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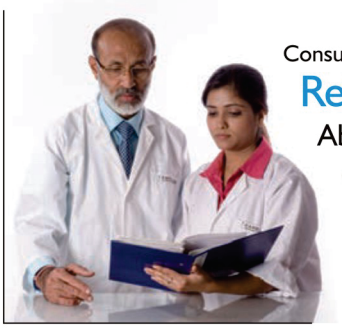

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From Editors Desk

Hello Friends,

This quarter was bad for the whole world as Pandemic Covid-18, spread from Wuhan to almost every part of the world. Almost whole world came to grinding halt and most of the countries declared lock down. It has definitely effected GDP of the whole world and in this quarter India's GDP went down to - 27%. The organizations remained closed from 24th March, 2020 onwards i.e., the date when lockdown was declared in India. This is a challenging time and this pandemic has changed life style, having far reaching ramifications. This year would be remembered for the changes it brought to the society like culture of work from home, increased digitization and increasing emphasis on hygiene have become the order of the day. All hopes are now on the development of Vaccine which may take 9 to 12 months to bring the life to near normal. As the Government pumped in money into economy, the demands in consumption increased and so was for edible oil too. This caused inflationary pressure also. New developments must be implemented to bring down the cost of the products keeping quality the prime focus. Consumption theme had gone pretty well in the lockdown period with increased impetus on the packed confectionary products.

Presently, newer technologies and processing techniques are being looked into by the processors. The important amongst them are confectionery fats. The Palm kernel oil and their fractions are used in confectionary fats. Due to high prices and shortage of cocoa butter, palm kernel oil and palm oil fractions are being extensively used due to flexibility of their use as cocoa butter alternatives such as cocoa butter equivalents (CBE), cocoa butter substitutes (CBS) and cocoa butter replacer (CBR) in which each one has its own different applications. Technological innovations in fractionation, inter-esterification and hydrogenation has given versatility to products from palm oil and palm kernel oil. Particularly, palm mid fraction is used for the purpose as palm oil has in its triglycerides POP, POS and SOS and in CBE, 70% of TG composition is of these triglycerides. Hydrogenated palm kernel oil is also used as a confectionary fat which renders good properties to the chocolate and at the same time price is also low.

In the end I would like to say stay safe and avoid the travel where you can, also influence people to do so and tell people to be hygiene conscious !!

(R.P. SINGH)

INDEX TO ADVERTISEMENTS / ANNOUNCEMENTS

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Deoiled *Simarouba glauca* Seed Cake : An Alternative Source for *N*-acyl Condensates – An Important Variety of Protein Based Surfactants

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Keywords : *Simarouba glauca seed oil, deoiled cake, protein isolation, hydrolysis of fatty acids, N-acyl condensates, surfactant properties*

ABSTRACT

In this present study, *N*-acyl condensates of protein hydrolysates were prepared from the protein isolated from *simarouba glauca* deoiled cake and their surfactant properties were evaluated. Protein was extracted from deoiled *simarouba* seed cake with aqueous sodium hydroxide solution and precipitated at isoelectric pH. *Simarouba glauca* protein hydrolysates were obtained by hydrolysis. These hydrolysates were then reacted with the mixture of fatty acids of coconut and *simarouba glauca* seed oils using thermal condensation reaction to obtain *N*-acyl condensates. The *N*-acyl condensates were evaluated for their surfactant properties like surface tension, foaming characteristics, emulsifying power, wetting power and calcium tolerance using standard analytical methods. The surfactant properties of the products were compared with commercial surfactant sodium lauryl sulphate (SLS). The superior results were observed for surface tension of sodium *N*-acyl condensates of coconut (22.77 mN/m) and *Simarouba glauca* (24 mN/m) fatty acid based protein hydrolysates compared to SLS (32.17 mN/m). In case of foaming ability, SLS exhibited better properties compared to coconut and *simarouba glauca* fatty acid based *N*-acyl condensates. The emulsion property of N-acyl condensates of both coconut and *simarouba glauca* were found to be better compared to SLS. The inferior results were found for the wetting properties of sodium *N*-acyl condensates (84 s for *Simarouba glauca* and 28 s for coconut) compared to SLS (2 s). The calcium tolerance of *N*-acyl condensates prepared from *simarouba glauca* were found to be much superior compared to SLS.

INTRODUCTION

In recent years, *simarouba glauca* has become a plant of renewed interest. Apart from the known medicinal uses, this is projected as one of the promising oil seed crops for various uses. Organized farming of this crop was started in last few years in many places of southern states like Tamilnadu, Karnataka and Andhrapradesh. *Simarouba gluaca* tree belongs to the family of *simaroubaceae.* The specific name *glauca* means covered with a bloom which refers to the bluish green plant leaves. The tree can be easily grown under diverse environmental conditions and different types of soils including the degraded soils. *Simarouba gluaca* tree is primarily a forest tree[1, 2]. *Simarouba gluaca* is an evergreen tree having 7-15 m height, which is mostly available in southern parts of India[3]. *Simarouba glauca* tree are popularly known for its medicinal properties in many countries. All parts of

simarouba glauca tree can be used for profitable applications, hence the tree can be considered as economically feasible to the industry[2]. The different types of pharmacological properties of bark and leaf extract of *Simarouba* are reported such as haemostatic, anthelmenthic, antiparasitic, antidysentric, antipyretic and anticancer activities[2, 4, 5]. The leaf, pulp of the fruit and seeds are known to possess pain relieving, antimicrobial, antiviral, vermifuse properties, stomachic and tonic[2]. It also contains active ingredients such as glaucarubin, quassinoids, ailanthinone benzoquinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol[2]. In recent years, a good amount of data was produced on *Simarouba glauca* seed oil. The most important by-product of oil extraction industry is the deoiled cake. No data is available on the use of deoiled cake of *simarouba glauca.* It was, therefore, decided to work on utilization of this major by-product.

Simarouba glauca trees can produce 2000-2500 kg seed in a hector in a year. In a study it was reported that, the *simarouba glauca* seeds are considered economically important as they contain high amount of oil (60-70%)[2]. The physicochemical properties of *simarouba glauca* seed oil was reported and found to contain FFA 1.3%, iodine value 53.9, saponification value 191.4 and unsaponifiable matter 0.4%[1]. It can be observed from iodine value, the oil contains the combination of saturated and unsaturated fatty acids. The fatty acid composition of the oil was determined and it was found that the major fatty acids present in the oil are palmitic 12.3%, stearic 27.3% and oleic acid 54.6%[6]. The oil extracted from the seeds can be used in manufacturing of vegetable fat or margarine. The reports in the peer-reviewed literature suggested that, *simarouba glauca* seed oil can have both edible as well as non-edible applications[2, 3, 7]. The seed oil which is mostly used for cooking purpose in tropical countries due to the presence of rich amount of vitamins[7, 8]. The reports are available on application of seed oil in the manufacturing of quality soaps, lubricants, paints, polishes, pharmaceuticals etc[2]. Another major advantage of this oil is higher saturated fat content and melting point being 29 C. This physical property of the oil shows its possibility to be used as a cocoa-butter substitute[9]. Thus, this seed oil can be used as a raw material in chocolate industry.

Sahoo*, et.al*., reported that, *simarouba glauca* seed oil can also be used for the preparation of biodiesel and lubricants[10]. Due to the scarcity of natural fossile fuels, people are looking for alternative fuels. Vegetable oils are the most important raw material for the production of biodiesel. The biodiesel prepared from *simarouba glauca* seed oil was subjected to engine testing as alternative to diesel and the results were very encouraging[11]. According to Patil *et. al*., the biodiesel prepared from *simarouba glauca* seed oil is a simple biodegradable, non-toxic fuel and essentially free from sulphur, aromatics. This can be used as fuel alone or even if blended with petroleum diesel[2,6].

The by-product of oil extraction/ biodiesel industry is the oilseed cake. In an earlier study it was reported that, the *simarouba glauca* oil seed cake contains 50-55% of protein[3].

The protein of SMG is currently not used for edible purpose because of the presence of high amount of toxic constituents like saponins, alkaloids, phenolics and phytic acid[3]. Currently, the deoiled cake obtained from biodiesel industry being used for low value applications. Primarily, the economy of the biodiesel industry depends on the utilization of huge quantity of deioled cake produced during the oil extraction. Possible value addition will always

help the overall economy of the biodiesel industry. The deoiled cakes can be used as protein source for various non-edible applications like household products, specialty chemicals, enzymes, pharmaceuticals, biological materials and surfactants[12, 13]. Deoiled seed cake of SMG is found to be rich in nitrogen (7.7 %), phosphorus (1.07%) and potash (1.24%). Because of this, the deoiled cakes are presently used as natural fertilizer[2].

Simarouba glauca bark and leaves also showed medicinal properties[2]. Some researchers also reported detailed work on characterization of *Simarouba glauca seed* oil[1,14]. However, there was no report on the specific application of deoiled seed cake of SMG as a source of protein. Hence the study was taken up for the utilization of deoiled cake of SMG as a good source of protein. Since, the oil seed cake contains some antinutritional components, initially non-edible applications were tried. The study was aimed for the preparation of SMG protein based surfactants by using deoiled cake. The protein based surfactants are reported to be more biodegradable and can be used in various surfactant based formulations for cosmetics and other applications. The surfactant prepared by using protein which is obtained from a low value byproduct deoiled cake is of considerable importance in economic stand point. *N*-acyl condensates can be synthesized by using pure fatty acid or mixture of fatty acids obtained from different oils with single amino acid or mixed amino acids/peptides[15, 16]. Sodium salts of N-acyl condensates of amino acids are group of surfactants which can be used in various types of shampoos, hair care products, hand cleaners and other industrial applications[17,18].

The synthesis of this group of surfactants from a very cheap raw material like *Simarouba* deoiled cake may bring economic advantage to the industry. The protein was initially isolated from the deoiled cake and hydrolyzed to get their protein hydrolysates. Further, the protein hydrolysates were reacted with the mixture of fatty acids of coconut and SMG seed oil fatty acids to obtain N-acyl condensates of SMG protein based surfactants. Coconut oil is having more lauric acid and hence was taken as another fatty acid source. The surfactant properties of the products of N-acyl condensates were evaluated and compared with SLS, a commonly used surfactant as a standard surfactant.

MATERIALS AND METHODS

Materials

Simarouba glauca (SMG) fruits were collected from Andhra Pradesh forest department, Hyderabad, India as a complimentary sample. The seeds were dried and the pulp part was separated. The dried seed was subjected to solvent extraction using soxhlet extraction system. The deoiled cake of *Simarouba glauca* seed was used further for the isolation of crude protein. The coconut oil (Manufactured by Marico Ltd, Mumbai) was purchased from local market in Hyderabad. Pre-coated thin layer chromatography (TLC) plates (silica gel 60 F_{254}) were procured from Merck, Darmstadt, Germany. Standards of fatty acid mixtures (C4-22) were purchased from M/s Sigma Chemicals, St Louis, USA for analytical use. Sodium lauryl sulfate (SLS) and other reagent grade chemicals and solvents were procured from M/s S D Fine Chemicals Pvt Ltd, Mumbai, India.

Methods

Extraction of Simarouba glauca seed oil: The dried *Simarouba glauca* seeds after separation of pulp were crushed to get powder. The seed oil was extracted by using soxhlet extractor with hexane as a solvent[19] using the method developed in this laboratory[18]. The extraction time was given about 6 hours for complete

extraction of oil. The solvent was then removed using rotary evaporator followed by complete drying under vacuum to obtain *Simarouba glauca* seed oil. The deoiled cake was air dried to remove the traces of solvent and was used for the isolation of protein from the deoiled cake. The Kjeldahl method was used for the estimation of the protein content in the deoiled cake of SMG[20].

Preparation of Fatty acid methyl esters: Fatty acid methyl esters *of Simarouba glauca* seed oil were prepared according to the method developed by this laboratory[21]. The oil (10-20 mg) was taken in round bottom flask and 20 ml of 2% of H_2SO_4 in methanol was added. The contents were refluxed for 4 h and the progress of the reaction was monitored by TLC. After complete conversion of methyl esters the solvent was partially removed and the mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with water until neutral. The extract was passed over anhydrous sodium sulfate and evaporated using rotary evaporator under reduced pressure to obtain fatty acid methyl esters. Similar procedure was used for the preparation of coconut oil fatty acid methyl esters.

Preparation of fatty acids: Free fatty acids of *Simarouba glauca* were obtained by the hydrolysis of *Simarouba glauca* seed oil. *Simarouba glauca* seed oil (20 g) was taken in 500 ml three necked round bottom flask connected with mechanical stirrer and kept in a heating mantle. The conventional alkaline hydrolysis method was followed according to a method reported by Srinivas et.al.[22]. Aqueous NaOH solution (2.28N, 50 ml) was added slowly to the oil while stirring. Then, the temperature of the reaction was raised to 90 C and the conditions were maintained for 4-5 h. The progress of the reaction was monitored by TLC. After complete hydrolysis, the contents were neutralized using dil. HCl solution to obtain free fatty acids. The fatty acid layer was washed with distilled water till free of acid and dried completely to obtain the mixtures of fatty acids (18 g, 90% yield). The similar procedure was followed for coconut oil to get fatty acid mixtures of coconut oil with an yield of 94.0%.

Gas Chromatography Analysis: Simarouba glauca and coconut oil fatty acid methyl esters were analyzed on Agilent 6890N Series Gas Chromatograph equipped with a flame ionization detector (FID), with a capillary column DB- 225 (30 m X 0.25 mm i.d. X 0.5 µm film thickness, J & W Scientific, USA). The injector and detector temperatures were maintained at 230 and 250C respectively. The oven temperature was programmed for 2 min at 160 C, further increased to 230 C at 5 C/min and finally maintained for 20 min at 230 C. The injection volume was 1 µl, with a split ratio of 50:1. Nitrogen was used as a carrier gas at a flow rate of 1.5 ml/minute. The fatty acids were identified by the comparison of retention times of co-rresponding commercial standard fatty acids.

Isolation of Simarouba glauca protein: Simarouba glauca deoiled cake was used for the isolation of crude protein by alkaline extraction method. The powdered deoiled cake (220 g) was taken in a 5 lit round bottom flask and 4.0 lit of aqueous NaOH solution (0.5 N) was added to it[16]. The contents were stirred for 3 h using mechanical stirrer at 30 C. The alkali solubles and insolubles were then separated by using centrifuge at 7500 rpm for 30 min. The alkali soluble portion was checked for isoelectric point of protein and accordingly the pH was adjusted to 4.0 using dilute hydrochloric acid solution to precipitate the crude protein. The crude protein precipitate was once again centrifuged at 7500 rpm for 30 minutes. The crude protein was collected by decanting the supernatant and washed with water and dried at 90-100 C using hot air oven to obtain 90.54 g. The similar procedure was repeated to collect the more amount of protein

from deoiled cake.

Preparation of Protein Hydrolysates: simarouba glauca protein (100 g) isolated from deoiled cake was taken in a 2 lit autoclave reactor and 430 ml of aqueous NaOH solution (1.7 N) was added while stirring and the reactor was closed. The temperature of the reaction was increased to 110 C and the pressure in the reactor was increased to 2.8 bars. The reaction was continued for 3 h by monitoring the hydrolysis of protein[23, 24]. The contents were then cooled and the material was discharged from the reactor. The pH of the solution was adjusted to 8.0 using dilute hydrochloric acid solution. The protein hydrolysates solution was found to contain 0.6 g solid/ ml of solution.

Preparation of N-acyl condensates of Simarouba glauca protein hydrolysates: N-acyl condensates of *Simarouba glauca* protein hydrolysates were prepared using *Simarouba glauca* and coconut oil fatty acid mixtures by thermal condensation reaction. *Simarouba glauca* protein hydrolysates (15.5 g, 22 ml) were taken in round bottom flask and the solution was concentrated up to 80% of solid. The fatty acid mixture (7.75 g, 1:0.5 ratio wt/wt of protein hydrolysates/fatty acids) of *Simarouba glauca* was added slowly at 120 C while stirring the contents. The reaction temperature was increased slowly to 210 C and maintained the conditions for 30 min[15, 25]. Then the reaction temperature was brought down to ~100 C and the pasty mass (21.2 g) was removed from the round bottom flask. The unreacted fatty acid mixture was extracted by using hexane from the reaction product. The similar procedure was followed for the preparation of *simarouba glauca* protein hydrolysates with the mixture of coconut oil fatty acids to obtain *N*-acyl condensates. *N*-acyl condensates of *Simarouba glauca* protein hydrolysates (20 g) with both the fatty acids were treated with the addition of 10 wt% of aqueous NaOH solution by maintaining pH 9.0 at 50°C for 30 min to obtain sodium *N*-acyl protein hydrolysates[23]. The reaction products were used for the evaluation of surfactant properties.

*Evaluation of surfactant properties: S*odium *N*-acyl condensates of *Simarouba glauca* protein hydrolysates with both the varieties of coconut and *Simarouba glauca* fatty acids were prepared in different concentration of aqueous solutions (1, 0.5 and 0.25 wt %) and their surfactant properties were evaluated. The instrument Kruss K100 tensiometer was used for the determination of surface tension of the surfactant solution. The emulsifying property was determined according to the method described by Subrahmanyam and Achaya[26]. The foaming property of surfactant was analyzed by using a pour foam apparatus[27]. The wetting power of the solutions were estimated by the method according to IS specification[28]. The calcium tolerance for the surfactants was also determined according to the reported method[29]. The similar concentrations of standard surfactant (SLS) solutions were prepared and their surfactant properties were compared.

RESULTS AND DISCUSSION

The *Simarouba glauca* seed oil was extracted from the seeds by using soxhlet apparatus with hexane and the oil content was found to be 56.8%.

The major objective of the present work is for the preparation of protein based surfactants by utilizing the low value by product deoiled cake of SMG. Genarally *N*-acyl condensates of protein based surfactants depend on which type of fatty acid mixtures were used for the preparation of surfactants. Coconut oil fatty acids are known as a suitable raw material for the preparation of superior surfactant as it contains more short and medium chain fatty acids[30, 31]. Therefore, in the present work, coconut oil fatty acids were used

for the preparation of protein based surfactants. In this study, SMG seed oil fatty acids were also used for preparation of *N*-acyl condensates.

The coconut oil fatty acid mixtures and *Simarouba glauca* fatty acid mixtures (SMGFA) were obtained by alkaline hydrolysis of coconut and SMG seed oils. The fatty acid compositions of both the fatty acid mixtures are reported in Table 1.

Table 1: Fatty acid compositions of *Simarouba glauca* and coconut oil fatty acids

Fatty acid	*Simarouba glauca* fatty acids	Coconut fatty acids
8:0	-	0.7
10:0	-	3.5
12:0	-	46.4
14:0	-	21.0
16:0	11.7	10.5
16:1	-	-
18:0	26.3	4.1
18:1	55.6	11.4
18:2	3.1	2.4
18:3	0.9	-
20:0	1.6	-
20:1	0.2	-
22:0	0.3	-
24:0	0.3	-

The data presented in Table 1 showed that, SMG mixed fatty acids contains oleic acid as major fatty acid (55.6%) followed by stearic acid (26.3%) and palmitic acid (11.7%). In coconut oil fatty acids, lauric acid is the major fatty acid (46.4%), followed by myristic (21%), oleic (11.4%) and palmitic acid (10.5%). Saturated fatty acids observed more in coconut oil compared to SMG fatty acids where as reverse in case of unsaturated fatty acids. Generally coconut oil fatty acids contain short, medium chain fatty acids where as *Simarouba glauca* mixture of fatty acids was found to contain long chain fatty acids. The mixture of

fatty acids of both coconut and SMG seed oils obtained by alkaline hydrolysis were used for the preparation of protein based surfactants.

Simarouba glauca protein was isolated from deoiled cake by alkaline extraction, precipitation method and the purity of the protein isolated was found to be 87.0%. Protein hydrolysates were obtained by alkali hydrolysis of the protein. The protein hydrolysates were then used to prepare *N*-acyl condensates using *Simarouba glauca* and coconut oil fatty acid mixtures by thermal condensation reaction. The reaction products of both the protein hydrolysates were further converted to their sodium salts. Different concentrations (1, 0.5 and 0.25%) of aqueous solutions of sodium N-acyl condensates were prepared and surfactant properties like foaming power, wetting property, surface tension, emulsion stability and calcium tolerance were evaluated. The surfactant properties of SMG protein hydrolysates were compared with the commercial surfactant SLS and the results are shown in Table 2 and 3.

Table 2: Surfactant properties of sodium *N*-acyl condensates of *Simarouba glauca*

Surfactants	Surface tension (mN/m)			Wetting time (s)			Foam height (cm)					
							Initial			After 5 min		
Conc. wt%	**1.0**	**0.5**	**0.25**	**1.0**	**0.5**	**0.25**	**1.0**	**0.5**	**0.25**	**1.0**	**0.5**	**0.25**
SMGFA *N*-acyl condensates	23.83	24.0	26.89	84	102	133	5.5	4.8	3.6	4.2	3.6	2.2
Coconut fatty acid *N*-acyl condensates	22.43	22.77	24.59	28	35	40	18.2	14.5	11.0	16.0	12.5	9.5
SLS	31.72	32.17	32.89	02	03	04	18.5	17.0	16.2	16.5	16.1	15.0

Table 3: Surfactant properties of sodium *N*-acyl condensates of *Simarouba glauca*

Surfactants	Emulsifying power (s)						Calcium tolerance (ppm)
	Time for 10 ml of separation			Time for 20 ml of separation			
	1.0% Conc.	**0.5% Conc.**	**0.25% Conc.**	**1.0% Conc.**	**0.5% Conc.**	**0.25% Conc.**	
SMGFA *N*-acyl condensates	290	195	155	608	388	315	>1000
Coconut fatty acid *N*-acyl condensates	202	167	138	384	259	192	815
SLS	162	149	122	415	365	312	79.8

The results clearly show that, the ability to lower the surface tension are much better for *Simarouba glauca* and coconut fatty acid based protein surfactants compared to commercial surfactant sodium lauryl sulphate (SLS). The surface tension of SMG and coconut oil fatty acid based *N*-acyl condensates was increased from 1.0% to 0.25% concentration of surfactant solution. Similar results were observed earlier for *Terminalia belerica* protein hydrolysates prepared by using *Terminalia* and coconut oil fatty acids[15]. It is quite evident that lowering of the surface tension decreases with the increase of fatty acid chain length.

The wetting ability of sodium *N*-acyl condensates of coconut oil fatty acids found to be 28 s and *simarouba glauca* 84 s, whereas it was observed for SLS 2 s (for 1% of concentration of solutions). In case of 0.25% concentration of solution, the wetting ability was observed for coconut 40 s, *simarouba glauca N*-acyl condensates 133 s and SLS 4 s. The wetting ability was observed to be better for coconut compared to *Simarouba glauca N*-acyl condensates. This behavior can be explained by the fact that, short and medium chain saturated fatty acid based *N*-acyl condensates generally exhibit better wetting ability compared to long chain fatty acid based *N*-acyl condensates[22]. The similar phenomina was followed for protein based surfactants of coconut and *Simarouba glauca* fatty acid based *N*-acyl condensates. It was reported that, the surfactants having good wetting properties can be used in textile industry[32]. Hence, the SMG protein based surfactants of coconut and SMG fatty acids can be an alternative source for textile industry.

The emulsion stability of *Simarouba glauca N*-acyl condensates were found to be superior compared to *N*-acyl condensates of coconut fatty acids. The emulsifying power and stability of the surfactants increases by increasing the fatty alkyl chain length[30, 33]. Due to the presence of long chain fatty acid in SMG fatty acid based *N*-acyl condensates, the emulsifying characteristics were better compared to coconut. The emulsifying property was enhanced by increasing the concentration of surfactant solution from 0.25 to 1.0%.

The SMG protein based surfactants prepared using SMG and coconut fatty acids showed better property compared to SLS. This property can be utilized in applications where emulsification and emulsion stability is important.

It was also observed that, the foaming property was enhanced by increasing the concentration of surfactant solution from 0.25 to 1.0%. The foaming properties of SMG *N*-acyl condensates were inferior compared to SLS. The foam stability for coconut fatty acid based *N*-acyl condensates was 16.0 cm, *simarouba glauca* fatty acid 4.2 cm and for SLS 16.5 cm for 1% concentration of surfactant solution. However, the coconut fatty acid *N*-acyl condensates (16cm) are comparable with SLS (16.5cm). Therefore, SMG and coconut oil fatty acid based *N*-acyl condensates can be used for industrial applications suggested by Azira, *et.al*[34].

The calcium tolerance for *simarouba glauca* was found to be >1000 ppm and coconut fatty acid based sodium *N*-acyl condensates 815 ppm, whereas for SLS it was found to be 79.8 ppm. The calcium tolerance was observed much higher for the surfactants prepared with SMG protein hydrolysates compared to SLS. This indicates that, the surfactants can function effectively in water having more hardness.

The *N*-acyl condensates of both coconut and *simarouba glauca* are more tolerant to calcium ions compared to SLS. The surfactants having calcium tolerance property

can be used in detergents formulations.

CONCLUSIONS

In the present work, attempts were made to utilize the deoiled cake of *simarouba glauca* to add value to this by-product of oil extraction/biodiesel industry. Accordingly, *N*-acyl condensates were prepared from *simarouba glauca* protein hydrolysates with both mixed fatty acids of *simarouba glauca* seed oil and coconut oil. It was observed that the properties of these surfactants were comparable with those of standard surfactant sodium lauryl sulphate (SLS). The surface tension lowering properties were found to be superior in case of *simarouba glauca* protein based *N*-acyl condensates in comparison to SLS.

REFERENCES

1. Anil D, S Yeshwant, M Harish, D Saroj, **J. Agric. Biol. Sci., 6**, 16, (2011).
2. Patil M S, D K Gaikwad, **J. Pharm. Sci. & Res., 3**,1195, (2011).
3. Govindaraju K, J Darukeshwara, A K Srivastava, **Food and chem. Toxicol., 47**, 1327, (2009).
4. Rivero, J F Cruz, **Phytother. Res., 19**, 136, (2005).\
5. Giron L M, V Freire, A Alonzo, A Caceres, **J. Ethnopharmacol., 34**, 173, (1991).
6. Mishra S R, M K Mohanty, S P Das, A K Pattanaik, **Res. J. Chem. Sci., 2**, 66, (2012).
7. Rout P K, Y R Rao, K S Jena, D Sahoo, A Shakir, **J. Food Sci. Technology., 51**, 1349, (2014).
8. Severen M L, **J. Am. Oil Chem. Soc., 30**, 124, (1953).
9. Jeyarani T, S Yella Reddy, **J. Am. Oil Chem. Soc., 78**, 3, 271, (2001).
10. Sahoo D, K S Jena, P K Rout, Y R Rao, **J. Food Sci. Technol., 39**, 552, (2002).
11. Praveen A H, D S Akshatha, G Manavendra, **Int. J. Innov. Res. Sci. Eng. Technol., 4**, 1059, (2015).
12. Ramachandran S, S K Singh, C Larroche, C R Soccol, A pandey, **Biores. Tech., 98**, 2000, (2007).
13. Moure A, J Sineiro, H Dominguez, J C Parajo, **Food Res. Intern., 39**, 945, (2006).
14. Jayashanthini S, K S Rathinam, R L Devi, R Sumathi, S Murugesan, N Senthilkumar, **Int. J. Pharm. & Biol. Sci. Arch., 7**, 16, (2019).
15. Ravinder T, K Swapnil, K Akhil, B Anjaneyulu, D Sudhan, P P Chakrabarti, **J. Lipid Sci. Technol., 47**, 3, 79, (2015).
16. Sreenu M, P P Chakrabarti, B V S K Rao, R B N Prasad, **J. Lipid Sci. Technol., 45**, 4, 164, (2013).
17. Sanders H L, M Nassau, **Soap Chem. Spec., 36**, 1, 57, (1960).
18. Toliwal S D, K Patel, **J. Sci. Ind. Res., 66**, 385, (2007).
19. Ravinder T, K Shiva Shanker, K Konda Reddy, S Kanjilal, B V S K Rao, S K Swain, R B N Prasad, **Int. J. Mod. Chem. Appl. Sci., 3**, 1, 293, (2016).
20. Official methods of Analysis of AOAC, Tenth Edition, Association of Official Analytical Chemists, Washington, DC, USA, pp.744, (1965).
21. Ravinder T, K Shiva Shanker, S Kanjilal, B V S K Rao, S K Swain, R B N Prasad, **Intern. J. Chem. Sci. Technol., 5**, 2, 32, (2015).
22. Sreenu M, B V S K Rao, R B N Prasad, P Sujitha, C Ganesh Kumar, **Eur. J. Lipid Sci. Technol., 116**, 193, (2014).

23. Prasad R B N, A Krishnamurty, A J Pantulu, G Lakshminarayana, **J. Oil. Technol. Assoc. India., 20**, 56, (1988).
24. Hoyle N T, J H Merritt, **J. Food Sci., 59**, 76, (1994).
25. Sokol P E, **J. Amer. Oil Chem. Soc., 52**,101, (1975).
26. Subrahmanyam V V R, K T Achaya, **J. Chem. Eng. Data., 6**, 1, 38, (1961).
27. Ross J, G D Miles, **Oil and Soap, 18**, 99-102, (1941).
28. BIS No: 1185, Bureau of Indian Standards, New Delhi, (1957).
29. Sreenu M, R R Nayak, R B N Prasad, Y Poornachandra, C Ganesh Kumar, **J. Dispers. Sci. Technol., 36**, 6, 765, (2015).
30. Mhaskar S Y, R B N Prasad, G Lakshminarayana, **J. Amer. Oil Chem. Soc., 67**, 1015, (1990).
31. Soo E L, A B Salleh, M Basri, R N Z Rahman, K Kamaruddin, **J. Biosci. Bioeng., 95**, 361, (2003).
32. Schramm L L, E N Stasiuk, D G Marangoni, **Phys. Chem., 99**, 3, (2003).
33. Szymanski A, B Wyrwas, E Bubien, T Kurosz, W Hreczuch, W Zembrzuski, Z Lukaszewski, **Water Res., 36**, 13, 3378, (2002).
34. Azira H, A Tazerouti, J P Canselier, **J. Surfact Deterg., 11**, 279, (2008).

Supercritical Carbon dioxide extraction of lipids from microalgae and its characterization

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Keywords: *Microalgae, organic solvent, Supercritical carbon dioxide, Polyunsaturated fatty acids (PUFAs)*

Abstract

In the present investigation, ultimate analysis, proximate analysis (moisture content, ash content, total carbohydrate content, total protein content, total fiber content), mineral analysis along with Fourier-Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA) was applied to study the characteristics of algae biomass. Also, different methods were implemented for the extraction of total lipids from microalgae. Comparative assessment of extraction methods, organic solvent extraction, and supercritical carbon dioxide extraction was carried out at different temperature and pressure. For the solvent extraction method, chloroform: methanol (1:1 v/v) showed better efficiency in producing lipid yield followed by ethanol and hexane. Lipid extraction yield with supercritical CO_2 increases with decreasing temperature from 60 C to 40 C. Fatty acid profile of extracted lipids obtained from GC-MS indicated that significant fractions of palmitic acid (C16:0), palmitoleic acid (C16:1n-7), oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) were present. Also, a small concentration of Eicosapentaenoic acid (EPA, C20:5n-3), Docosahexaenoic acid (DHA, C22:6n-3) and arachidonic acid (AA, C20:4n-6) were present at higher temperature and pressure. This suggests that supercritical CO_2 is more effective and provides higher selectivity for triglycerides, though the total lipid yield extracted was less than the organic solvent extraction method.

1. Introduction

Algae are photosynthetic eukaryotic organisms. They vary significantly in size, 3-10μm unicellular to 70m multicellular. They have high aerial productivity and can be cultivated in open ponds. They absorb atmospheric CO_2 as a carbon source for their growth, thus reducing greenhouse gas emissions from anthropogenic activities (Wang et al., 2008). It also requires light, temperature (20-30°C), inorganic salts (nitrogen, phosphorous), and wastewater or saline water for its multiplication. The utilization of wastewater contaminants such as nitrogen, phosphorous, and metals as nutrients for algae cultivation helps in wastewater treatment (Wang et al., 2008). They are classified as two types, microalgae and macroalgae (also known as seaweeds). They are subcategorized as cyano-bacteria, diatoms, dinoflagellates, red algae, green algae, and brown algae. They are a potential source of bioactive compounds such as lipids, proteins, polysaccharides, carotenoids, polyphenols, minerals, and vitamins. The Chemical composition of a few algae is given in Table 1. Microalgae contain 40-80% of lipid, which can be used to produce different types of renewable biofuels such as biohydrogen, biodiesel, methane, ethanol, etc. (Chisti, 2007). Due to the presence of high-value products in algae, it has various commercial applications such as food additives, pharmaceuticals, cosmeceuticals, aquaculture feeds, and biofertilizers (Mata et al., 2010). The transformation of algae to biofuels and high-value products by sustainable processing way is shown in figure 1.

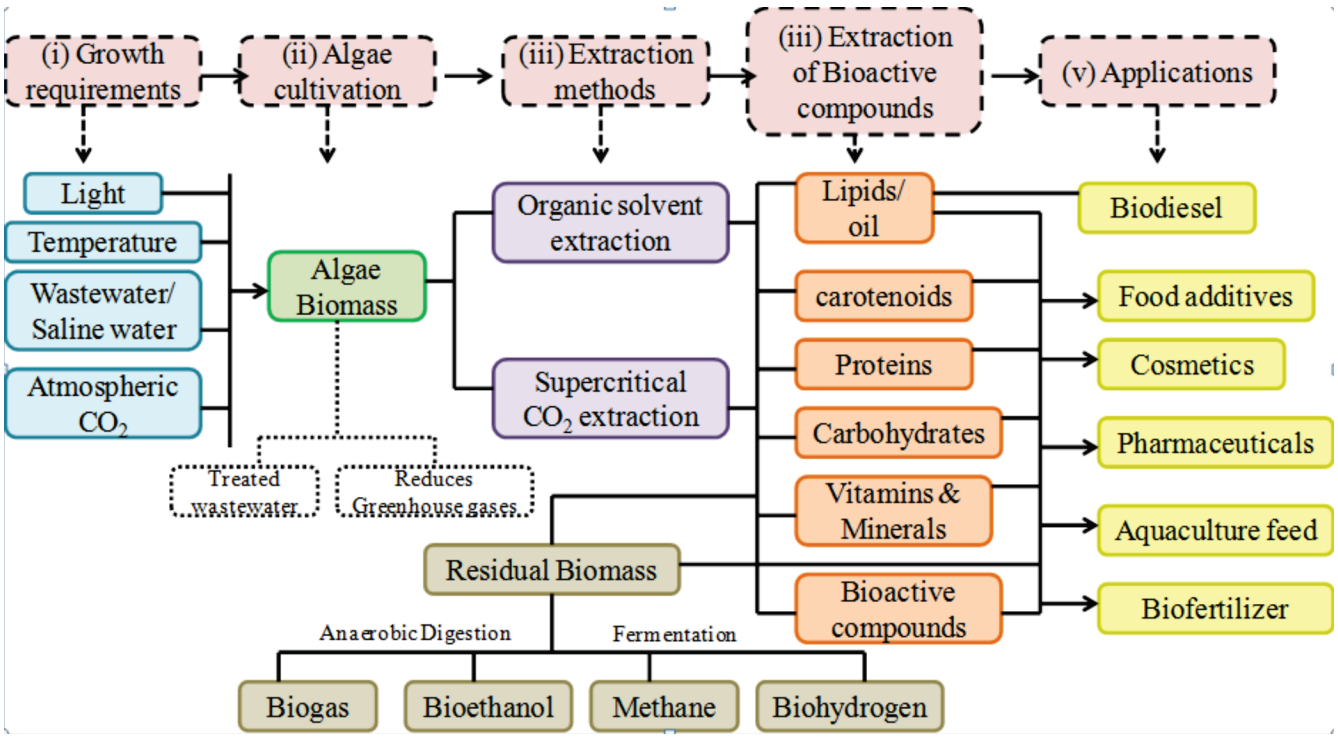


Figure 1 : Transformation of algae to biofuels and High- value products

Microalgal lipids or crude lipids are hydrophobic biomolecules which are soluble in organic non-polar solvents. Lipids are classified as fatty acids and triglycerides. Fatty acids do not have any ester functional group; they are carboxylic acids with long hydrocarbon chains that are either saturated or unsaturated (Sahena et al., 2009). Triglycerides are esters that are transesterified with alcohol to produce fatty acid methyl esters (FAME) and glycerol in the presence of a catalyst (Pragya et al., 2013). Lipid fraction mainly consists of polyunsaturated fatty acids (PUFAs) such as ω-3 fatty acids [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)] and ω-6 fatty acids [γ-linoleic acid (GLA), arachidonic acid (AA)] (Yen et al., 2015; Michalak and Chojnacka, 2014). They have an essential role in human health as it helps in regulating blood pressure or blood clotting and develop functions of the brain and nervous system (Ibañez at al., 2012). They help prevent diseases like arthritis, diabetes, obesity, and cardiovascular diseases (Wall et al., 2010).

Various methods are employed to extract microalgal lipids such as conventional based solvent extraction method and supercritical CO_2 extraction method. The traditional solvent method is time-consuming, and it needs a high volume of toxic and flammable solvents, which have an adverse effect on human health and the environment. It is operated at high temperatures due to which polyunsaturated fatty acids and other bioactive compounds are degraded (Careri et al., 2001). The supercritical carbon dioxide method for the extraction of lipids is an alternative process for the solvent extraction method. It has many advantages such as it uses carbon dioxide as a solvent, which is non-toxic, inflammable, cheap, eco-friendly, inert, odorless, and can easily be removed from the product. It allows the extraction of thermally labile compounds even at low

temperatures (Michalak et al., 2015). In addition, carbon dioxide has low critical temperature, i.e., 31.1 C and low critical pressure, i.e., 73.9 bar, which allow the extraction of thermolabile compounds without loss of their activities (Herrero et al., 2006).

Table 1: Compositional analysis of few Microalgae and Macroalgae (% dry weight)

Algae	Lipid	Protein	Carbohydrate	Applications	References
MICROALGAE					
Chlorella vulgaris	14-22	51-58	12-17	Food supplements, Cosmetics, pharmaceuticals	Wang et al.,2010
Dunaliella salina	6	57	32	Food, energy, pro-vitamin A	Mendiola et al., 2008
Spirulina platensis	4-9	46-63	8-14	Food and Health, Cosmetics Immunofluorescence technique , Antibody labels	Furuki et al, 2003
Botryococcus braunii	33	40	2	Cosmetic mask Energy Food additives	Lee et al., 2010
Synechoccus sp.	11	63	15	Food and Health	Viskari and colyer, 2003
Scenedesmus dimorphus	16-40	8-18	21-52	Food	Miranda et al., 2012
Porphyridium cruentum	9-14	28-39	40-57	Nutritional supplements	Román et al., 2002
MACROALGAE					
Enteromorpha compressa	11.45	7.26	24.75	Food additives, cosmetics, drugs	Parthiban et al., 2013
Ulva lactuca	9.6-11.4	11.4-12.6	11.6-13.2	Food and Health	Ortiz et al., 2006
Chaetomorpha linoides	12	9.45	27	Energy	Aresta et al., 2005
Caulerpa cupressoides	10.97	7.43	51.75	Pharmaceuticals	Lin et al., 2012
Hypnea valentiae	9.6-11.6	11.8-12.6	11.8-13.0	Food	Manivannan et al., 2009
Cladophora fascicularis	15.70	15.53	49.50	Wastewater treatment	Kumar et al., 2010
Acanthophora spicifera	10-12	12.0-13.2	11.6-13.2	Food	Kaliaperumal et al., 2002

In this study, the physicochemical analysis of algae biomass was investigated. Also, Mineral analysis, Fourier-transform infrared spectroscopy (FTIR), and Thermogravimetric analysis (TGA) was determined for the characterization of algae biomass.

Lipids were extracted from microalgae using organic solvent and supercritical carbon dioxide at different temperature and pressure. Fatty acid methyl ester (FAME) of crude lipids were performed and quantified by gas chromatography-mass spectrometry (GC-MS).

2. Materials and methods

2.1 Raw materials

The algal biomass used in this study was grown in a 1000L tank, explicitly designed to cultivate algae (Figure 2). The biomass was grown in tap water medium containing Na_2CO_3 (0.02 g L^{-1}); $NaNO_3$ (0.15 g L^{-1}); KH_2PO_4 (0.0125 g L^{-1}), at a temperature in the range of 30-35 C and with atmospheric aeration. The biomass consisted of *Chlorella* and *Phormidium* consortium with predominant *Chlorella* species. The cultured biomass was harvested by centrifugation, and then wet biomass was sun-dried. The dried flakes of biomass obtained were grounded manually using a grinder and were stored at room temperature for further use.

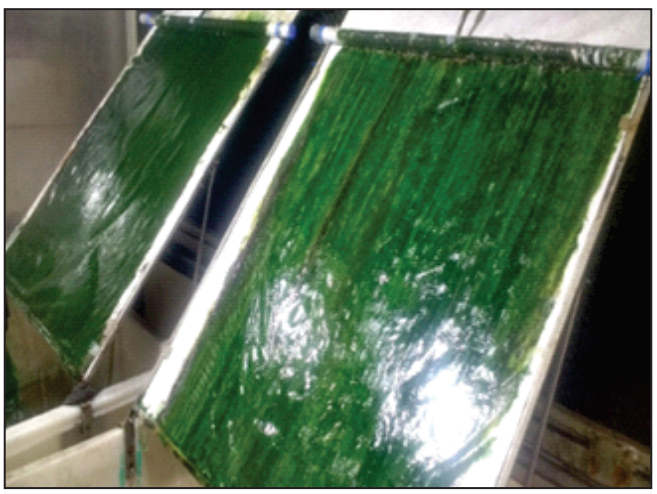


Figure 2: Cultivation of Algae

2.2 Chemicals

Extraction was carried out with high purity carbon dioxide (99.7%) purchased from Laser gases (New Delhi). All solvents used were of HPLC grade, with purity 99.5% (Sigma Aldrich).

2.3 Analysis of algae solid residue

2.3.1 Physiochemical analysis

The elemental compositions of algal biomass were determined using vario EL III Element Analyzer (Elementar India Pvt. Ltd., India) to quantify carbon, hydrogen, and nitrogen percentage. The proximate composition of algal biomass collected was analyzed to obtain moisture, ash, carbohydrate, protein, and crude fiber content. The moisture content was determined by using a moisture analyzer. The determination of ash content was done according to the National Renewable Energy Laboratory (NREL) procedure (Van Wychen and Laurens, 2016). The total carbohydrate was determined by the NREL method (Van Wychen and Laurens, 2013). Spectrophotometric estimation of total protein was done by Bradford assay (Bradford, 1976). The total fiber content was assessed according to AOAC 991.43 enzymatic gravimetric method (Lee et al., 1992).

2.3.2 Mineral analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

A sample was treated with a mixture of hydrochloric acid and nitric acid (3:1) using a microwave oven for the complete digestion of biomass. The operational condition of the microwave process was: a ramp time of 5 min to reach 450W, a hold time of 10 min at 650W and cooling down to room temperature. The digested solution was diluted to 50 ml with Milli-Q water and carefully filtered using syringe filters before analysis (Ahmed et al., 2017). Metal concentration in the sample was then determined by Agilent ICP-MS 7900 with Ultra High Matrix Introduction (UHMI) technology.

2.3.3 Fourier-transform infrared spectroscopy (FTIR)

A qualitative and preliminary analysis of the main functional groups on the surface of biomass was analyzed and recorded by the FTIR Nicolet iS05 instrument (Thermo Scientific). FTIR spectrum was obtained by the potassium bromide (KBr) pellet method. About 0.1g of biomass was mixed with 0.1g of KBr and compacted in pellet form. FTIR spectra were then recorded in the wavelength range of 400 and 4000 cm^{-1} with the spectral resolution of 0.5 cm^{-1}.

2.3.4 Thermogravimetric analysis (TGA)

Thermogravimetric analysis of algal biomass was conducted by a thermogravimetric analyzer (Perkin Elmer). Approximately 20mg of algal biomass was placed in alumina pan. The sample was heated from room temperature to 105 C in the atmospheric flow of argon for 10 minutes and then raised to 900 C for 30 min. After completion, it is cooled down to 600 C, where the gas flow was switched from argon to air and then kept for 60 min to burn off fixed carbon. Thermogram was obtained by the weight loss of biomass due to the temperature increment (Ong et al., 2019).

2.4 Extraction methods

2.4.1 Organic solvent extraction

The extractable lipid was determined using soxhlet extraction method (AOAC, 2000). The pre-dried sample was weighed accurately into a soxhlet thimble. The thimble was transferred to the extraction chamber of the soxhlet apparatus. Lipids were selectively extracted into the solvents during percolation. The solvents chosen were hexane, ethanol, and a mixture of chloroform and methanol (1:1 v/v). Solvents were heated according to their boiling point in soxhlet extractor equipment. The extractions were conducted for 10-15 h until the filtrate was colorless. All the extractions were carried out in duplicates. A mixture of solvent and extracted lipids was concentrated using a vacuum rotary evaporator and weighed gravimetrically to obtain the crude lipid of microalgae. The mass of total lipid extract was expressed as a percentage of the lipid extract obtained for each solvent to the original weight of the biomass.

2.4.2 Supercritical CO_2 extraction

The experiment was carried out in Waters Prep SFE system equipped with thermostat 500 ml extraction cell unit, a heating chamber, a high-pressure pump, and a back pressure regulator capable of operating at a pressure of up to 400 bars (Figure 3).

All the components of the extractor are fully automatic and are controlled by computer software.

In this study, lipid extraction by supercritical CO_2 was conducted at a temperature of 40 C and 60 C and a pressure of 150-350 bar. The flow rate of CO_2 was maintained constant at $30gmin^{-1}$ for all the experiments.

Approximately 50g of dried algal biomass was loaded in the extraction vessel unit. Carbon dioxide was liquefied by the chiller and pressurized to operational pressure conditions by using a high-pressure pump.

The pressurized carbon dioxide was heated to operation temperature conditions and pumped into the extraction cell.

After 90 min of extraction run, the lipid extract was collected in a glass tube.

At the end of the experiment, ethanol is used to wash the extract off from collecting vessels and pipelines and mixed with the extracted solution.

Yellowish microalgae lipid was collected after evaporating the ethanol by vacuum rotary evaporator.

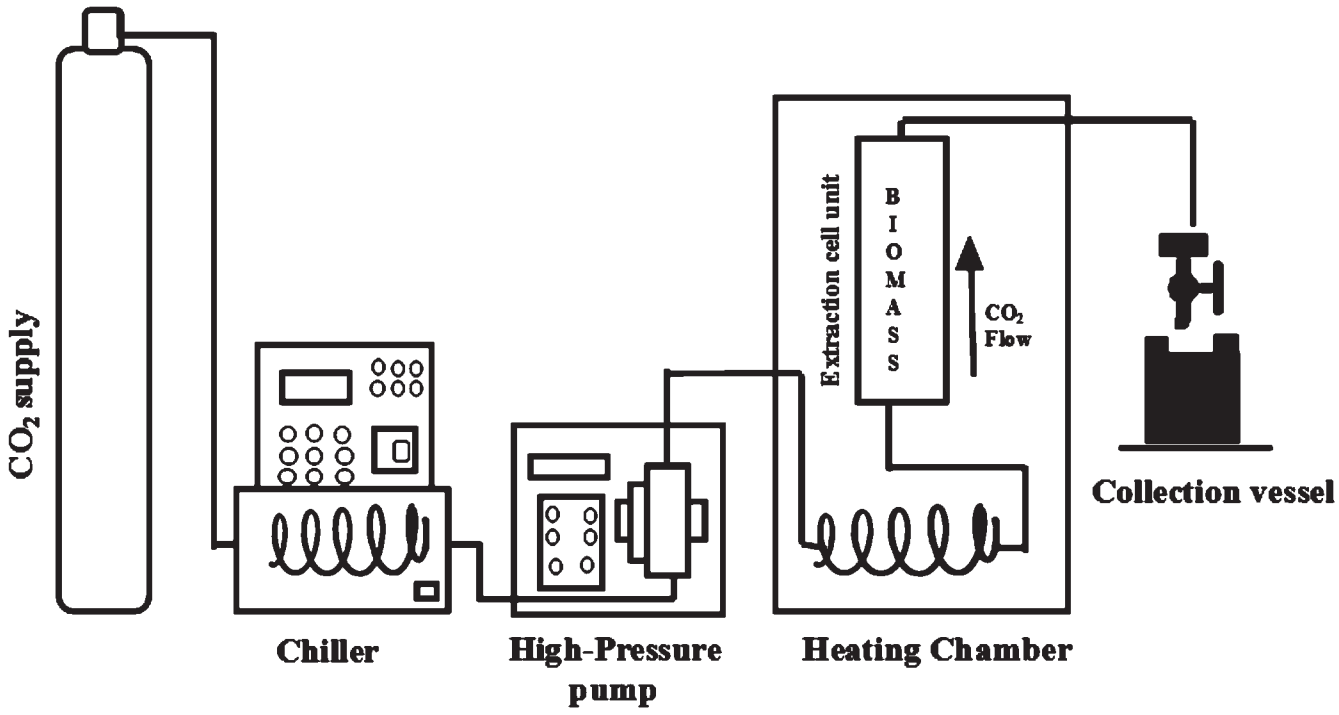


Figure 3: Supercritical CO_2 apparatus

2.5 Compositional analysis of fatty acids in microalgal lipids

2.5.1 Fatty acid methyl esters (FAME)

Fatty acid methyl esters are commonly prepared by transesterification of microalgal lipids by method followed by Fallon (O'fallon et al., 2007). Approximately 0.5g of lipid was mixed with 0.7ml of 10N KOH in water and 5.3 ml of methanol. It is then incubated in a water bath at 55 C for 90 min with vigorous handshaking of tubes for 5 sec after every 20 min to properly hydrolyze the sample. After incubation, the mixture was cooled down to room temperature, and then a total of 0.58ml of 24 N H_2SO_4 is added. This mixture is again incubated for 90 min at 55 C with handshaking of tubes for 5 sec after every 20 min. After synthesis of FAME, tubes were cooled down to room temperature. 3ml of hexane is added in a mixture tube and vortex for 5 min. The tube was centrifuged at 5000rpm for 10 minutes, and the hexane layer containing FAME was collected and placed at -20 C until GC-MS analysis.

2.5.2 Gas chromatography-mass spectrometry (GC-MS) analysis

Fatty acid composition of microalgal lipids was identified and quantified by Gas Chromatography Mass spectrometer (Shimadzu QP-2010 Plus with Thermal Desorption System TD 20) with a capillary column (DB-23, 60m X 0.25mm ID X 0.15µm). The GC temperature was maintained at 140 C for 5 min, subsequently increased to 280 C at a heating rate of 4 C/min, and then held for 10 min. The GC injector and detector temperature were set at 260 C. The flowing rate of carrier gas helium was 1.21ml/min, with a split ratio of 10:1.

3. Results and discussion

3.1 Analysis of algae biomass

Microalgae were sun-dried for 5-7 days and then milled to powder using a grinder for further experiments. Various parameters, including physicochemical properties, proximate composition, and mineral content, were analyzed. The ultimate and proximate analysis results of the microalgae sample tested are presented in Table 2. The composition of major elements was carbon (36.7 ± 0.2%), hydrogen (5.7 ± 0.1%), oxygen (51.5 ± 0.3) and nitrogen (4.9 ± 0.3) due to presence of protein. Moisture content was observed to be 8.6 ± 0.1. In general, the ash content represents the total mineral content in the microalgae. The ash content of microalgae was found to be 14.2 ± 0.2g/g of DW. Analysis of fiber content shows the total dietary fiber content, which was 29.7 ± 0.2% of DW in microalgae biomass. Recent studies have demonstrated that algal fiber exhibits important functional activities such as antioxidant, anticoagulant, and antitumor activities (de Morais et al., 2015). These fibers are resistant to digestion and act as a binding site for the ion-exchange of organic molecules (Ruperez et al., 2001). The Bradford method determined the amount of total protein content and was found to be 17.7 ± 0.3g/g DW. Total carbohydrate content was 59.1 ± 0.3g/g DW, which was calculated by the anthrone method.

Microalgae are rich in essential minerals and trace elements that are required for human nutrition. The mineral content in the biomass was evaluated by ICP-MS (Table 2), and results reveal that it contains a high amount of sodium (Na) (15.5 ± 0.1ppm) and potassium (K) (6.49 ± 0.007ppm).

Both sodium and potassium maintains fluid balance in the body, helps in sending nerve impulses, benefits bones, and blood pressure (Aguilera-Morales et al., 2005). Calcium (Ca) and magnesium (Mg) were abundant in biomass, and their concentration was 3.78 ± 0.1ppm and 5.27 ± 0.02ppm, respectively.

Magnesium and calcium help regulate blood pressure and blood sugar and enable muscles to contract (Capelli and Cysewski, 2010). The concentration of aluminium (Al) was 0.8 ± 0.007ppm and iron (Fe) was 1.0 ± 0.01ppm. Apart from these elements, microalgae biomass possesses

trace elements such as Manganese (Mn) (0.04 ± 0.0ppm), nickel (Ni) (0.01 ± 0.0ppm), copper (Cu) (0.06 ± 0.003ppm), zinc (Zn) (0.7 ± 0.05ppm), lead (Pb) (0.01 ± 0.0ppm), strontium (Sr) (0.3 ± 0.03ppm) in negligible amount. Trace elements are important for cell functions at biological, chemical, and molecular levels.

Table 2: Ultimate, Proximate and mineral composition of microalgae

Ultimate analysis[a]				
Carbon (C)	Hydrogen (H)	Nitrogen (N)	Sulphur (S)	Oxygen (O)
36.7 ± 0.2	5.7 ± 0.1	4.9 ± 0.3	-	51.5 ± 0.3

Proximate analysis[a]	
% Moisture content (% DW*)	8.6 ± 0.1
% Ash content (g/100g$_{dry\ biomass}$)	14.2 ± 0.2
Total carbohydrate (g/100g$_{dry\ biomass}$)	59.1 ± 0.3
Total protein (g/100g$_{dry\ biomass}$)	17.7 ± 0.3
Total crude fiber (%DW)	29.7 ± 0.2

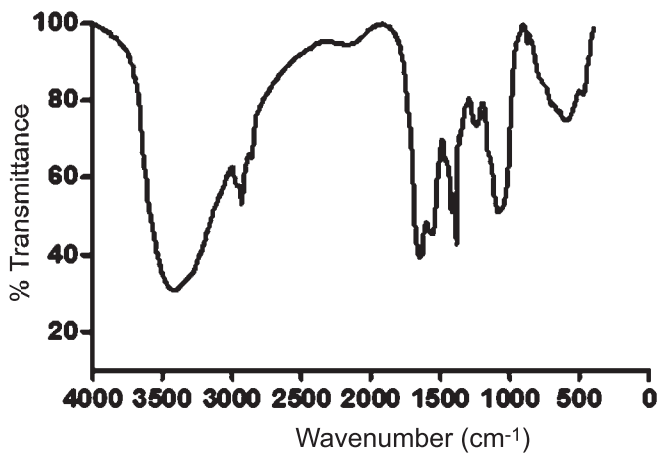
Mineral analysis[a] **(ppm)**											
Na	Mg	Al	K	Ca	Mn	Fe	Ni	Cu	Zn	Pb	Sr
15.5 ± 0.1	5.27 ± 0.02	0.8 ± 0.007	6.49 ± 0.007	3.78 ± 0.1	0.04 ± 0.0	1.0 ± 0.01	0.01 ± 0.0	0.06 ± 0.003	0.7 ± 0.05	0.01 ± 0.0	0.3 ± 0.03

[a] Results are expressed as Mean ±SD (n=2) * DW= Dry Weight

FTIR transmittance of microalgae biomass reveals the presence of –OH, -NH, -C=C, and -CH organic compound groups (Figure 4). The band at 3389 cm^{-1} is due to strong O-H stretching of hydroxyl functional groups in carboxylic, phenolic and alcoholic compounds. This range can also be attributed to the medium N-H stretching of protein. The weak band at 2920 cm^{-1} is due to the presence of =C-H, C-H stretching vibration due to the lipid and carbohydrate content in microalgae. The peak at 1630 cm^{-1} and 1380 cm^{-1} represents alkene and aldehyde groups, respectively. These peaks confirm the presence of fatty acids. The band at 1040 cm^{-1} and 569 cm^{-1} depicts alcohol and halo compounds.

Figure 4: FTIR transmittance spectra of microalgae

A:FTIR spectra of biomass



B:IR spectrum table by frequency range

Wavenumber (cm^{-1})	Group	Compound class
3550-3200	O-H	Carboxylic acid, Alcohol
3400-3300	N-H	Amine groups
1648-1638	C=C	Alkene (monosustituted)
1390-1380	C-H	Aldehyde
1085-1010	C-O	Primary alcohol
995-985	C=C	Alkene
850-550	C-Cl	Halo compounds

The effect of heating rate on the thermal behavior of microalgae biomass was investigated using TGA. For this analysis, the experiment was conducted at up to 800°C. The weight loss curve obtained for the algal biomass is shown in Figure 5.

At the first stage, there is a weight loss due to the moisture removal or some light volatile matters from 70 C to 100 C (Gai et al., 2013). At the second stage, from 200-600 C, the vaporization of organic matter takes place. The mass loss of microalgae between the ranges of 180-270 C attributes to the decomposition of carbohydrate, and from 320-450 C ascribes degradation of proteins (Carpio et al., 2019).

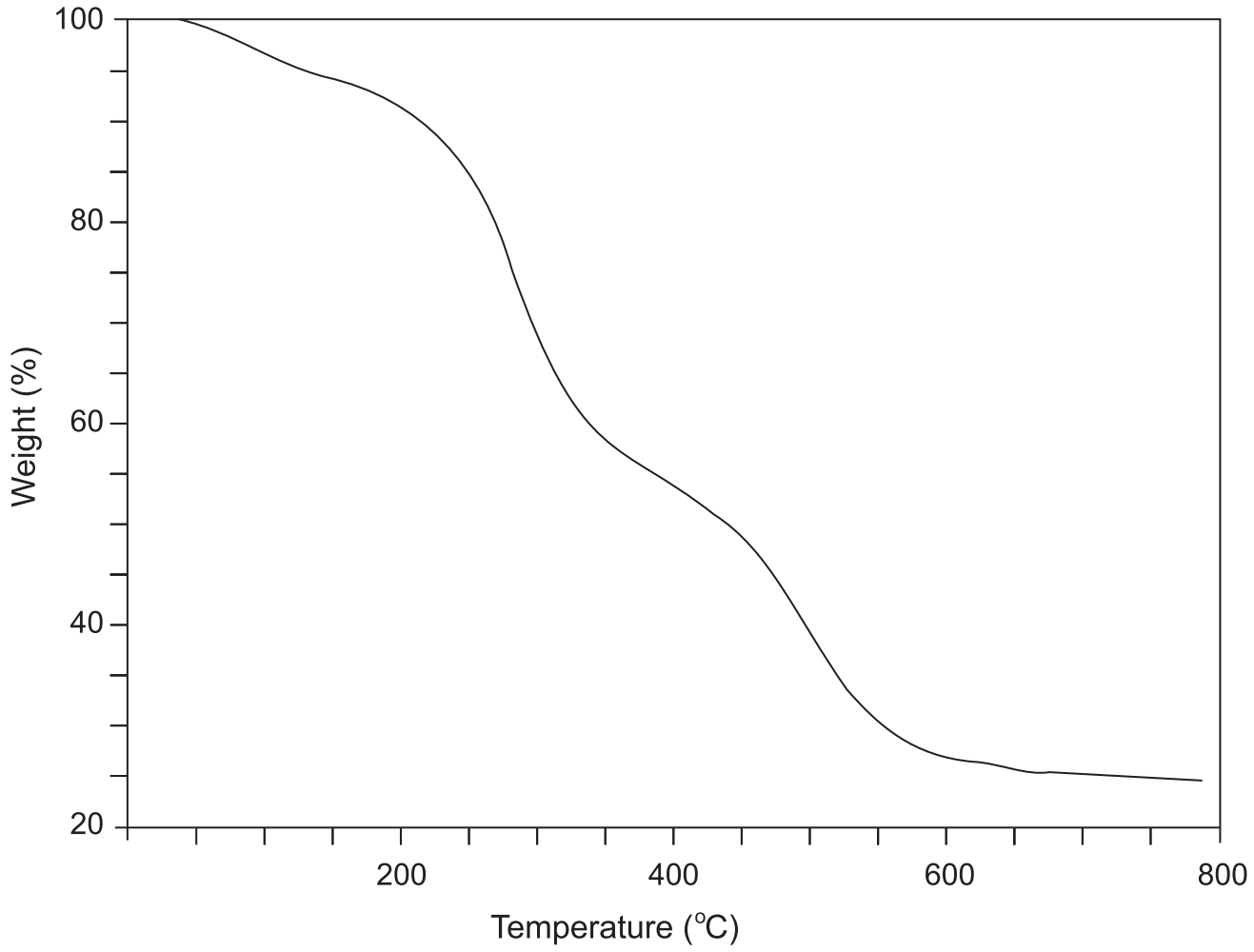


Figure 5: TGA analysis of algal biomass

3.2 Total lipid yield extracted with an organic solvent and supercritical CO_2

Different organic solvents (Chloroform: Methanol, hexane, ethanol) and supercritical carbon dioxide were investigated to extract the lipids from microalgae under different temperatures and pressure conditions. The amounts of lipids obtained from dried algal biomass have been tabulated in Table 3. Total lipid content determined by solvent extraction depends on the solvent's polarity used to carry out the extraction. The highest value of lipid extract was 28.2 ± 2.3% achieved by extraction with chloroform: methanol (1:1) in an extraction time of approximately 15 h. The amount of lipids extracted from the hexane solvent was 1.8 ± 0.5% in approx. 10 h of extraction time.

The crude lipid yield obtained by ethanol solvent extraction was 18.0 ± 1.1% in approx. 12 h of

extraction time. Chloroform: methanol proved to have the highest efficiency for the extraction of lipids from microalgae. It may be contributing to weakening the cell wall, thus assisting towards a more intense extraction of lipids from microalgae. The low recovery of lipids was observed by hexane, maybe due to its non-polar character and low selectivity towards microalgal lipids.

Supercritical carbon dioxide extraction of lipids from microalgae was investigated at 40 C and 60 C temperature and 150-350 bar pressure at a constant flow rate of 30g/min carbon dioxide. The cumulative yield of lipids at 40 C and 60 C is shown in Table 3. The yield of lipids was 3.0 ± 0.4% at 40 C and 1.9 ± 0.4% at 60 C in extraction time of 90 min. It is found that yield of algal lipids increases significantly with decreasing extraction temperature from 60 C to 40 C. This can be because high temperature reduces the CO_2 density, which in turn decreases the solubility of the solute. The yield of lipid was maximum at 250bar at 40 C, and at 50 C, it was maximum at 200bar. However, the yield of lipid increased significantly with increasing extraction pressure from 150bar to 250bar, but further increasing the pressure to 350bar resulted in a significant decrease in lipid yield.

Table 3: Comparison of lipid extraction yield of microalgae biomass for different extraction methods[a]

	Methods				
	Soxhlet extraction			**Supercritical CO_2 extraction**	
	Chloroform: Methanol (1:1)	Hexane	Ethanol	SC-CO_2 40°C	SC-CO_2 60°C
Extraction time	□ 15 hours	□ 10 hours	□ 12 hours	90 minutes	90 minutes
Crude lipid yield (wt%)	28.2 ± 2.3	1.8 ± 0.5	18.0 ± 1.1	3.0 ± 0.4	1.9 ± 0.4

3.3 Comparison of Fatty acids profile of microalgae

The fatty acid composition of extracted lipids using organic solvent and supercritical CO_2 at different temperatures and pressures are presented in Table 4. The result shows that biomass composed of saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Fatty acid composition in the present study varied considerably, with 8.57-23.36% of SFA, 15.95-52.65% of MUFAs, and 0.57-20.45% of PUFAs. Among the SFA, Palmitic acid (C16:0; 5.06-18.45%) and myristic acid (C14:0; 0.60-4.81%) were the predominant fatty acids. As for MUFAs, palmitoleic acid (C16:1n-7; 9.11-37.66%) and oleic acid (C18:1n-9; 3.53-30.39%) were the leading fatty acids. The most abundant PUFA were linoleic acid (C18:2n-6; 0.42-20.34%) and arachidonic acid (C20:4n-6; 0.11-1.15%).

Out of all the extraction methods, ethanol extraction had the highest relative concentration of SFA in their total lipid content. Supercritical CO_2 at 40 C and 350bars had the highest level of MUFAs. The most top content of PUFAs was present in Supercritical CO_2extraction at 40 C and 150bar. The health beneficial Eicosapentaenoic acid (EPA, C20:5n-3) was found at a higher temperature (60°C), with 1.62% of total fatty acids as the highest concentration at 350bars. Another physiologically essential long-chain PUFA, Docosahexaenoic acid (DHA, C22:6n-3) and arachidonic acid (AA, C20:4n-6) were found in low concentration.

When comparing the fatty acid profile of lipids extracted with the organic solvent and supercritical CO_2, PUFAs composition was highest at lower temperature and pressure conditions of supercritical CO_2 (40 C and 150bar). This gives the highest composition of MUFAs, representing 52.65% of total fatty acids, 20.45% of PUFAs, and 8.57% of SFA. The primary polyunsaturated fatty acid was linoleic acid (C18:2n-6), represent 99% of total PUFAs. The main monosaturated fatty acids were palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9), which represents 41% and 58% of total

MUFAs, respectively. The primary saturated fatty acid was palmitic acid (C16:0), representing 59% of total SFA.

Therefore, Supercritical extraction has advantages of shorter extraction time and rich product quality despite a lower lipid yield.

Table 4: Fatty acid composition (% of total fatty acids) of microalgae

Fatty acids (Shorthand notation)	Organic solvent extraction			Supercritical carbon dioxide extraction			
	Chloroform: Methanol (1:1)	Hexane	Ethanol	$SC\text{-}CO_2$ 40°C/ 150bar	$SC\text{-}CO_2$ 40°C/ 350bar	$SC\text{-}CO_2$ 60°C/ 150bar	$SC\text{-}CO_2$ 60°C/ 350bar
Saturated Fatty acids (SFA)							
C12:0	-	-	-	0.08	-	0.15	-
C13:0	-	-	-	-	-	-	0.18
C14:0	0.60	2.63	2.66	2.3	3.41	3.31	4.81
C15:0	0.39	0.72	0.96	0.33	0.82	1.68	1.47
C16:0	18.45	14.85	17.11	5.06	6.99	12.84	15.14
C17:0	-	-	0.17	-	0.29	0.79	1.26
C18:0	1.23	1.99	0.57	0.53	0.13	-	-
C20:0	-	-	0.44	0.27	0.54	0.46	0.28
C22:0	-	-	0.46	-	0.15	0.35	0.22
Total SFA	**20.67**	**20.19**	**22.37**	**8.57**	**12.33**	**19.58**	**23.36**
Monounsaturated Fatty acids (MUFAs)							
C16:1n-7	12.17	37.66	30.5	21.69	28.36	9.11	13.86
C18:1n-9	3.53	8.97	13.72	30.39	27.88	18.09	23.61
C24:1n-9	0.25	-	0.17	0.57	0.14	0.15	-
Total MUFAs	**15.95**	**46.63**	**44.39**	**52.65**	**56.38**	**27.35**	**37.47**
Polyunsaturated Fatty acids (PUFAs)							
C18:3n-3 (α-LA[a])	0.12	0.22	0.61	-	-	-	0.3[b]
C20:5n-3 (EPA[c])	-	-	-	-	-	0.35	1.62
C22:6n-3 (DHA[d])	-	-	-	-	-	-	0.32
C18:2n-6	-	0.42	0.73	20.34	-	4.84	4.36
C18:3n-6	0.14	0.54	1.04	-	-	-	-
C20:4n-6 (AA[e])	0.76	1.15	1.10	0.11	0.57	0.17	0.59
Total PUFAs	**1.02**	**2.33**	**3.48**	**20.45**	**0.57**	**5.36**	**7.19**

[a]α-LA- alpha-linolenic acid, [b]Gamma linolenic acids (GLA), [c]EPA- Eicosapentaenoic acid, [d]DHA- Docosahexaenoic acid , [e]AA- Arachidonic acid

4. Conclusion

The nutritional composition, along with proximate analysis of microalgae biomass, was investigated in this study. Total carbohydrate content, total protein content, total fiber content, and mineral composition was analyzed. The FTIR analysis indicates the presence of lipid groups, alcoholic groups, and carboxylic groups. The thermal stability of biomass was characterized by thermo-gravimetric analysis. Total crude lipid from biomass was determined using organic solvents and supercritical CO_2. Fatty acid profile of extracted lipids indicated major fractions of palmitic acid, palmitoleic acid, oleic acid, and linoleic acid. Lipid yield was maximum by chloroform: methanol (1:1) organic solvent, but most of the important fatty acids were recovered at low temperature and pressure conditions of supercritical CO_2. Some of the health beneficiary fatty acids, such as Gamma-linolenic acids (GLA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and Arachidonic acid (AA) were present at a higher temperature and pressure conditions of supercritical CO_2. This indicates that, as pressure and fluid

density increases, the solubility of triglycerides with more unsaturated fatty acids increases. Based on this study, it can be concluded that the supercritical CO_2 extraction of lipids is an effective and attractive alternative to conventional organic solvent extraction. It is becoming a more popular and promising method for the extraction of lipids, especially for the food processing and pharmaceutical industries.

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References

Aguilera-Morales, M., Casas-Valdez, M., Carrillo-Domınguez, S., González-Acosta, B., & Pérez-Gil, F. Chemical composition and microbiological assays of marine algae Enteromorpha spp. as a potential food source. **Journal of food composition and analysis**, **18(1), 79-88, (2005).**

Ahmed, M., Chin, Y. H., Guo, X., & Zhao, X. M. Microwave assisted digestion followed by ICP-MS for determination of trace metals in atmospheric and lake ecosystem. **Journal of Environmental Sciences, 55, 1-10 (2017).**

AOAC. Association of official analytical chemists. **Official methods of analysis (Washington, DC, USA, (2000).**

Aresta, M., Dibenedetto, A., Carone, M., Colonna, T., & Fragale, C. Production of biodiesel from macroalgae by supercritical CO 2 extraction and thermochemical liquefaction. **Environmental Chemistry Letters, 3(3), 136-139 (2005).**

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry, 72(1-2), 248-254, (1976).**

Capelli, B., & Cysewski, G. R. Potential health benefits of spirulina microalgae. **Nutrafoods, 9(2), 19-26, (2010).**

Careri, M., Furlattini, L., Mangia, A., Musci, M., Anklam, E., Theobald, A., & Von Holst, C. Supercritical fluid extraction for liquid chromatographic determination of carotenoids in Spirulina Pacifica algae: a chemometric approach. **Journal of Chromatography A, 912(1), 61-71, (2001).**

Carpio, R. B., Zhang, Y., Kuo, C. T., Chen, W. T., Schideman, L. C., & de Leon, R. L. Characterization and thermal decomposition of demineralized wastewater algae biomass. **Algal Research, 38, 101399, (2019).**

Chisti, Y. Biodiesel from microalgae. **Biotechnology advances, 25(3), 294-306 (2007).**

de Morais, M. G., Vaz, B. D. S., de Morais, E. G., & Costa, J. A. V. Biologically active metabolites synthesized by microalgae. **BioMed research international, (2015).**

Furuki, T., Maeda, S., Imajo, S., Hiroi, T., Amaya, T., Hirokawa, T., ... & Nozawa, H. Rapid and selective extraction of phycocyanin from Spirulina platensis with ultrasonic cell disruption. **Journal of Applied Phycology, 15(4), 319-324 (2003).**

Gai, C., Zhang, Y., Chen, W. T., Zhang, P., & Dong, Y. Thermogravimetric and kinetic analysis of thermal decomposition characteristics of low-lipid microalgae. **Bioresource technology, 150, 139-148, (2013).**

Herrero, M., Cifuentes, A., & Ibañez, E. Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. **Food Chemistry, 98(1), 136-148, (2006).**

Ibañez, E., Herrero, M., Mendiola, J. A., & Castro-Puyana, M. Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. **In Marine bioactive compounds, Springer, Boston, MA 55-98, (2012).**

Kaliaperumal, N., Ramalingam, J. R., Kalimuthu, S., & Ezhilvalavan, R. Seasonal changes in growth, biochemical constituents and phycocolloid of some marine algae of Mandapam coast. **Seaweed**

Research and Utilisation, 24(1), 73-77 (2002).

Kumar, J. I., Kumar, R. N., Kaur, M. A., Bora, A., & Chakraborty, S. Variation of Biochemical Composition of Eighteen Marine Macro Algae Collected from Okha Coast, Gulf of Kutch, India. **The Journal of Indian Botanical Society, 89(1and2), 105-110 (2010).**

Lee, J. Y., Yoo, C., Jun, S. Y., Ahn, C. Y., & Oh, H. M. Comparison of several methods for effective lipid extraction from microalgae. **Bioresource technology, 101(1), S75-S77 (2010).**

Lee, S. C., Prosky, L., & Vries, J. W. D. Determination of total, soluble, and insoluble dietary fiber in foods—Enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. **Journal of AOAC international, 75(3), 395-416, (1992).**

Lin, H. C., Chou, S. T., Chuang, M. Y., Liao, T. Y., Tsai, W. S., & Chiu, T. H. The effects of Caulerpa microphysa enzyme-digested extracts on ACE-inhibitory activity and in vitro anti-tumour properties. **Food chemistry, 134(4), 2235-2241 (2012).**

Manivannan, K., Thirumaran, G., Karthikai Devi, G., Anantharaman, P., & Balasubramanian, T. Proximate composition of different group of seaweeds from Vedalai Coastal waters (Gulf of Mannar): Southeast Coast of India. **Middle-East Journal of scientific research, 4(2), 72-77 (2009).**

Mata, T. M., Martins, A. A., & Caetano, N. S. Microalgae for biodiesel production and other applications: a review. **Renewable and sustainable energy reviews, 14(1), 217-232 (2010).**

Mendiola, J. A., García-Martínez, D., Rupérez, F. J., Martín-Álvarez, P. J., Reglero, G., Cifuentes, A., ... & Señoráns, F. J. Enrichment of vitamin E from Spirulina platensis microalga by SFE. **The Journal of Supercritical Fluids, 43(3), 484-489 (2008).**

Michalak, I., & Chojnacka, K. Algal extracts: Technology and advances. **Engineering in life sciences, 14(6), 581-591, (2014).**

Michalak, I., Dmytryk, A., Wieczorek, P. P., Rój, E., Łęska, B., Górka, B., ... & Schroeder, G. Supercritical algal extracts: a source of biologically active compounds from nature. **Journal of Chemistry, (2015).**

Miranda, J. R., Passarinho, P. C., & Gouveia, L. Pre-treatment optimization of Scenedesmus obliquus microalga for bioethanol production. **Bioresource technology, 104, 342-348 (2012).**

O'fallon, J. V., Busboom, J. R., Nelson, M. L., & Gaskins, C. T. A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. **Journal of animal science, 85(6), 1511-1521, (2007).**

Ong, M. Y., Syahira Abdul Latif, N. I., Leong, H. Y., Salman, B., Show, P. L., & Nomanbhay, S. Characterization and Analysis of Malaysian Macroalgae Biomass as Potential Feedstock for Bio-Oil Production. **Energies, 12(18), 3509, (2019).**

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., ... & Rios, A. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica. **Food chemistry, 99(1), 98-104 (2006).**

Parthiban, C., Saranya, C., Girija, K., Hemalatha, A., Suresh, M., & Anantharaman, P. Biochemical composition of some selected seaweeds from Tuticorin coast. **Advances in applied science research, 4(3), 362-366 (2013).**

Pragya, N., Pandey, K. K., & Sahoo, P. K. A review on harvesting, oil extraction and biofuels production technologies from microalgae. **Renewable and Sustainable Energy Reviews, 24, 159-171, (2013).**

Román, R. B., Alvarez-Pez, J. M., Fernández, F. A., & Grima, E. M. Recovery of pure B-phycoerythrin from the microalga Porphyridium cruentum. **Journal of Biotechnology, 93(1), 73-85 (2002).**

Ruperez, P., & Saura-Calixto, F. Dietary fiber and physicochemical properties of edible Spanish seaweeds. **European Food Research and Technology, 212(3), 349-354, (2001).**

Sahena, F., Zaidul, I. S. M., Jinap, S., Karim, A. A., Abbas, K. A., Norulaini, N. A. N., & Omar, A. K. M. Application of supercritical CO2 in lipid extraction–A review. **Journal of Food Engineering, 95(2), 240-253, (2009).**

Van Wychen, S., & Laurens, L. Determination of total carbohydrates in algal biomass. **Contract, 303, 275-3000, (2013).**

Van Wychen, S., & Laurens, L. M. *Determination of Total Solids and Ash in Algal Biomass: Laboratory Analytical Procedure (LAP)* (No. NREL/TP-5100-60956). **National Renewable Energy Lab.(NREL), Golden, CO, United States, (2016)**.

Viskari, P. J., & Colyer, C. L. Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. **Analytical biochemistry, 319(2), 263-271 (2003).**

Wall, R., Ross, R. P., Fitzgerald, G. F., & Stanton, C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. **Nutrition reviews, 68(5), 280-289, (2010).**

Wang, B., Li, Y., Wu, N., & Lan, C. Q. CO 2 bio-mitigation using microalgae. **Applied microbiology and biotechnology, 79(5), 707-718 (2008).**

Wang, H. M., Pan, J. L., Chen, C. Y., Chiu, C. C., Yang, M. H., Chang, H. W., & Chang, J. S. Identification of anti-lung cancer extract from Chlorella vulgaris CC by antioxidant property using supercritical carbon dioxide extraction. **Process Biochemistry, 45(12), 1865-1872 (2010).**

Yen, H. W., Yang, S. C., Chen, C. H., & Chang, J. S. Supercritical fluid extraction of valuable compounds from microalgal biomass. **Bioresource technology, 184, 291-296, (2015).**

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[Contributed by Dr. K.V. PADMAJA]

Synthesis and Characterization of Polyethylene Glycol Diesters from Estolides Containing Epoxides and Diols

Estolides from oleic acid, 12-hydroxystearic acid, and methyl ricinoleate were synthesized and converted to polyethylene glycol (PEG) diesters by Isbell etal. Oleic estolide was synthesized from oleic acid as a homo-oligomeric material using perchloric acid in 68.8% yield and an estolide number (EN) value of 1.29. Estolides from 12-hydroxy-stearic acid were homo-oligomers made by heating under vacuum at 150 C for 24 hours to give a quantitative yield of estolide with an EN value of 2.55. Oleic acid-based estolides and 12-hydroxystearic acid-based estolide were esterified with PEG-200 diol to form PEG 200 diesters. Ricinoleate estolides was capped with lauric acid or 12-hydroxystearic estolide by reacting methyl ricinoleate with the corresponding fatty acids at 150 C using tin(II) octoate as a catalyst. The corresponding estolides were transesterified with PEG-200 diol to form the diesters. The residual olefin of ricinoleate was then epoxidized and underwent ring opening hydrolysis to form the corresponding diol [J. Amer. Oil Chem. Soc. 97 (4), 409-423(2020)]. NMR spectroscopy (^{1}H, ^{13}C, distortionless enhancement by polarization transfer, heteronuclear single quantum correlation, heteronuclear multiple bond correlation, and correlated spectroscopy) was used to characterize the products.

Viscometric Properties of Polyethylene Glycol di-Esters of Estolides

Polyethylene glycol (PEG) diesters prepared by Isbell et al. from estolides of oleic acid, ricinoleic acid, and 12-hydroxy stearic acid were used up to 5 wt% additive in petroleum-derived base oils: 100N, 220N, 600N, PAO 2cSt, PAO 4cst, and PAO 8cSt. The viscosity indices of the petroleum base oils were the lowest (VI = 104–108); the polyalpholefin (PAO) synthetic petroleum-derived base oils were better (VI = 124–136) and the vegetable-derived oils were the best (VI = 111–205). 12-Hydroxystearic estolide PEG 400 diester gave the largest increase in viscosity index in 5% w/w admixtures with PAO 2cSt, 4cSt, 220N, and 600 N base oils with a 12.3–16.3% increase in viscosity index. Single fatty chain esters had the least impact on viscosity index. As the molecule bulk increased, the viscosity index also increased.

This viscosity index effect was demonstrated for the series of monomer to oleic estolide to oleic estolide PEG-200 diester, which gave viscosity indices of 110, 111–122, respectively, in 5% admixtures with 100N base oil. The larger the size of the molecule coupled with the polar PEG moiety gave the largest impact on viscosity index improvement where a 5 wt% admixture of 12-hydroxystearate estolide PEG 200 diester gave a 25.8% increase in 100 C viscosity in PAO 2cSt.

Incorporation of a dihydroxyl moiety into the molecule at a central side chain location in laurate-capped ricinoleate estolide PEG-200 diester did not increase viscosity index of the base oil but greatly reduced its solubility to less than 0.5 wt% [J. Amer. Oil Chem. Soc. 97 (4), 425-435(2020)].

Synthesis and Characterization of Potential Bio-Based Lubricant Basestocks *via* Epoxidation Process

Chemical modifications of vegetable oils may be applied for the purpose of improving their physicochemical properties in their usage for the bio-based lubricants. The vegetable oils with a high percentage of oleic acid, such as soybeans and rapeseed oils, are important raw materials to obtain the biolubricants. In this particular study by Marques et al., the oleic

acid was esterified with 1-octanol, followed by epoxidation. The oxirane ring opening reaction was performed using different alcohol structures (linear, branched, and cyclic), in order to evaluate their influence on the final physicochemical properties with the synthesized samples. These aforesaid reaction steps were followed by ^{1}H nuclear magnetic resonance and the main physicochemical properties in the intermediate and final samples were assessed. The highest oxidative stability was observed for the samples obtained, using a cyclic alcohol at the oxirane ring opening reaction (230 min), followed by the linear alcohols with the branched alcohol presenting the lowest oxidative stability (124 min). The pour point values for the samples synthesized with the branched alcohol were slightly better than those with the linear and cyclic alcohols. All the synthesized samples showed high viscosity indexes (>130) and kinematic viscosities at 40C, ranging from 30 to 33 cSt (application degree ISO VG-32), which are adequate for their use in the formulation of bio-based lubricants [J. Amer. Oil Chem. Soc. 97 (4), 437-446(2020)].

Catalytic Cracking of Rapeseed Oil with Binary Oxide Systems: An Alternative to Production of Petrochemicals

The aim of this work by Przekop et al., is to evaluate analytical tools for fast assessment of the catalysts' ability to conduct a catalytic cracking process with the use of vegetable oils. The practical context of the presented concept relates to the use of cracking reaction products as valuable chemical raw materials. The proposed analytical tools allow for quick assessment of reaction products, indication of the molecular weight range, or the presence of specific functional groups. We want to emphasize that vegetable oils can not only be raw products for biofuels but also an alternative to petrochemicals. The study was undertaken to determine the influence of acid–base properties of catalysts on the rapeseed oil conversion process at 500 C. The effect of these properties on the character of the process and quality of the products obtained was shown to be very high [J. Amer. Oil Chem. Soc. 97 (5), 543-550(2020)]. Basic correlations between the formation of coke, gaseous products and dehydrogenation products, and acid–base parameters of the individual catalysts have been observed. The use of spectroscopic methods (FTIR - Fourier Transform Infrared Spectroscopy, ^{1}H NMR-Nuclear Magnetic Resonance) for fast qualitative analysis of the products is described.

Synthesis of Edible Vegetable Oils Enriched with Healthy 1,3-Diglycerides Using Crude Glycerol and Homogeneous/Heterogeneous Catalysis

Commercial edible vegetable oils in which part of their triglycerides are substituted with 1,3-diglycerides are healthier for human consumption than the original oils. This is because the human metabolism of 1,3-diglycerides is believed to occur through a distinct pathway with less probability of being deposited as fat in the body tissues. To obtain these enriched oils, conversion of triglycerides into diglycerides was carried out by Dosso etal., by glycerolysis using commercial crude glycerol containing dissolved alkali cations that homogeneously catalyze the reaction. The addition of a food production-compatible MgO as a supplementary solid basic catalyst, shortens the reaction time by half due to a combination of homogeneous and heterogeneous catalysis processes. In either homogeneously or homogeneous-heterogeneously catalyzed glycerolysis, the increase of the reaction temperature in the range of 453–493 K increases the final 1,3-diglyceride content. Furthermore, in both glycerolysis processes the triglyceride content can be decreased in more than 60% with the consequent increase of total diglycerides to

50%, 70% of which are the 1,3-isomers. The glycerolysis reaction proceeds without altering the fatty acid distribution of the original oil [J. Amer. Oil Chem. Soc. 97 (5), 551-561(2020)].

Determination of Physicochemical Properties, Fatty Acid, Tocopherol, Sterol, and Phenolic Profiles of Expeller–Pressed Poppy Seed Oils from Turkey

In this study by Ozbek et al., the aim was to characterize the physicochemical properties and some bioactive compounds of expeller-pressed oils of five registered poppy seed varieties (TMO–1, Ofis–8, Ofis–96, Ofis–95, Ofis–3) grown in Turkey. The amounts of total carotenoids, chlorophylls, phenols, and antioxidant activities of oils ranged between 0.08–0.24 mg $100g^{-1}$, 0.03–9.04 mg pheophytin a kg^{-1}, 3.41–8.57 mg gallic acid equivalent 100 g^{-1}, and 5.60–7.33 mM Trolox equivalent 100 g^{-1}, respectively. The most abundant fatty acid in poppy seed oils was linoleic acid (69.85–74.02%), followed by oleic acid (13.98–16.99%), and palmitic acid (8.51–9.75%). In addition, poppy seed oils were rich in β–sitosterol (133.47–153.42 mg 100 g^{-1}), campesterol (45.36–58.60 mg 100 g^{-1}), and δ–5–avenasterol (28.21–39.40 mg 100 g^{-1}). High amounts of γ–tocopherol and α–tocopherol were detected [J. Amer. Oil Chem. Soc. 97 (5), 591-602(2020)]. This research is the first study, which identified and quantified the polyphenol, β–carotene, and lutein compounds of expeller–pressed poppy seed oils by HPLC. Tyrosol, apigenin, syringic acid, 3–hydroxytyrosol, luteolin, *p*–coumaric acid, quercetin, ferulic acid, sinapic acid, and veratric acid were detected in expeller-pressed poppy seed oils

Interesterification of mafura butter and camellia oil for cosmeceutical formulations: Chemical composition and physicochemical properties of products

Mafura butter (MB) obtained from seeds of *Trichilia emetica* Vahl is widely used in traditional cosmetic formulations throughout Southern Africa. It is gaining increasing popularity in the modern cosmetic industry due to growing consumer demand for natural cosmetics. However, the butter has a high melting point and low spreadability, which limits its emollient properties. In the present study by Poojary et al., MB was chemically and enzymatically interesterified with camellia oil (CO, *Camellia oleifera* C.Abel) at different ratios (90:10, 80:20, 70:30, 60:40 and 50:50 w/w) to produce formulations with improved physicochemical and cosmeceutical properties. Chemical interesterification (CI) was performed using sodium methoxide catalyst, while enzymatic interesterification (EI) was carried out with three different immobilized enzymes, including Lipozyme® TL IM, Lipozyme® RM IM and Lipozyme® 435. The original and interesterified blends were examined for fatty acid (FA) and triglycerides compositions, slip melting point (SMP), solid fat content (SFC), tocopherol and sterol contents, toxic heavy metal contents, radical scavenging activity (RSA) and *in vitro* ultraviolet radiation protection ability. Both CI and EI reduced SMP and SFC of interesterified products, resulting in products with improved consistency. Interesterification distributed cosmeceutically relevant unsaturated FA such as oleic acid, linoleic acid and linolenic acid in the products, depending on the ratios of MB and CO. The tocopherol and phytosterol contents in MB was 495.08 ± 19.02 and 842.61 ± 35.77 µg/g, respectively, while it was 438.6 ± 20.89 and 163.57 ± 20.47 µg/g, respectively in CO step [Ind. Crops & Products 147, 112178 (2020)]. The CI dramatically reduced the tocopherol contents up to 50 % in the products, while EI did not affect its content significantly. The ICP-MS analysis revealed that MB, CO and interesterified products does not contain toxic metals such as Sn and Hg, while Cr (< 0.18 ppm) and Pb (< 0.14 ppm) were present within the acceptable limits. Interesterified products showed promising RSA (with IC50 values in the range of 10.15 ± 0.79–12.30 ±

1.15 mg/mL), however, had a low *in vitro* sun protection factor (SPF < 0.2).

Oleogels Based on Palmitic Acid and Safflower Oil: Novel Formulations for Ocular Drug Delivery of Voriconazole

The gelation of the vegetable oils using fat crystals has gained significant attention in recent years. These formulations have been explored for food and pharmaceutical applications. The alteration in the properties of palmitic acid (20–40% w/w) and safflower oil oleogels is extensively studied by Mohanti etal., at microscopic and macroscopic levels. The thermal and mechanical stability of the oleogels is improved when the proportion of the palmitic acid content is increased. However, under stress, the fat crystal network junction zones of the oleogels with higher proportions of palmitic acid undergo disruption. The changes in the properties of the oleogels are due to the alteration in the molecular packing, crystallite size, and lattice strain of the fat crystal network.

The alteration in the properties is governed by the changes in the extent of inter- and intramolecular hydrogen bonding within the components of the oleogels [Eur. J. Lipid Sci. Technol. 122 (4), 1900288 (2020)]. The oleogels can demonstrate the ability to deliver the drug, voriconazole, across the corneal tissue. Further, the prepared oleogels are biocompatible to murine fibroblast cells and do not elicit adverse reactions when instilled within the ocular sac of rabbits. The results suggest that the oleogels can be tried as ocular delivery vehicles.

Chia Seed Oil Prevents High Fat Diet Induced Hyperlipidemia and Oxidative Stress in Mice

Hyperlipidemia is a common cardiovascular disease. At present, the influence of high fat diet (HFD) on this is being explored. Recently, vegetable oils rich in omega-3 have been reported that can treat hyperlipidemia caused by HFD. However, the effects of chia seed oil (CSO) on HFD-induced hyperlipidemia and oxidative stress are poorly studied. Hence, in this study by Han et al. The effects of CSO on hyperlipidemia and oxidative stress induced by HFD in mice are analyzed by various commercial kits, section staining, and protein expression. The results show that CSO decreases body weight and organ index. Meanwhile, CSO reduces serum lipid levels of total cholesterol, triglyceride, and low-density lipoprotein cholesterol. It can also elevate superoxide dismutase and glutathione peroxidase activities and reduce malondialdehyde content in serum and liver. The results of histopathological analysis prove that CSO improves hepatic steatosis and reduces lipid deposition. Further, the results of western blot demonstrate that CSO upregulates the expression of peroxisome proliferator-activated receptor alpha and carnitine palmitoyltransferase 1a in the liver. As a result, CSO may be a potential lipid-lowering oil to prevent and treat HFD-induced hyperlipidemia and oxidative stress oleogels [Eur. J. Lipid Sci. Technol. 122 (4), 1900443 (2020)].

Sea Buckthorn Oil Tocopherol Extraction's By-Product Utilization in Green Synthesis of Polyurethane Coating

The focus of the present study is to utilize a by-product obtained during extraction of tocopherols, a valuable vitamin E compound, from sea buckthorn (SBT) oil and in doing so find a reliable alternative to petrochemical based polyols. Bio-based polyurethane (PU) is prepared by Prabhudesai et al., using SBT oil based fatty acid methyl ester polyesteramide polyols (SBTPEP) with toluene diisocyanate (TDI). The fatty acid methyl ester is converted to the corresponding fatty amide by reaction with diethanolamine. The formed fatty amide is then esterified with phthalic anhydride to synthesize polyesteramide polyol.

Characterization techniques used to evaluate polyesteramide polyol are Fourier-transform infrared spectroscopy (FTIR) and NMR.

The cured PU coating is also put through various mechanical tests to analyze the physical properties. The cured PU coating shows good surface and mechanical properties. It shows a gloss value of 87.4 and passes impact, adhesion, and chemical resistance tests. It is hydrophobic which is evident from its contact angle of 100.2°. It has good thermal stability which is evident by its glass transition temperature of 53.9 C. Use of phthalic anhydride contributes to the bio-based characteristics of synthesized PU [Eur. J. Lipid Sci. Technol. 122 (4), 1900387 (2020)].

Continuous Transesterification Reaction of Residual Frying Oil with Pressurized Ethanol Using KF/Clay as Catalyst

The efficiency of a heterogeneous potassium fluoride (KF)/clay catalyst in continuous ester production from residual frying oil using pressurized ethanol is evaluated. Reactions without catalysts are conducted to determine the effect of the catalyst on ester yield. To verify the performance of the catalyst, the reactions are conducted by Zempulski etal., for 3 h with determination of the ester yield every 30 min and characterization of the catalyst after each reaction. The influence of the temperature, catalyst mass used in the catalytic bed, and the residence time are evaluated. KF/clay is found to be efficient in ester synthesis and provides better results compared with non-catalytic reactions and the formation of esters is favored on increasing the temperature. The ester yields remain stable over time at 275 and 300 C but at 225 and 250 C the yields decrease by 48.42% and 38.40%, respectively. This may be due to the lower diffusion coefficient at these temperatures, implying that the reaction occurs preferentially at the catalyst surface [Eur. J. Lipid Sci. Technol. 122 (5), 1900315 (2020)]. There is an increase in yield with an increase in the catalyst mass up to 2 g. The catalyst maintains its morphological characteristics after the reaction and the average mass loss in each reaction is <8%.

NMR Spectroscopy: Determination of Peroxide Value in Vegetable and Krill Oil by Using Triphenylphosphine as Tagging Reagent

Hydroperoxides are formed as the primary product during lipid oxidation, being analyzed as the peroxide value to detect the degradation level of oils and fats. As an alternative to the classical titration method according to Wheeler, a ^{1}H-{^{31}P}decoupled NMR method is developed using triphenylphosphine (TPP) as a tagging agent by Hafer etal.. TPP reacts with peroxides to form TPP oxides. The quantification of the peroxide value is performed by comparing the amount of reacted TPP oxide and non-reacted TPP. This approach eliminates the requirement for an additional internal standard. Low-oxidized oils (peroxide value < 3 meq/kg) and high-oxidized oils with peroxide values of 150 meq/kg are precisely quantified with an relative standard deviation (RSD) of 4.90% and 0.16%, respectively [Eur. J. Lipid Sci. Technol. 122 (5), 1900442 (2020)]. A total number of 108 oil samples are examined using the newly-developed ^{1}H-{^{31}P}decoupled NMR method, indicating the applicability for vegetable oils and krill oils.

Analysis of Reaction Kinetics of Edible Oil Oxidation at Ambient Temperature by FTIR Spectroscopy

Edible oils are studied to analyze their reaction kinetics during oxidation and predict shelf life at ambient temperature by Wang et al. Rapeseed oil (RO), soybean oil (SO), linseed oil (LO), and peanut oil (PO) are detected via Fourier transform infrared spectroscopy with a mesh cell as spectral acquisition accessory. The reaction kinetics of ROOH bonds, C=O bonds, *trans* double bonds (TDBs), and carbon chain skeletons (CCSs) are determined by

absorbance changes in their characteristic absorption peaks. Prediction models for shelf life based on various characteristic absorption peaks are converted via the reaction kinetics equations of the oils. Results show that first-order reaction kinetics are used to describe absorbance changes of ROOH bonds in PO, SO, and LO, while zero-order reaction kinetics are used to describe the absorbance changes in RO. Both C═O bonds and CCS absorbance changes in the four oils satisfy first-order reaction kinetics. TDBs absorbance changes in PO, SO, and LO are in accordance with zero-order reaction kinetics [Eur. J. Lipid Sci. Technol. 122 (6), 1900302 (2020)]. The regression equations of reaction kinetics exhibit good fitting degree (coefficient of determination, R^2 > 0.9000) and statistical significance (p < 0.001), indicating that the proposed method can be used to analyze the reaction kinetics of oil oxidation and predict shelf life.

Detection of Soft-Deodorized Olive Oil and Refined Vegetable Oils in Virgin Olive Oil Using Near Infrared Spectroscopy and Traditional Analytical Parameters

The European Parliament identifies virgin olive oil (VOO) as one of the foods which are often subject to fraudulent activities. Possibilities of adulteration are the application of illegal soft deodorization of extra virgin olive oil (EVOO) or the commercialization of blends of EVOO with soft-deodorized EVOO or refined vegetable oils. Despite the search for possibilities to prove the illegal soft deodorization of EVOO or the addition of cheaper vegetable oils to EVOO, suitable methods are still missing. Therefore, the aim of the study by Gertz et al., is to develop a new analytical and statistical approach addressing detection of mild deodorization or addition of refined foreign oils. For this purpose, VOOs are treated in lab-scale for 1 h up to 28 days at different temperatures (20, 50, 60, 80,100, 110, and 170 C) in order to simulate and study the effect of heat treatment on known analytical parameters by near infrared spectroscopy (NIR) [Eur. J. Lipid Sci. Technol. 122 (6), 1900355 (2020)]. A logit regression model enabling the calculation of the probability for a heat treatment is developed. This new methodology allows detecting both soft deodorized olive oils and blends of EVOO with cheaper full refined vegetable oils. Adding only 10% of full refined oil could be detected in extra VOO.

Formation of Polar Compounds During Deep-frying—Determination by ^{1}H NMR and ESR

The present study by Li et al., aims to elucidate the factors influencing the generation of polar compounds in oils during deep-frying. Oils with different fatty acid compositions, including palm oil (PO), refined palm kernel oil (RPKO), and refined coconut oil (RCO), are applied in successive frying processes. ^{1}H NMR spectra reveal that heated PO has a higher percentage of allyl acyl group and is more prone to formation of non-polar dimeric triglycerides as compared to other types of oils. In addition, electron spin resonance (ESR) spectra indicate that alkyl radicals are more predominant than alkoxy radicals in heated PO. In contrast, RPKO and RCO are inclined to generation of alkoxy radicals during the thermal treatment. The results reveal that oils with high unsaturated fatty acid content are more prone to generation and oxidation of non-polar dimeric triglycerides [Eur. J. Lipid Sci. Technol. 122 (6), 1900363 (2020)].

Grape Seed Oil Characterization: A Novel Approach for Oil Quality Assessment

The amount of organic pomace, left behind agricultural processes, is continuously rising in accordance with industrial progress. Grape pomace, generated in the wine industry all over the world, represents a raw material for obtaining valuable products. Grape seeds are especially rich in oil containing bioactive compounds that can have various health-related effects. In this study Dabetic et al., compared the quality of seed oils obtained

from six white grapes, including two Serbian autochthonous varieties.

Linoleic acid, associated with numerous health benefits, is the major fatty acid in all samples (≈ 66% of total); α-tocopherol is the main tocopherol homologue. Total polyphenol content ranges from 73.4 to 104.3 mg of gallic acid equivalents per 100 g. In order to provide comprehensive information about antioxidant capacity of grape seed oil (GSO), three tests are performed (ferric ion reducing antioxidant power; 2,2'-diphenyl-1-picrylhydrazyl, and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulpho nic acid) radical scavenging). Antimicrobial activity is investigated against different strains; however, GSO inhibits the growth of *staphylococcus aureus* and *candida albicans* triglycerides [Eur. J. Lipid Sci. Technol. 122 (6), 1900447 (2020)]. Obtained results are used to develop a novel approach for oil quality assessment. Calculated oil quality scores (OQS) reveal no significant difference between international and autochthonous varieties, although Smederevka stands out as the most potent one.

Synthesis of Functionalized High-Oleic Soybean Oil Wax Coatings and Emulsions for Postharvest Treatment of Fresh Citrus Fruit

High-oleic soybean oil is chemically functionalized by Levya-Gutirerrez et al., in order to mimic the structure and physical properties of hydrogenated castor oil (HCO). The resulting wax-like material is evaluated for use as an alternative to other commercial wax coatings for the postharvest treatment of fresh citrus fruit. The racemic nature of the material inhibits ordered crystalline arrangement and negatively affects its relative crystallinity (17.7%), hardness (0.59 ± 0.04 mm^{-1}), and melting profile (44–46 C), with respect to HCO oil (37.7%, 5.33 ± 0.01 mm^{-1}, 83–87 C). Nevertheless, compounding the new material with carnauba wax (CAR) imparts a very attractive gloss and prevents moisture loss significantly better than polyethylene, shellac, and CAR-based coatings [Eur. J. Lipid Sci. Technol. 122 (6), 2000005 (2020)]. Compounding the hydroxy-functionalized high-oleic soybean wax may potentially reduce dependence on imported CAR and other ingredients used in citrus coating emulsion formulations.

Chemical Synthesis and Isolation of *Trans*-Palmitoleic Acid (*Trans*-C16:1 n-7) Suitable for Nutritional Studies

Trans-palmitoleic acid (*trans*-9 C16:1, or *trans*-C16:1 n-7, TPA) is typically found in ruminant-derived foods (milk and meat). Of note, previous epidemiological studies associated high levels of circulating TPA with a lower risk of type 2 diabetes and metabolic syndrome in humans. At the current time, TPA intakes in humans are ensured by ruminant-derived foods. However, due to the very low commercial availability of TPA, there are no supplementation studies carried out so far. Therefore, the ability for dietary TPA to prevent type 2 diabetes and metabolic syndrome has never been experimentally assessed. Here, a method (among others) to get dozens of grams of pure TPA as ethyl ester, to perform dedicated supplementation studies, is reported by Guillocheau et al.. For that purpose, it starts from food sources containing high amounts of *cis*-palmitoleic acid (*cis*-9 C16:1, or *cis*-C16:1 n-7, CPA), dealing with fatty acids ethyl esters all along the experiment. CPA is purified with flash liquid chromatography, then submitted to a *cis*/*trans* isomerization step. Finally, TPA is separated from CPA by low-temperature crystallization in methanol. The final product is fully characterized by ^{1}H and ^{13}C nuclear magnetic resonance spectrometry. It is possible to produce ≈ 70 g of 85%-purity TPA suitable for nutritional studies coatings [Eur. J. Lipid Sci. Technol. 122 (6), 1900409(2020)].

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