitiator-free uv coatings

Qing Wang Guangxue *et al* reported castor oil based biothiol (CO-SH) as a highly stable and self-initiated oligomer for photoinitiator (PI)-free UV coating. CO-SH was synthesized via esterification reaction between castor oil (CO) and 3-mercaptopropionic acid (MPA) **[ACS Sustainable Chem. Eng, 5**, 376–381, (2017)]. The results of both proton nuclear magnetic resonance (^{1}H NMR) and Fourier transform infrared spectroscopy

(FTIR) revealed that the targeted oligomer was successfully prepared. The oligomer with the number-average molecular weight of 2040 g mol^{-1} exhibited a narrow molecular weight distribution (PDI = 1.23). CO-SH could be stored at 80 °C for 7 days with only a light C=C conversion (≈6%); hence, the steric effect of the vegetable oil molecule gave the primary biothiol an excellent temporal stability. Furthermore, real-time FTIR results indicated that excellent photopolymerization profiles were obtained, and the final conversions of double bond and thiol were 100% and 77% in the absence of PI, respectively, showing the rapid self-initiated photoactivity. In addition, the self-initiated photopolymerization mechanism of CO-SH was investigated. Finally, the UV-curable films of CO-SH also showed good physical and chemical properties

Fattyacidsand sterols composition, and antioxidant activity of oils extracted from plant seeds

Mariola Kozłowska *et al* determined and compared the contents of bioactive components in plant seed oils extracted with *n*-hexane (Soxhlet method) and chloroform/methanol (Folch method) from coriander, caraway, anise, nutmeg and white mustard seeds **[Food Chemistry 213**, 450-456, (2016)]. Oleic acid dominated among unsaturated fatty acids in nutmeg and anise seed oils while petroselinic acid was present in coriander and caraway oils. Concerning sterols, β-sitosterol was the main component in seed oils extracted with both methods. The content of total phenolics in nutmeg, white mustard and coriander seed oils extracted with chloroform/methanol was higher than in their counterparts prepared with *n*-hexane. The seed oil samples extracted according to the Folch method exhibited a higher ability to scavenge DPPH radicals compared to the oil samples prepared with the Soxhlet method. DPPH values of the methanolic extracts derived from oils produced with the Folch method were also higher than in the oils extracted with *n*-hexane.

Revisiting the Enzymatic Epoxidation of Vegetable Oils by Perfatty Acid: Perbutyric Acid Effect on the Oil with Low Acid Value

Enzymatic epoxidation of vegetable oils in the presence of free fatty acids has been well studied in recent years, by mainly using long chain fatty acids (*e.g.*, stearic acid) as the active oxygen carrier. However, for the previous enzymatic processes, the acid value (AV) of final epoxidized oils using long chain fatty acids is high, and the free fatty acid is not easily removed in the post treatment with water. Aiming at developing a more sustainable process, enzymatic epoxidation of sunflower oil was revisited by **Wei Liu *et al.*** using different free fatty acids catalyzed by Novozym 435 (lipase B from *Candida antarctica*, provided by Novozymes, Bagsvaerd, Denmark). When long chain stearic acid was introduced into the epoxidation in toluene solvent, the epoxy oxygen group content (EOC) of 6.41 ± 0.19 % was obtained. Due to the poor water solubility of stearic acid, the AV of the final epoxidized oil product was very high (53.40 ± 1.34) after it was washed with water. Alternatively, current study shows that the epoxidation process using short chain butyric acid produced the final epoxidized oil with lower AV of 2.57 ± 0.11. When the enzymatic epoxidation of sunflower oil was optimized in the presence of butyric acid and Novozym 435, EOC of 6.84 ± 0.21 % was obtained, reaching an oxriane conversion of 96.4 ± 3.0 %. Therefore, introducing short chain butyric acid as an active oxygen carrier will provide an alternative to the present enzymatic epoxidation process and produce the desired epoxidized oil products with much lower AV only after simple water-treatments, which will make the enzymatic epoxidation more attractive **[**J. Amer. Oil Chem. Soc**.93**, p. 1479-1486 (2016)].

Waste Frying Oils as Substrate for Enzymatic Lipolysis: Optimization of Reaction Conditions in O/W Emulsion

Waste frying oils (WFO) can be both environmental pollutants and a source of valuable products. **Miguel García-Román *et al.*** explored the conversion of WFO into surface-active substances, such as FFA and partial glycerides, through enzymatic hydrolysis in O/W emulsion. Two different WFO and three lipases of different origin were tested. In addition, we optimized the conditions for the production of O/W emulsions to be used as reaction medium as well as several reaction parameters, such as the type of enzyme and its concentration, the pH, and the presence of Ca^{2+}ions. Gum arabic-stabilized emulsions with an oil fraction of 0.15 proved to be the most adequate owing to their high interfacial area and short-term stability **[**J. Amer. Oil Chem. Soc**. 93**, p. 1487-1497(2016)]. The physicochemical characterization of both WFO revealed the presence of an increased amount of surface-active matter relative to food-grade vegetable oils. These substances, mainly FFA, can interfere with lipase action and reduce the reaction rate. However, the extent of hydrolysis was only slightly affected by them, and remained fairly similar to that achieved with a control mixture of food-grade vegetable oils. The product distribution depended on the enzyme used. *Pseudomonas fluorescens* lipase was found to be particularly suitable for the production of partial glycerides (mono- and diacylglycerols), which are in great demand by the food industry. In any case our results demonstrate that WFO are a good substrate for enzymatic hydrolysis, comparable to food-grade vegetable oils, providing an alternative route for the valorization of this waste.

Development of High-Strength Soy Protein Adhesives Modified with Sodium Montmorillonite Clay

The high strength of a soy protein adhesive system with good flowability at high protein concentration was investigated by **Xiuzhi Susan Sun *et al*.** Sodium montmorillonite (Na MMT), the most widely used silicate clay, was incorporated into viscous, cohesive soy protein adhesives at concentrations ranging from 1 to 11 % (dry basis, w/w). Hydroxyethyl cellulose was used as a suspension agent to stabilize the soy protein and nano clay to be the dispersion system. The interaction between soy protein and Na MMT was characterized by XRD, FTIR, Zeta potential and DSC. Results indicated that soy protein molecules were adsorbed on the surface of the interlayer of Na MMT through hydrogen bonding and electrostatic interaction. The soy protein/Na MMT adhesives had the intercalation structure with Na MMT contents ranging from 1 to 11 %. Adhesion strength, specifically wet adhesion strength, of soy protein adhesives at isoelectric point (p*I*) was significantly improved by the addition of Na MMT **[**J. Amer. Oil Chem. Soc**. 93**, p. 1509-14517 (2016)]. It is believed that the physical cross-linking reactions between soy protein and Na MMT mainly contribute to the improved adhesion performance of soy protein adhesives. Wet adhesion strength increased from 2.9 MPa of control soy protein adhesive to 4.3 MPa at 8 % Na MMT. An increase of pH beyond p*I* value resulted in decreased adhesion strength due to increased surface charges of soy protein and slightly reduced affinity of soy protein on the nano clay surface.

Characterisation of minor components in vegetable oil by comprehensive gas chromatography with dual detection

The profile of minor compounds, such as alcohols, sterols, free and alkyl fatty acids, waxes, etc., was investigated **by Giorgia Purcaro *et al*** in different vegetable oils by a comprehensive gas chromatographic system, coupled with a simultaneous dual detection (flame ionisation detector and mass spectrometer) for quantitative and qualitative purposes **[Food Chemistry, 212**, 730-738, (2016)]. Such a system generated a unique two-dimensional chromatogram to be used as a chemical fingerprint. Multi-level information, due not only to a more “comprehensive” preparation technique, but also thanks to the exploitation of a more powerful and sensitive analytical determination allowed the extrapolation of diagnostic information from the minor components profile of different vegetable oils, along with their characteristic profile. Furthermore, an admixture of an extra virgin olive oil with a low amount of sunflower and palm oils was evaluated, attesting to the powerful diagnostic information provided by the proposed approach.

A detection method of vegetable oils in edible blended oil based on three-dimensional fluorescence spectroscopy technique

Edible blended vegetable oils are made from two or more refined oils. Blended oils can provide a wider range of essential fatty acids than single vegetable oils, which helps support good nutrition. Nutritional components in blended oils are related to the type and content of vegetable oils used, and a new, more accurate, method is proposed to identify and quantify the vegetable oils present using cluster analysis and a Quasi-Monte Carlo integral. Three-dimensional fluorescence spectra were obtained at 250–400 nm (excitation) and 260–750 nm (emission) **by Jing Xu *et al***. Mixtures of sunflower, soybean and peanut oils were used as typical examples to validate the effectiveness of the method **[Food Chemistry, 212**, 72-77, (2016)].

Determination of theacidvalues of edible oils via FTIR spectroscopy based on the OH stretching band

A new method for determining the acid values (AVs) of edible oils based on the OH stretching band was developed **by Xiuming Jiang *et al***. The oil sample was diluted with carbon tetrachloride and was placed in a quartz cuvette with a thickness of 1 cm to record the FTIR spectrum **[Food Chemistry, 212**, 585-589, (2016)]. The peak at 3535 cm^{-1}, which corresponds to the OH stretch of the carboxyl group in free fatty acids, together with the peak valley at 3508 cm^{-1} and the spectral data in the range of 3340–3390 cm^{-1} were used to determine the AV of the edible oil. The excellent linear relationship between the AVs measured in this work and those measured using a titration method, with a correlation coefficient (R) of 0.9929, indicates that the present procedure can be applied as an alternative to the classic method for determining the AVs of edible oils.

Mechanism of formation of 3-chloropropan-1,2-diol (3-MCPD) esters under conditions of the vegetable oil refining

Jan Smidrkal *et al* carried out work on 3-MCPD esters are contaminants that can form during refining of vegetable oils in the deodorization step. It was experimentally shown that their content in the vegetable oil depends on the acid value of the vegetable oil and the chloride content **[Food Chemistry, 211**, 124-129, (2016)]. 3-MCPD esters form approximately 2–5 times faster from diacylglycerols than from monoacylglycerols. It has been proved that the higher fatty acids content in the oil caused higher 3-MCPD esters content in the deodorization step. Neutralization of free fatty acids in the vegetable oil before the deodorization step by alkaline carbonates or hydrogen carbonates can completely suppress the formation of 3-MCPD esters. Potassium salts are more effective than sodium salts.

Improvement of the Flavor and Oxidative Stability of Walnut Oil by Microwave

Walnut oil is in great demand due to its high nutritional value. However, it is easily oxidized and often loses its typical flavor. **Dong Pei *et al***. studied the role of microwave pretreatment in improving the flavor and oxidative stability of walnut oil, and also investigated the effects of microwave pretreatment on unsaturated fatty acids (oleic, palmitoleic, linoleic, and linolenic acids) and antioxidant components (tocopherols and phytosterols). The results indicate that microwave pretreatment is effective in generating pyrazine compounds. The typical 'roasted' flavor was present when pretreatment for 2 min or more was applied. Meanwhile, compared with the control sample, only the highly treated sample (microwave-pretreated for 4 min) showed higher oxidative stability. Only small changes were found in the composition of the unsaturated fatty acids, while the levels of tocopherols and phytosterols significantly decreased with increasing duration of microwave treatment ($P < 0.05$) [J. Amer. Oil Chem. Soc**. 93**, p. 1563-1572 (2016)]. The results suggest that the Maillard reaction caused the improvement of oxidative stability, since this reaction can also generate antioxidant products (melanoidins) in addition to pyrazines. Moreover, microwave pretreatment was found to be effective for enhancing the oil yield during pressing. Therefore, despite its adverse effects on tocopherols and phytosterols, microwave pretreatment could be used to improve the flavor and oxidative stability of walnut oil.

Lipase-Catalyzed Production of Biodiesel by Hydrolysis of Waste Cooking Oil Followed by Esterification of Free Fatty Acids

Biodiesel is conventionally produced by alkaline-catalyzed transesterification, which requires high-purity oils. However, low-quality oils can be used as feedstocks for the production of biodiesel by enzyme-catalyzed reactions. The use of enzymes has several advantages, such as the absence of saponification side reactions, production of high-purity glycerol co-product, and low-cost downstream processing. **Paulo Waldir Tardioli *et al***. produced biodiesel from lipase-catalyzed hydrolysis of waste cooking oil (WCO) followed by esterification of the hydrolyzed WCO (HWCO) [J. Amer. Oil Chem. Soc**. 93**, p. 1615-1624 (2016)]. The hydrolysis of acylglycerols was carried out at 30 °C in salt-free water (WCO/water ratio of 1:4, v/v) and the esterification of HWCO was carried out at 40 °C with ethanol in a solvent-free medium (HWCO/ethanol molar ratio of 1:7). The hydrolysis and esterification steps were carried out using immobilized *Thermomyces lanuginosus* lipase (TLL/WCO ratio of 1:5.6, w/w) and immobilized *Candida antarctica* lipase B (10 wt%, CALB/HWCO) as biocatalysts, respectively. The hydrolysis of acylglycerols was almost complete after 12 h (ca. 94 %), and in the esterification step, the conversion was around 90 % after 6 h. The purified biodiesel had 91.8 wt% of fatty acid ethyl esters, 0.53 wt% of acylglycerols, 0.003 wt% of free glycerol, viscosity of 4.59 cP, and acid value of 10.88 mg KOH/g. Reuse hydrolysis and esterification assays showed that the immobilized enzymes could be recycled five times in 10-h batches, under the conditions described above. TLL was greatly inactivated under the assay conditions, whereas CALB remained fully active. The results showed that WCO is a promising feedstock for use in the production of biodiesel.

Enantiomeric separation of triacylglycerols containing polyunsaturated fatty acids with 18 carbon atoms

Regioisomers and enantiomers of triacylglycerols (TAGs) containing any combination of stearidonic (18:4n-3) and octadecapentaenoic (18:5n-3) acids were prepared **by Tomas Rezanka *et al*** by organic synthesis**[Journal of Chromatography A, 1467**, 261-269, (2016)]. Gradient polar organic liquid chromatography/high resolution atmospheric pressure chemical ionization-tandem mass spectrometry (NARP-LC/HRMS^2-APCI) and chiral liquid chromatography were used for the separation and identification of molecular species of these TAGs. Further, NARP-LC and chiral LC were used to separate natural mixtures of TAGs obtained from the haptophyte alga *Coccolithophora* sp. cultivated in a salinity range from 7.5 to 60‰. The ratio of regioisomers and enantiomers was found to change with increasing salinity of the culture medium. This can be explained by variable activity of acyltransferases in cells exposed to salt stress.

Microwave enhanced alcoholysis of non-edible (algal, jatropha and pongamia)oilsusing chemically activated egg shell derived CaO as heterogeneous catalyst

Microwave enhanced fast and efficient alcoholysis (methanolysis and ethanolysis) of non-edible oils (*algal*,*jatropha* and *pongamia*) is achieved by **Girdhar Joshi *et al*** by using chemically activated waste egg shell derived CaO (*i.e.*CaO(cesp)) as heterogeneous catalyst [**Bioresource Technology, 219**, 487-492, (2016)]. CaO(cesp) was extracted from waste chicken egg shell and further activated chemically by supporting transition metal oxide. The maximum conversion was achieved using 3 wt% catalysts under 700 W microwave irradiation and 10:1 alcohol/oil ratio in 6 min. Alcoholysis using ZnO activated CaO(cesp) catalyst has shown higher reaction yields in comparison to other modified catalysts. Methanolysis has shown better biodiesel conversion in comparison to ethanolysis. The catalyst has shown longer lifetime and sustained activity after being used for four cycles. Due to more saturated fatty acid content; algal biodiesel has shown improved fuel

properties in comparison to other biodiesels.

Pressurized liquid extraction and chemical characterization of safflowerоil: A comparison between methods

Rogerio Conte *et al* investigated the extraction process of safflower oil using pressurized ethanol, and compares the chemical composition obtained (in terms of fatty acids) with other extraction techniques. Soxhlet and Ultrasound showed maximum global yield of 36.53% and 30.41%, respectively (70 °C and 240 min) **[Food Chemistry, 213**, 425-430, (2016)]. PLE presented maximum global yields of 25.62% (3 mL min^{-1}), 19.94% (2 mL min^{-1}) and 12.37% (1 mL min^{-1}) at 40 °C, 100 bar and 60 min. Palmitic acid showed the lower concentration in all experimental conditions (from 5.70% to 7.17%); Stearic and Linoleic acid presented intermediate concentrations (from 2.93% to 25.09% and 14.09% to 19.06%, respectively); Oleic acid showed higher composition (from 55.12% to 83.26%). Differences between percentages of fatty acids, depending on method were observed. Results may be applied to maximize global yields and select fatty acids, reducing the energetic costs and process time.

Synthesis and Characterization of Phosphonates from Methyl Linoleate and Vegetable Oils

Synthesis of phosphonates on a medium scale (~200 g) from three lipids—methyl linoleate (MeLin), high-oleic sunflower oil (HOSO) and soybean oil (SBO), and three dialkyl phosphites—methyl, ethyl and *n*-butyl, using a radical initiator was reported by **Grigor B. Bantchev *et al*.** A staged addition of the lipid and the initiator was used to achieve good yields. Good results were observed with MeLin (94–99% conversions of the double bonds, as determined by NMR, and 83–99% isolated yields) and HOSO (99–100% NMR conversions, 87–96% isolated yields) using *tert*-butyl perbenzoate as the initiator. With SBO, benzoyl peroxide was used as the initiator, due to its capability to generate radicals at a higher rate at slightly lower temperatures, and thus to shorten the reaction time. Conversions of 91–93% (by NMR) and isolated yields of 80–94% were achieved. The progress of the reaction was monitored with GC–MS [J. Amer. Oil Chem. Soc**. 93**, p. 1671-1682 (2016)]. The products were characterized using ^{1}H, ^{13}C and ^{31}P NMR, IR and gel permeation chromatography. A prolonged reaction led to some transesterification between the carboxylic and phosphite ester groups. Conditions favoring higher reaction rates led to the formation of more oligomers and benzoate fatty ester byproducts. The benzoate fatty ester byproducts were formed by the attack of a benzoate radical on a double bond. The more double bonds that were present per lipid molecule, the more oligomers were formed: MeLin 2–8%, HOSO 3–9% and SBO 8–29%. Optimization of alcoholic soybeanoilextraction as a step towards developingin-situ transesterification for fatty acid isopropyl esters

As a step towards developing *in-situ* transesterification (*in-situ* TE) for seed-to-biodiesel production, four solvents (methanol, ethanol, isopropanol and acetone; neat and in blends) were investigated by **Nattapong *et al*** for their potential in soybean oil extraction. Neat isopropanol compared favorably with the other solvents tested in terms of oil yield and properties of biodiesel product; thus, isopropanol was selected for process optimization of liquid-solid ratio (L-S ratio), shaking speed, extraction time and temperature **[Industrial Crops and Products, 94**, 189-196, (2016)]. L-S ratio had the most significant effect on oil yield, and an increase in extraction temperature was able to reduce the solvent loading and improve the oil yield. Finally, *in-situ* TE was accomplished by applying the optimal conditions of isopropanol soybean oil extraction together with use of sodium catalyst. Applying optimal catalyst concentration and other process conditions, the yield of fatty acid isopropyl ester was 86.7% of the maximum theoretical yield. Moreover, the biodiesel had a low cloud point with −10 °C. This promising biodiesel yield, simple process and superior cold flow property suggests *in-situ* TE may be feasible as a small decentralized process for rural areas

Solid acid as catalyst for biodiesel productionvia simultaneous Esterification and transesterification of macaw palm oil

Heterogeneous catalysis applied to esterification and transesterification of non-edible oil offers a strategy to the clean synthesis of the biodiesel and is driving research interested into the development of acid catalysts for efficient conversion of low quality vegetable oils into fuels to meet future societal demands. Thus, sulfated niobium oxide catalyst was synthesized by the impregnation method and used as a heterogeneous catalyst aimed at biodiesel production via macaw palm oil through high free fatty acid content transesterification with ethanol by Heizir F. de Castro **et al.** The effect of two reaction parameters, molar ratio of ethanol to macaw palm oil and reaction temperature, on ester content and viscosity was studied by the response surface methodology (RSM). The ester content was determined by GC. The catalyst shows excellent activity (99.2% ester content and 4.5 mm^2/s viscosity) towards biodiesel production. Its optimum reaction conditions were: 120:1 molar ratio of ethanol to macaw palm oil at 250 °C reaction temperature. The catalysts characterization was carried out by using the X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), and N_2Adsorption-desorption and Surface Acidity Analyses [Industrial crops and Products, 89, p. 416–424 (2016)].

Biolubricant production from castoroilin a magnetically

stabilized fluidized bed reactor using lipase immobilized on Fe3O4nanoparticles

Candida rugosa lipase was covalently immobilized onto functionalized magnetic nanoparticles (MNPs) by **Mohamad Hajar and Farzaneh Vahabzadeh**, and the particles were structurally characterized by TEM, XRD, FTIR, and VSM analyses. The MNPs-immobilized lipase (MNPs-IL) possessed a specific activity of 2.4 U/mg protein when the protein loading and immobilization efficiency were 38 mg protein/g MNPs and 95%, respectively. The enzymatic conversion of castor oil to biolubricant in a magnetically stabilized fluidized bed reactor (MSFBR) was studied [**Industrial Crops and Products, 94**, 544-556, (2016)]. The performance of MNPs-IL in the reactor was affected by the magnetic field strength and liquid flow rate. The effect of other reaction variables, such as enzyme amount, substrate molar ratio, and temperature, were also determined. A 96.9% methyl ester yield was obtained after 24 h reaction under the optimum conditions.

The biocatalysis was modeled with reference to the ping-pong bi-bi mechanism and considering substrate inhibition, and the following kinetic parameters were obtained: V_{max} = 6.64 mmol/L min,$K_{m,oil}$ = 219.16 mmol/L, $K_{m,methanol}$ = 881.34 mmol/L, $K_{i,oil}$ = 1305.74 mmol/L, and$K_{i,methanol}$ = 623.15 mmol/L. The data were statistically analyzed, and the adequacy of the suggested model was confirmed. In a reusability test of the MNPs-IL in the MSFBR, 87% of the initial activity was retained after eight consecutive runs.

Modeling systems relevant to the biodiesel production using the CPA equation of state

CPA parameters for heavy esters, glycerides, organic acids, and glycerol are presented by **Ioannis Tsivintzelis *et al*** , together with trends of these parameters against the van der Waals volume. Such trends allow the prediction of parameters for compounds for which data are not available **[Fluid Phase Equilibria, 430**, 75-92, (2016)]. Pure fluid parameters were estimated by adjusting model predictions to recent DIPPR correlations and carefully selected literature data. Then, the performance of CPA was evaluated in correlating the vapor – liquid and liquid – liquid equilibrium of binary systems containing fatty acids and their esters, glycerides, water, alcohols and/or glycerol. Satisfactory correlation results were obtained using one (water-acids, alcohols/water - glycerol) or two (systems containing fatty acid esters with water, alcohols or glycerol and mixtures containing glycerides and alcohols) interaction parameters. Moreover, the interaction parameters show smooth trends with carbon chain length, permitting extrapolation for systems for which data are not available. Finally, the estimated parameters and correlations were used for the prediction of liquid-liquid equilibrium of one ternary and one multicomponent mixture. The results showed that accurate predictions are feasible, however indicated the need of more accurate data, at least for important binary mixtures, such as the systems with glycerol.

Unusually low pour point of fatty acid methyl esters with low saturated fatty acid content

Many oilseeds grown in Canada have unusual fatty acid profiles including Cruciferous oilseed varieties that have very low levels (<3%) of saturated fatty acid. Different oil samples were collected by **Martin J.T. Reaney *et al***. from five cultivars of *Brassica rapa* L.*,* seven cultivars of *Brassica napus* L. and five cultivars from other oilseeds species namely *Brassica juncea* L., *Sinapis alba* L., *Camelina sativa* L. and *Linum usitatissimum* L. The fatty acid profile, cloud point (CP) and pour point (PP) were determined for each oil sample. Fatty acid methyl esters (FAME) were synthesized from each oil sample [**European Journal of Lipid Science and Technology, 118, p. 1486-1494 (2016)]**. The saturated fatty acid content was highly correlated (R^2 = 0.93) with PP in methyl esters prepared from *Brassica* sp. oils. In addition, we identified three publications where PP was measured on fatty acid methyl esters and found that the correlation of PP with saturated fat content was consistent with data presented herein. Since *B. rapa* oil exhibited superior low temperature properties, it was chosen for further studies of oxidative stability. *B. rapa* cultivars, with less than 3.5% saturated fat and less than 20% polyunsaturated fat, can be an excellent feedstock for production of oils with improved cold fluidity and oxidative stability.

Selective hydrogenation using palladium bioinorganic catalyst

Palladium bioinorganic catalyst (bio-Pd) was manufactured by **Ju Zhu *et al*** using bacteria (*Desulfovibrio desulfuricans and Escherichia coli*) *via* the reduction of Pd(II) to bio-scaffolded Pd(0) nanoparticles (NPs) **[Applied Catalysis B: Environmental, 199**,108-122, (2016)]. The formed Pd NPs were examined using electron microscopy and X-ray powder diffraction methods: a loading of 5 wt% Pd showed an average particle size of ~4 nm. The catalytic activities of the prepared bio-Pd NPs on both bacteria were compared in two hydrogenation reactions with that of a conventionally supported Pd catalyst (Pd/Al_2O_3). Concentration profiles of the different hydrogenation products were fitted using a Langmuir-Hinshelwood expression. In 2-pentyne hydrogenation, 5 wt% $Pd_{E.coli}$ achieved 100% of 2-pentyne conversion in 20 mins and produced 10.1 ± 0.7 × 10^{-2} mol L^{-1} of desired *cis*-2-pentene; in contrast 5 wt% Pd/Al_2O_3 yielded 6.5 ± 0.4 × 10^{-2} mol L^{-1} of *cis*-2-pentene after 40 mins. In the solvent-free hydrogenation of soybean oil, the use of 5 wt% $Pd_{E.coli}$ yielded *cis*-C18:1 of 1.03 ± 0.04 mol L^{-1} and *trans*-C18:1 of 0.26 ± 0.03 mol L^{-1} (~50% less of the latter than 5 wt% Pd/Al_2O_3) after 5 h. Similar results were obtained using bio-$Pd_{E.coli}$ and bio-$Pd_{D.desulfuricans}$. Bio-Pd was

concluded to have the advantage of a lower *cis-trans*isomerisation in hydrogenation of alkyne/alkenes. Hence biomanufacturing is an environmentally attractive, scalable and facile alternative to conventional heterogeneous catalyst for application in industrial hydrogenation processes. *D. desulfuricans* is inconvenient to grow at scale but wastes of *E. coli* are produced from various industrial processes. 'Second life' (i.e. recycled from a pilot scale biohydrogen production process) *E. coli* cells were used to make bio-Pd catalysts. Although 'bio-$Pd_{secondlife'}$ gave a slower conversion rate of 2-pentyne and soybean oil compared to bio-Pd from purpose-grown cells it showed a higher selectivity to the *cis*-isomer product.

Multiple response optimization to reduce exhaust emissions and fuel consumption of a diesel engine fueled with olive pomaceoilmethyl ester/diesel fuel blends

I. Lopez *et al* with the aim of selecting the optimal operating conditions of a diesel engine (with regard to load and biodiesel blend) besides reducing both main exhaust emissions and *BSFC* (brake-specific fuel consumption*)* a methodology based on multiple response optimization has been proposed [**Energy, [117**, Part 2, 398-404,(2016)]. *BSFC* and emissions (NO_x, SO_2 and CO) using three different load conditions (50%, 75% and 100%) using similar olive pomace methyl esters/diesel blends have been evaluated. Engine testing was followed by the design of the global desirability function in terms of both engine load and biodiesel blends. Eventually, a direct correlation between emissions and load was found. Finally, to ensure minimum values of *BSFC* under three levels of emissions reduction (10%, 20% and 40%) optimum engine operating ranges, in terms of load rate and biodiesel blends, are studied.

Castor oil-based waterborne polyurethanes with tunable properties and excellent biocompatibility

Biopolymers materials with tunable properties are the spotlight in the polymer molecules design and synthesis. **Songping Zhang *et al***. synthesized a series of castor oil based-waterborne polyurethanes (CWPUs) with highly tunable properties by adjusting the content of 2,2-dimethylolbutanoic acid (DMBA), a hydrophilic chain extender. The size of the polyurethane dispersions (PUDs) decreases from 120 nm to 20 nm, when DMBA content increases from 5 to 9 wt%. Infrared spectrum of the corresponding CWPUs films shows that the hydrogen bonds between hard segments have an enhancement as DMBA content increases. The thermal and mechanical properties of the CWPUs films have an obvious change due to this enhanced hydrogen bond interactions and behave like soft elastomer or rigid plastic. For example, the glass transition temperature (T_g) of the CWPUs films increases from 47.8 to 92.9 °C and meanwhile the tensile strength increases from 9.6 to 20.0 MPa. In addition, the CWPUs films are enzymatically hydrolysable due to the surface hydrophilicity. Importantly, those films exhibit excellent biocompatibility to mouse fibroblast (L-929) cells and a relative cell viability of 76% compared to tissue culture polystyrene (TCPS) plates is obtained [**European Journal of Lipid Science and Technology, 118, p. 1512-1520 (2016)]**.

Catalytic valorization of oil-derived fatty esters via cross-metathesis with nitriles

The cross-metathesis of methyl oleate with 3-pentenenitrile to obtain 2-undecene, methyl 9-undecenoate, methyl 11-cyano-9-undecenoate, and 3-dodecenenitrile was studied by Carlos R. Apesteguía et al. at 323 K in a batch reactor using second-generation using Hoveyda–Grubbs (HG) catalysts. Self-metathesis of methyl oleate was the main undesired secondary reaction. For a 3-pentenenitrile/methyl oleate reactant ratio (R3PN/MO) of one, the yield ( ) and selectivity ( ) to cross-metathesis products were 47 and 63%, respectively. The formation of cross-metathesis products increased with increasing 3-pentene initial concentrations, essentially because the reaction equilibrium was shifted to higher methyl oleate conversions. Thus, and were 74 and 83%, respectively, at R3PN/MO = 5. Nevertheless, the HG complex was significantly deactivated when high R3PN/MO values were employed [European Journal of Lipid Science and Technology, 118, p. 1722-1729 (2016)].

Biodiesel production from oleaginous yeasts using livestock wastewater as nutrient source after phosphate struvite recovery

Microbial oils, which can be converted to biodiesel, could be produced by oleaginous yeast. To reduce the overall cost, which is a limitation of heterotrophic cultivation, the livestock wastewater (LW) was examined by Jane Chung et al as a feedstock [Fuel, 186, 305-310, (2016)]. It was found that the LW needed pretreatment, and hydrodynamic cavitation (HC) was effective, surpassing sonication and shaking. The nature of HC made it possible to disintegrate cells and release nutrients like phosphorus; it also rendered ammonia stripping and struvite formation, when MgO was added. The pretreated LW with the HC supported the growth of the two yeasts: C. pseudolambica had 3.6 h of doubling time, 2.19 g/L of DCW, and 35.3% lipid per dry mass; and I. occidentalis had 2.9 h of doubling time with 6.54 g/L of final DCW, and 28.9% lipid per dry mass. All this showed the LW could turn into a valuable resource, as long as a suitable pretreatment.

Process intensification of NaOH-catalyzed transesterification for biodiesel production by the use of bentonite and co-solvent (diethyl ether)

A novel transesterification process for biodiesel production has been developed by Lian Wu et al. Bentonite was used as a water adsorbent to remove moisture from the biodiesel synthesis reaction system [Fuel, 186, 597-604, (2016). Diethyl ether (DEE) was used as a co-solvent to improve the mutual solubility of oil and methanol. The influence of concentration of NaOH, DEE/methanol molar ratio, methanol/oil molar ratio, amount of bentonite, agitation rate, reaction temperature and reaction time was investigated. Response surface methodology was applied to optimize the main process parameters. The maximum FAME yield of 98.35 ± 0.69% was achieved under the optimized conditions of 0.56:1 of DEE/methanol molar ratio, 1.07 wt% concentration of NaOH and 5.65:1 methanol/oil molar ratio. The kinetics and thermodynamic analysis for this process was also carried out. The activation energy for the process was 23.73 kJ mol−1, and the thermodynamic analysis reveals that the net Gibbs energy change for the process was almost same as that for the bentonite-enhanced transesterification process.

Modeling the effects of cosolvents on biodiesel production

In this study, a mathematical model is developed by Aliakbar Roosta and Iman Sabzpooshan for predicting the effect of cosolvents on the biodiesel production, by taking into account the fact that the process consists of two liquid phases [Fuel, 186, 779-786, (2016)]. The proposed model consists of a detailed chemical kinetic reaction mechanism and the phase equilibrium of the two phases. The experimental data for estimating the reaction rate constants consists of 1580 dynamic concentration data related to cosolvent-free systems. Then, the calculated reaction rate constants are used to model the biodiesel production process in systems containing cosolvents to investigate the cosolvent effects on the process. The average absolute relative deviation of 1.0% for all the experimental data points is a testament to the accuracy of the predictive model. Hence, the model was used to investigate the effect of methanol to oil ratio, the ratio of cosolvent to methanol, and the amount of catalyst on the biodiesel production rate and yield, in the presence of cosolvents tetrahydrofuran and diethyl ether. According to the results, the use of cosolvent leads to increase the reaction rates. In the other words, the process time is reduced; however, the final yield of biodiesel does not change. Comparison between two cosolvents shows that diethyl ether is more appropriate than tetrahydrofuran, because, in the use of diethyl ether, less cosolvent to methanol ratio is needed. Also, the effect of catalyst concentration is similar to the effect cosolvent.

A critical review on microalgae as an alternative source for bioenergy production: A promising low cost substrate for microbial fuel cells

The environmental pollution caused by the excess of human energy consumption and the foreseeable depletion of fossil fuels underline the need for new eco-friendly, sustainable and cost effective energy sources. Recent research works on microalgae have identified this new bio-material as a promising technology for bioenergy production, wastewater treatment, the development of high value added products and CO2 capture. Microalgae can be used to produce biodiesel, bioethanol, methane or hydrogen. However, one of the newest applications of this bio-material is its use in microbial fuel cells (MFCs). The resulting microalgae-MFC systems can produce electricity using the electrons released to the anode during microalgae degradation. Furthermore, microalgae can be grown in the cathode chamber, capturing the CO2therein and using light as power source. Z. Baicha et al reviewed an overview of new applications of microalgae for bioenergy production [Fuel Processing Technology, 154, 104-116, (2016)]. It includes as a novelty the use of microalgae for electricity generation in microalgae-MFCs and capturing the CO2 emissions of these systems, their advantages, limitations and future prospects

Reactive desorption of fatty acid adsorbed on γ-alumina using supercritical methanol

A novel method for regeneration of fatty acid-adsorbed γ-alumina using supercritical methanol was developed by Hee Suk et al. This method is based on a difference in the affinity of a fatty acid and the corresponding fatty acid methyl ester (FAME) for γ-alumina [Ind. Eng. Chem. Res, 55, 10420–10426, (2016)]. Palmitic acid was selected as a model fatty acid. Batch-type reactors were used to investigate the effect of the operating parameters (temperature, methanol to γ-alumina weight ratio, and reaction time) on supercritical methanol regeneration. Almost all of the adsorbed palmitic acid was desorbed at temperatures above 300 °C, or when the weight ratio of methanol to γ-alumina was higher than 75:1, and/or if the reaction time was longer than 15 min. The crystal structure and BET surface area of γ-alumina were unchanged after supercritical methanol regeneration. The developed technique requires relatively lower operating temperature, is ecofriendly, and generates fuel, although the process is more complex than the thermal regeneration method.

Clean enzymatic preparation of oxygenated biofuels from vegetable and waste cooking oils by using sponge like ionic liquids technology

The biocatalytic synthesis of oxygenated biofuels (fatty acid solketal esters, FASEs) and biodiesel (fatty acid methyl esters, FAMEs) was carried out by Pedro LozanoCelia et al by both the direct esterification of fatty

acids (i.e., lauric, myristic, palmitic, and oleic acids, respectively) with solketal or methanol, and the transesterification of vegetable oils (i.e. sunflower, olive, cottonseeds, and waste cooking oil) with the same alcohols, in hydrophobic ionic liquids (ILs) based on cations with long alkyl side-chains (e.g., octadecyltrimethylammonium bis(trifluoromethylsulfonyl)imide [C18tma][NTf2]) [ACS Sustainable Chem. Eng, 4, 6125–6132, (2016). These hydrophobic ILs are temperature switchable ionic liquid/solid phases that behave as sponge-like system. As liquid phases, they are excellent monophasic reaction media for proposed biotransformations with all the assayed fat substrates, e.g. near to 100% yield of fatty acids solketyl esters (FASEs) and fatty acid methyl esters (FAMEs) in 6 h at 60 °C. By using waste cooking oil mixed with free fatty acids as substrate, green biofuels containing either both FAMEs and FASEs (e.g., aprox. 80% FAMEs and 20% FASEs, etc.) can easily be prepared. Moreover, the reaction mixture can be easily fractionated by iterative centrifugations at controlled temperature into three phases, i.e. solid IL, water, and FAMEs + FASEs mixture leading to a straightforward and clean approach allowing the full recovery of the biocatalyst/IL system for further reuse and the simple product isolation. Furthermore, the enzyme did not shown any loss in activity during reuse in these reaction systems after six operation cycles.

Developments and challenges in biodiesel production from microalgae: A review

The imminent depletion of fossil fuels and the surging global demand for renewable energy have led to the search for nonconventional energy sources. After a few decades of trial and error, the world is now testing the sources of the third generation of fossil fuels, which contain for most parts microalgae. With more than 80% oil content, being adaptable in growth parameters and highly versatile, microalgae are highly promising sources of biofuels in the present time. The present article by Tanvi Taparia et al makes a sweeping attempt to highlight the various methods employed for cultivation of microalgae, techniques to harvest and extract biomass from huge algal cultures, as well as their downstream production and processing procedures [Biotechnology and Applied Biochemistry, 63, 715–726, (2016)] . The advantages, limitations, and challenges faced by each of them have been described to some extent. Major concerns pertaining to biofuels are supposed to be their environmental sustainability and economic viability along with their cost effectiveness. This would require a great deal of empirical data on existing systems and a great deal of optimization to generate a more robust one. We have concluded our article with a SWOT analysis of using algae for biodiesel production in a tabulated form.

JOURNAL FOR LIPID SCIENCE AND TECHNOLOGY (JLST)
Guidelines for Authors

About Journal

The *Journal of Lipid Science and Technology (JLST)* is a quarterly published journal by Prof. R. P. Singh, on behalf of the Oil Technologists' Association of India (OTAI), C/o OTAI Building, Harcourt Butler Technical University, Kanpur-208002, UP (INDIA). It aims to provide a central path for exchanging and disseminating new ideas and technologies in the fields of vegetable oils and their derivatives, alternative fuels (especially bio fuel), surface coatings, lubricants, detergents, polymers, foods and food products. The JLST publishes the original research paper, review paper, general article, newsletters and short communication on various aspects such as new process development for storage, production and refining, by-products utilization, environmental impact and economical aspects of above mentioned fields.

The technology for vegetable oils include harvesting and storage of oil bearing materials, extraction of oil, refining, packaging and supply chain management for both edible as well as non-edible oils. Vegetable oil derivatives include confectionery products, green polymer, surfactants, etc. If new ideas are outstanding, but do not belong to above fields, will also be considered for publication.

Audience

Vegetable oil processing researchers, energy researchers, engineering and technology students, surfactants and interfacial scientist and engineers, chemists, bio scientists, polymer researchers, food researchers, environmentalists, effluent treatment researchers and companies working in these fields.

Guidelines for Author

You may submit your paper either PDF or Word document file during submission stage. After getting your paper accepted, you will be asked to submit the correct format of paper. You may be asked some additional data if required by the editorial board.

We keep the same sequence of authors provided in the manuscript submitted by the author (s). The journal will not make any correction even after your request, therefore, we request you to write the order on which all authors must agree before submitting the article. We will coordinate with only one corresponding author. It is his responsibility to convey all message/queries with respect to manuscript during the peer-reviewing.

Submission Check List for Author

Before submitting the article, authors are requested to check the following requirements.

Address check lists

1. Email address of corresponding author – spell checked.
2. Correspondence address of the same – spell checked.

Manuscript

1. The manuscript should be written in either “Times new roman” or “Arial” format with font size 14 for headings and 12 for the body and subheadings.

2. The abstract must not be exceeding 200 and 300 words for original and other than original research papers respectively. Include all keywords and keywords should be in *italic*.

3. Use number 1, 2, 3 etc. for figure as well as table numbers. Figure caption should be below the figure while table caption must be above table. Table must be submitted as editable text. And whenever you refer the figure or table in text use the same table or figure number. Avoid the use of above or below word in referring. For appendices use A.1, A.2 and so on.

4. Before using abbreviation for any particular, first use the full form along with its abbreviation even for standard abbreviation. You should also write a footnote for all abbreviations on the first page of the paper. Use the full form only once for any abbreviation and after that make uniformity by using only abbreviation.

5. Use different colour along with different shapes of legend if the figure contains two or more lines or

area or any other plots.

6. Article as well as grammar must be checked.

References Requirements

If something is not original and taken from some other research, credit must be given by referring them into your article. **All references should be arranged in alphabetical order**. If your reference papers having only one or two authors, no need to write *et al.*, write as (author (s) name, year). If there are more than two authors, write the reference when you refereeing it – (first author *et al.*, year). If one author has published more than one paper in a year – differentiation must be done by putting 1, 2, 3 etc. as a superscript on year. There is also **no need to bulletize the references using any numeric or alphabet**. The maximum number of citations from a single journal article should not exceed 4-6. The minimum number of references must be more than 15 and 50 for original research and review papers respectively. For completely new ideas, paper can be accepted for further process without meeting the minimum requirement of references, but reviewers must be convinced to do so. You can also use the Google scholar to get the references. Writ you references as following on reference pages.

Author (s). Year. Article title. Journal Title volume (issue), volume, pages.

Example: Hussain, S., Mahmood, Z., Ahmad, I., Mahmood, Q., Ahmad, Z., Khan, J., Farooq, A., Rashid, N., and Wu, D., 2010, "Low temperature conversion of plastic waste into light hydrocarbons," Journal of Hazardous Materials **179**, 15–20.

Note: Reference must contain all authors name.

Subscription

Annual Subscription of the Journal:

Rs. 500 (India)

Rs. 60 US$ (Overseas)

For more information, contact the editor at journalotai@gmail.com, otaihbtu@gmail.com, editorinchief@otai.org

Review Process

After accepting your paper for peer review, you will be informed through email. After that, our peer-review team will go through your paper to appraise the importance as well as innovation in your work. During peer-reviewing, you may be asked for some queries and justifications. Please keep checking your email after sending your paper because for all queries there will be a deadline after that your paper may be rejected. After peer-review, your paper will go through at least 2-3 more deep review rounds at editorial board level before publishing it. We do this level of reviewing to maintain our quality and meet the standards. We also check the plagiarism using our own software package.

Acknowledgements

You can acknowledge to whom so ever you want, it is completely up to you. No authors will come under acknowledgement section.

Author Inquiries

For more details, you can visit to our website-www.otai.org. For any other information, you can write us at *journalotai@gmail.com, otaihbtu@gmail.com editorinchief@otai.org*

All the members of OTAI are requested to update their membership details and send the information to their respective zonal secretaries

Name : .....

Membership Type : .....

Membership Number : .....

Address : .....

.....

.....

Phone : .....

E-mail : .....

The e-mails of zonal secretaries are given below:

Central Zone : otaicz@gmail.com

Northern Zone : otainz@gmail.com

Eastern Zone : otaiez@gmail.com

Southern Zone : otaisz@gmail.com

Western Zone : otaiwz@gmail.com

Please mark a copy to : journalotai@gmal.com, editorinchief@otai.org
otaihbtu@gmail.com, rpshbti@rediffmail.com

MEMBERSHIP OF

OIL TECHNOLOGISTS' ASSOCIATION OF INDIA

There are seven categories of Membership:

- Patrons
- Honorary Fellows
- Life Members
- Corporate Members
- Fellow Members
- Affliate Members
- Student Members

For details, see *www.otai.org*

Journal of Lipid Science and Technology

(Formerly The Journal of the Oil Technologists' Association of India)

ADVERTISEMENT TARIFF

Back Cover (Multi-color)	**Rs 35,000/- per insertion (Rs 1,00,000/- per four insertions)**
Inside Cover (Multi-color)	**Rs 25,000/- per insertion (Rs 75,000/- per four insertions)**
Full Page (Multi-color)	**Rs 35,000/- per insertion (Rs 1,00,000/- per four insertions)**
Full page (Black & White)	**Rs 12,000/- per insertion (Rs 40,000/- per four insertions)**
Half page (Black & White)	**Rs 8,000/- per insertion (Rs 25,000/- per four insertions)**

MECHANICAL DATA

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

All correspondence to:

Dr. R. P. Singh
Ex-Director
H.B.T.I., Kanpur-208002
Mo.: + 91- 9415405642
Phone: + 91- 9721456005
Correspondence Address:
7/105-E, Ratan Kunj, Villa-3,
Swaroop Nagar, Kanpur-208002
E-mail: journalotai@gmal.com,
editorinchief@otai.org,
rpshbti@rediffmail.com

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

This Quarterly periodical is published by Mr. C.S. Joshi on behalf of Oil Technologists' Association of India, C/o Lipid Science & Technology Division, CSIR-Indian Institute of Chemical Technology, Hydrabad-500 007, Telephone No. 91-40-27193370 and Printed by him at Bhakti Prakashan (Designers & Printers), Shri Ram Krishna Pragya Bhawan, 8/87, Arya Nagar, Kanpur - 208002. Ph. 9336270570. Editor : Prof. R.P. Singh

Registered with the Registrar of Newspapers for India Under No. RN 18734/69

ADVANCE
Home & Personal Care Ltd.

T: +91 11 25060804
F: +91 11 25068604
A: 511/2/1. Rajokri, New Delhi 38
globalsales@advancegroup.biz

Home Care

Dish Wash:
- *Liquids*
- *Powders*
- *Bars*
- *Gels*
- Rinse Agents

Cleaners:
- *Floor*
- *Glass*
- *Toilet*
- *Hard Surfaces*
- De-odorants

Personal Care

- Face Wash
- Hand Wash
- Body Wash
- Toilet Soaps
- Shampoos
- Hair Condiioners
- Hair Oils
- Tooth Pastes
- Talcum Powders

Fabric Care

- Laundry Powders
- Laundry Liquids
- Laundry Bars
- Fabric Softners
- Stain Removers
- Oil Based Laundry Bars

A formidable contract-manufacturing partner

Providing manufacturing as a service

Manufacturing HPC products since 1939

Leaders in formulation and manufacturing technology

Excellence in operations management

Pan India presence for logistic and fiscal advantages

Enabling brand owners to focus on their core

Reliance fresh | bharti WAL*MART | VISHAL MEGA MART — Manufacturing to Retailing — A Unit of Vishal Retail Ltd. | spencer's Smart ways to shop. | SRS VALUE BAZAAR

MUEZHEST
Concept to Performance

A LEADING TECHNOLOGY PROVIDERS & TURNKEY PLANT & MACHINERY SUPPLIERS

OFFERING ENERGY EFFICIENT & ENVIRONMENT FRIENLDY PROJECTS GLOBALLY

EXTRACTION

- PRE - TREATMENT
- EXTRACTION
- DISTILLATION
- DESOLVENTIZATION

OLEOCHEMICALS

- FAT SPLITTING
- GLYCERINE DISTILLATION
- PURIFICATION / EVAPORATION
- TRANSESTERIFICATION

FAT MODIFICATION

- ESTERIFICATION
- INTERESTERIFICATION
- HYDROGENATION

REFINING

- ☐ PHYSICAL
- ☐ CHEMICAL
- DEGUMMING
- BLEACHING
- WINTERIZATION
- DE-WAXING
- DEODORIZATION
- FRACTIONATION

VALUE ADDED PRODUCTS

- LECITHIN
- MARGARINE
- BAKERY SHORTENING







MUEZ-HEST INDIA PRIVATE LIMITED

230 & 231, Blue Rose Industrial Estate., W.E. Highway, Borivali (East), Mumbai - 400 066, INDIA.
Tel/Fax: +91 22 2854 1758 / 28701752, Visit us: - www.muezhest.com, Email: info@muezhest.com