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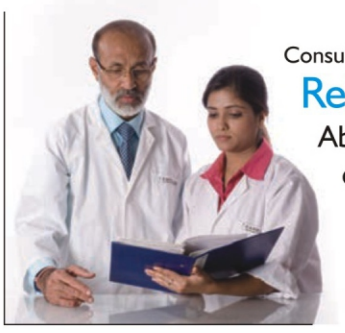

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From Editors Desk

Hello Friends,

We were facing challenging time of Covid-19 pandemic in the last 6 to 7 months and continue to face the bad time even today as there are roughly one lac cases of corona per day in our country which has totally changed the life style of the individuals. Still businesses are running at very slow pace, although vegetable oils being essential commodity, this industry was permitted to run with a lot of restrictions. This will enormously effect country's GDP as first quarter was totally lost due to lock down. This period has taught every one to live in hygeinic conditions, increased digitization as well as work from home culture. The demand for ready to eat `RTE' segment has decreased tremendously however, " Take Home" delivery has started picking up gradually. Moreover, we attended a lot many webinars and online meetings in this time which has become a new normal and I think some of these will last permanently. We pray that vaccine comes out sooner than expected and that too at affordable price so that a large population of our country can be vaccinated in shorter time for which the Govt. must be thinking in terms of infrastructure creation, we are aware that several pharmaceutical companies are trying to work on vaccine trials.

Now new challenge is coming for the technocrats working in the oil industry to bring down the level of trans content in the oils and fats to below 3% level from existing 5%. Further, it has been decided a long time ago by regulatory body i.e., FSSAI to bring it below 3% by 1st January 2021 and to 2% by January 2022. There is another issue of 3-MCPD which should be below 20 micro gram as per European Commission Regulation and generally people talk of this in palm oil. These levels may be even higher in some Indian oils also as Rice Bran Oil also has higher FFA and requires extensive processing. Therefore, our organization must take up the research work in above two areas to control these levels and I think that the FSSAI regulation must be there on the level of 3MCPD as well as glycedyl esters. We must debate these issues In open forums of the Oil Technologists' Association of India by way of seminars, discussions in our scientific panel, and come out with our conclusions.

In the end I would like to say that working in the capacity of the Editor-in-Chief for the Journal of Lipid Science and Technology (JLST) in the last five years has given immense satisfaction as we could do away with backlog of journals in spite of pandemic period of almost six months. I would like to thank my editorial team and particularly Dr. K. V. Padmja and Dr. Praveen Yadav who were associate editors and extended their full cooperation during these years. I am also thankful to Mr. R. C. Arora for keeping the accounts meticulously. I welcome the new editorial team under the leadership of Dr. Rakesh K Trivedi and hope that they will enhance the reach and quality of Journal.

R. P. Singh

(R.P. SINGH)

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Production and characterization of cocoa butter equivalents using palm mid fraction and kokum butter

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Keywords: *palm mid fraction, kokum butter, cocoa butter equivalents, blends*

Abstract

Palm mid fraction is considered as a by-product which is generated in large quantities during the fractionation process of crude palm oil in palm oil refinery. Kokum butter is a rich source of stearic acid. Palm mid fraction (PMF) and kokum butter (KB) were blended in different ratios to obtain cocoa butter equivalents (CBEs). All the prepared blends were characterized for their slip melting point, iodine value, fatty acid composition, triacylglycerol composition and melting profile. Three blends of PMF/KB (30/70, 80/20 and 90/10) possessed slip melting point (34.8 to 36.1°C), iodine value (38.74 to 41.70 g I_2/ 100 g fat), fatty acids, triglycerides and melting profile similar to commercial CBEs. These blends can be further recommended for use as CBEs, due to their similar properties as CBEs.

1. Introduction

Cocoa butter is an edible fat extracted from the mature beans of *"Theobroma cacao"* plant. It is obtained as by-product during the processing of cocoa beans. It is the primary ingredient in confectionery and chocolates. The main fatty acids present in cocoa butter are palmitic acid, stearic acid, oleic acid and linoleic acid. The other minor fatty acids present are myristic acid and lauric acid. The major triglycerides present in cocoa butter are: glycerol-1,3-distearate-2-oleate (SOS, 23.8-31.2%), glycerol-1-palmitate-2-oleate-3-stearate (POS, 33.7-40.5%) and glycerol-1,3-dipalmitate-2-oleate (POP, 13.6-15.5%) (Bootello *et al*., 2012). Cocoa butter shows polymorphism, having multiple crystalline forms of varying melting points. The hardness of chocolates is attributed to the polymorphism and high melting point of cocoa butter which lies in the range of 34-38°C (D'Souza *et al*., 1990). As compared to other edible fats and oils available in the market cocoa butter has high market demand and low supply which makes it quite expensive; manufacturers are therefore looking for alternatives to CB. Cocoa butter alternatives (CBAs) are three types: Cocoa butter equivalents (CBEs), Cocoa butter replacers (CBRs) and Cocoa butter substitutes (CBSs) (Naik *et al*., 2014).

CBEs are prepared from the non-lauric fats having different polymorphism. After hydrogenation of edible oils, CBRs are obtained. The fatty acid and triacylglycerol composition of cocoa butter equivalents are similar to cocoa butter which imparts similar physical properties. The slip melting point of CBEs is 35-37°C and iodine value is about 34-40. The glycerol-1-palmitate-2-oleate-3-stearate (POS) (40%), glycerol-1,3-distearate-2-oleate (SOS) (27%) and glycerol-1,3-dipalmitate-2-oleate (POP) (21%) are the major triglycerides present in cocoa butter equivalent (Undurraga *et al.,* 2001). There are different fat alternatives which are blended in various ratios to obtain physical and chemical properties similar to cocoa butter. During chocolate manufacturing, CBEs which are produced from low-cost edible oils are blended with cocoa butter providing an alternative to expensive cocoa butter (Shukla, 2005). According to the

European Union Chocolate Directive, upto 5% CBEs are allowed in chocolate and related products, (Stewart *et al*., 2004).

To produce CBEs fats rich in SOS are blended with fats rich in POP (Kaphueakngam *et al*; 2009; Salas *et al*., 2011). Palm mid fraction (PMF) is obtained during the multi-stage fractionation of palm oil (*Elaeis guineensis*). It is a POP rich fraction. PMF with the melting temperature in the range of 9.8°C-33°C is favoured for the blending process (Bootello *et al*., 2012). 51.8% POP is present in PMF in comparison to the other triglycerides (Biswas *et al*., 2017). It is considered as a good alternative in the manufacture of confectionaries and other food products as it crystallizes in the β' form (Sonwai *et al*., 2014). Apart from this, PMF has a steep Solid Fat Content (SFC) melting curve similar to cocoa butter (Biswas *et al*., 2017).

Kokum fat or butter (KB) is derived from the seeds of *Garcinia indica*. It has a light yellow appearance with a faint odour and has a slip melting point of 39°C-42°C. The major fatty acid composition of kokum butter includes 50%-60% stearic acid and 36-40% oleic acid. The major triglyceride present is SOS (upto 70%) which makes it suitable for the production of cocoa butter equivalents (Reddy and Prabhakar, 1994). Due to high content of stearic acid and low content of palmitic acid it has health benefits over other fat alternatives.

The different fat alternatives available for the production of cocoa butter alternatives do not have similar triacylglcerol composition in comparison to cocoa butter. Therefore, to obtain triacylglcerol profile similar to cocoa butter, these fat alternatives need to be blended. Blending means the technique to mix different edible oils and fats in a defined ratio to produce cocoa butter alternatives. The alternatives produced by the process of blending must have similar triacylglycerol and fatty acid composition similar to cocoa butter. Different CBEs with similar melting profile produced by the process of blending have been reported (Kaphueakngam *et al*., 2009). In this study different blends of palm mid fraction and kokum butter were prepared by the process of blending and their characterization was done. Iodine value, slip melting point, fatty acid composition, triacylglycerol composition and melting profile analysis was carried out. These blends can be further used as cocoa butter equivalents (CBEs) in different confectionery products.

2. Materials and methods:

Palm mid fraction (PMF) was procured from B.L Agro Industries Ltd., Bareilly, India. Unrefined and raw kokum butter (KB) was purchased from Urban Platter, Delhi, India. All the chemicals were purchased from Sigma Aldrich (St. Louis, US) and Himedia Ltd. India of AR grade. All the solvents were purchased from Rankem of AR grade.

2.1 Preparation of blends

Palm mid fraction was blended with kokum butter in the ratios of PMF/KB: Blend 1 (90:10), Blend 2 (80:20), Blend 3 (70:30), Blend 4 (60:40), Blend 5 (50:50), Blend 6 (40:60), Blend 7 (30:70), Blend 8 (20:80) and Blend 9 (10:90). A total of nine blends were prepared. All the blends were melted completely at 60°C using water bath before blending.

2.2 Determination of Slip melting point (SMP) and Iodine value (IV)

The slip melting point and iodine value were determined according to the methods of Bureau of Indian Standard (BIS) - IS:548-1964 (2007).

2.3 Determination of Fatty Acid Composition

The fatty acid composition was determined by Gas chromatography (GC). 100-200 mg of sample was weighed in the 26 ml glass tube. 5 ml of Boron trifluoride: Toluene: Methanol (1:1:1) was added to the sample. The mixture was vortexed for 30 seconds and was placed at 60°C for 45 min in water bath. The mixture was cooled down to room temperature. 5 ml of n-hexane and 5 ml of distilled

water were added and vortexed the mixture for 30-40 seconds. 1 g anhydrous sodium sulphate was added. The mixture was added and held down to stand. The upper layer was added in the GC vial for analysis. Agilent GC-FID (8890) was used for the analysis of the fatty acids.

2.4 Determination of Triacylglycerol (TAG) composition

Agilent GC-FID (8890) was used for the identification of the triglycerides in the blends. The TAG analysis was done according to the method of AOAC 986.19, 20th edition, 2006.

2.5 Determination of Melting profile

The melting behaviour was determined by Differential Scanning Calorimeter using Netzsch-DSC 204FI Phoenix 240-12-0109L. Each sample (5-20 mg) was weighed and sealed in a 30µl capacity pan of aluminium. Nitrogen was used as the purge gas. In the analysis, the melting profiles of the samples were determined by heating the samples from 25°C to 80°C.

2.6 Statistical analysis

All experiments were performed in triplicates and were recorded as mean ± standard deviation. For statistical analysis (IBM SPSS Statistics 20.0 Inc. Chicago, IL, USA) was used. For determination of the statistical differences (Oneway ANOVA test and Duncan's test, 5% level of probability, $p<0.05$) were used.

Table 1 : Slip melting point (SMP) and Iodine value (IV) for all blends

	Melting point(°C)	Iodine value (g I_2/100 g fat)
PMF	39.0±0.00	47.07±0.03
KB	35.3±0.57	39.61±0.35
Blend 1	39.1±0.28	48.52±0.48
Blend 2	39.1±0.28	47.43±0.39
Blend 3	39.1±0.28	45.62±0.25
Blend 4	38.3±0.28	44.57±0.53
Blend 5	38.0±0.00	43.28±0.33
Blend 6	37.1±0.28	43.64±0.46
Blend 7	36.1±0.28	41.70±0.30
Blend 8	35.3±0.28	40.82±0.69
Blend 9	34.8±0.28	38.74±0.69

3. Results and Discussion

3.1 Slip melting point (SMP)

The slip melting point (SMP) of palm mid fraction, kokum butter and blends are shown in Table 1. SMP were in the range of 34.8°C to 39.1°C. The melting points of blends were similar to cocoa butter. The slip melting point reported for cocoa butter was 27°C-40°C (Gunstone *et* al., 2011) and 29°C-40°C (Jahurul *et* al., 2013). Small variations were observed in the SMP due to different ratios for blending. The main factors for SMP variation were short and unsaturated chain of fatty acids. The slip melting point of palm mid fraction was 39°C and kokum butter was 35.3°C. Blend 1 had the highest SMP due to high amount of palm mid fraction. A decrease in the slip melting point was observed with increase in the concentration of kokum butter.

Cocoa butter remains brittle and hard at room temperature which is a function of high SMP. More than 50% commercial cocoa butter available in the market melts completely within the temperature range of 27°C-35°C. Blend no. 6 to 9 have desirable melting point of 37.0°C to 34.8°C, which is in the range of melting point of cocoa butter.

3.2 Iodine value (IV)

Iodine value reflects the chemical composition (unsaturation of fats). It is also an important parameter in determination of the thermal properties (Traitler *et al*., 1985). The iodine value of palm mid fraction was 49.07 g I_2/100 g fat which is in the range of the iodine value reported by Jin *et al*., 2018. The iodine value of kokum butter was 39.61 g I_2/100 g fat.

The iodine value of the different PMF and kokum butter blends ranged from 38.7 to 48.5 g I_2/100 g fat. Table 1 shows iodine value of all the blends formulated from palm mid fraction and kokum butter. The lowest iodine value was reported for the blend (10:90). Low iodine value of the blend could be due to the presence of high amounts of saturated fatty acids. The highest iodine value was reported for the blend (90:10) due to the presence of high unsaturated fatty acids (Jahurul *et al*., 2018). With increase in the amount of kokum butter, the iodine value decreased.

The unsaturation of the blends was affected with increase and decrease in the amounts of palm mid fraction and kokum butter. With increase in the amount of kokum butter in the blends the iodine value was closer to the cocoa butter. The iodine value of cocoa butter ranged from 34.40-38.65 g I_2/100 g fat (Chaiseri and Dimick, 1989).

The iodine value reported for Mexican cocoa butter was 35.74 g I_2/100 g fat, Nigerian cocoa butter was 37.33 g I_2/100 g fat, Peruvian cocoa butter was 37.94 g I_2/100 g fat and Brazilian cocoa butter was 37.46 g I_2/100 g fat. Cocoa butter replacers prepared by blending palm oil and palm kernel oil had iodine value in the range of 32.8-42.1 g I_2/100 g fat. Cocoa butter with higher iodine value is softer (Chaiseri and Dimick, 1989).

Table 2 : Fatty acid composition of palm mid fraction, kokum butter and all blends.

	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
PMF	1.07±0.05	48.05±0.70	4.76±0.06	37.56±0.29	8.53±0.18
KB	-	23.09±0.05	36.62±0.05	36.64±0.06	3.60±0.05
Blend 1	-	45.78±0.06	8.22±0.06	37.42±0.04	8.02±0.09
Blend 2	1.06±0.06	42.86±0.06	10.67±0.04	38.10±0.06	7.41±0.06
Blend 3	-	41.29±0.28	14.36±0.09	36.42±0.05	7.12±0.03
Blend 4	-	38.91±0.03	17.25±0.06	36.34±0.06	6.74±0.05
Blend 5	1.11±0.05	34.63±0.05	21.15±0.05	37.71±0.04	5.40±0.05
Blend 6	-	34.45±0.06	22.18±0.08	36.81±0.03	6.07±0.05
Blend 7	-	30.52±0.05	27.37±0.06	35.88±0.07	5.19±0.08
Blend 8	-	27.68±0.07	30.33±0.06	37.93±0.05	4.13±0.06
Blend 9	-	24.94±0.08	34.18±0.07	35.56±0.06	4.19±0.08

3.3 Fatty acid composition

The fatty acid composition of different mixtures of PMF/KB is shown in Table 2. Both unsaturated and saturated fatty acids are present in palm mid fraction and kokum butter. PMF is a rich source of palmitic (48.05%) and oleic acids (37.56%). The other minor fatty acids present are linoleic (8.53%), stearic acids (4.76%). Kokum butter is rich in oleic (36.64%) and stearic acids (36.62%). The other fatty acids present in kokum butter are palmitic acid (23.09%) and linoleic acids (3.60%). The fatty acid composition of PMF and kokum butter individually was not similar to cocoa butter or cocoa butter equivalent. Therefore, blends of PMF and kokum butter in different ratios were prepared to form CBEs. With increase in the ratio of kokum butter, the percentage of palmitic acid decreased from 45.78% to 24.94%. The stearic acid content increased from 8.22% to 34.18% and oleic acid was in the range of 35 to 37%. The variations in the fatty acid composition were observed due to the solubilisation and dilution of different fatty acids in the blends prepared from two different fat sources. The fatty acid composition of cocoa butter reported by Kang *et al*., 2013 was palmitic acid (26.7%), stearic acid (36.8%) and oleic acid (32.2%). The percentage of different fatty acids reported by Ghazani *et al*., 2018 was palmitic acid (27.27%), stearic acid (37.27%), oleic acid (32.90%) and linoleic acid (2.55%). According to Biswas *et al*., 2017 the percentage of fatty acids in cocoa butter were palmitic acid (25.69%), stearic acid (36.15%) and oleic acid (33.24%).

Cocoa butter equivalents (CBEs) are formed through different methods. Blending is one of the common methods for the formation of CBEs. Ghazani *et al*., 2018 reported the fatty acid composition of CBEs. The fatty acid composition of Algal Butter A reported was palmitic acid (3.33%), stearic acid (58.84%) and oleic acid (35.17%) and of Algal Butter B was palmitic acid (3.17%), stearic acid (58.70%) and oleic acid (35.14%). The major fatty acids present in CBE according to Kang *et al*., 2013 were palmitic acid (23.6%), stearic acid (39.5%) and oleic acid (33.5%). The blend (70:30) contains palmitic acid (30.52%), stearic acid (27.37%) and oleic acid (35.88%). The blends 8 and 9 have almost similar fatty acid composition to cocoa butter. The palmitic acid content in the blends was slightly higher than the reported content of palmitic acid in commercial cocoa butter. The stearic acid and oleic acid content of the blends was significantly similar to the cocoa butter.

Table 3 : Triacylglycerol composition of palm mid fraction, kokum butter and all blends.

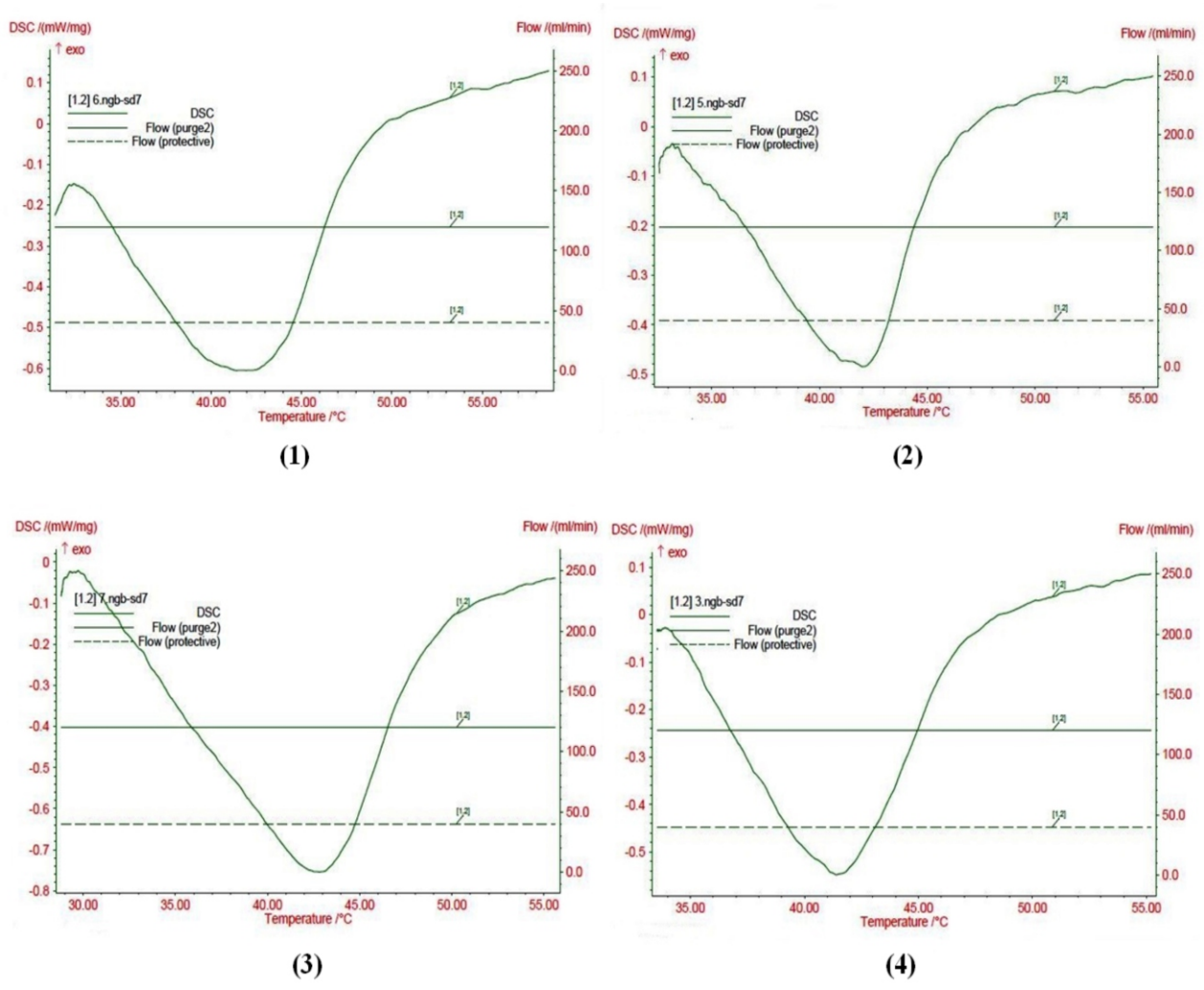
	POP	POS	SOS	POO	SOO
PMF	63.31±0.56	26.07±0.28	0.62±0.09	6.95±0.48	1.90±0.05
KB	16.31±0.44	5.10±0.77	67.02±0.91	2.12±0.15	7.21±0.17
Blend 1	59.11±0.71	24.22±0.30	5.81±0.19	7.12±0.10	2.65±0.08
Blend 2	56.65±0.56	22.70±0.52	10.55±0.41	6.43±0.26	2.84±0.18
Blend 3	51.64±0.23	21.88±0.36	16.54±0.47	6.09±0.10	3.44±0.12
Blend 4	48.24±0.51	20.55±0.55	22.26±0.38	5.39±0.17	4.11±0.10
Blend 5	44.40±0.69	18.19±0.28	28.20±0.17	5.18±0.17	4.53±0.35
Blend 6	39.12±0.10	16.45±0.30	34.92±0.12	4.25±0.22	5.15±0.12
Blend 7	34.22±0.18	13.15±0.15	43.17±0.05	3.37±0.12	5.72±0.07
Blend 8	29.55±0.52	11.33±0.06	49.95±0.09	3.05±0.05	6.12±0.05
Blend 9	23.38±0.07	9.50±0.02	57.43±0.11	2.95±0.04	7.26±0.06

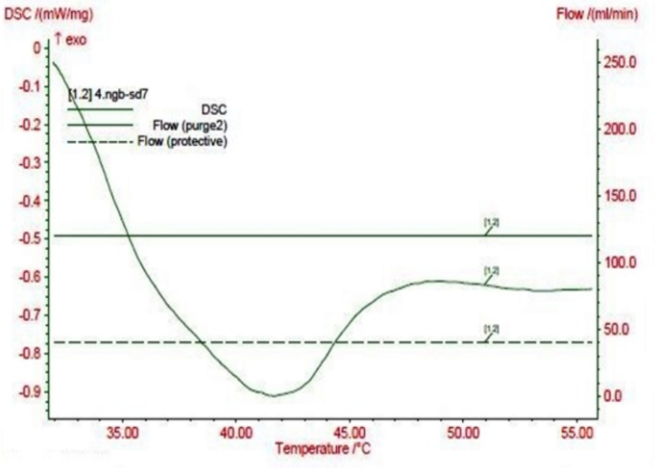
3.4 Triacylglycerol Composition

The triglycerides present in the blends were analyzed using Gas Liquid Chromatography (GLC) and the composition is represented in Table 3. The three major triglycerides identified were POP, POS and SOS. Triglycerides determine the physical attributes of cocoa butter majorly the β polymorphism and melting profile for its applications in chocolate formation. The triacylglycerol composition of cocoa butter reported was POP (15.18%), POS (38.30%) and SOS (27.81%) (Ghazani *et al*., 2018). In commercial cocoa butter, POP (16.9%), POS (42.7%) and SOS (26.7%) had been reported.

Kang *et al*., 2013 reported the triacylglycerol composition of commercial cocoa equivalent (CBE): POP (34.3%), POS (12.1%) and SOS (35.3%). The TAG composition of blends 7 to 9 was quite similar to the TAG profile of the reported CBEs. The POP, POS and POO content decreased significantly with increase in the kokum butter amount in the blends. POP content decreased from 59.11% to 23.38%, POS content decreased from 24.22% to 9.50% and POO content decreased from 7.12% to 2.95%. On the other hand, the SOS and SOO content increased. A 14.72% increase in the POS content was observed and SOO content increased from 2.65% to 7.26%. The POP, POS, POO and SOO content in blends 7, 8 and 9 were almost similar to the triglycerides of CBEs reported by Ghazani *et al*., 2018. The SOS content was slightly higher in blends 7, 8 and 9 in comparison to CBEs.

Figure 1 : Melting profiles of blends (1-9) and cocoa butter (CB)

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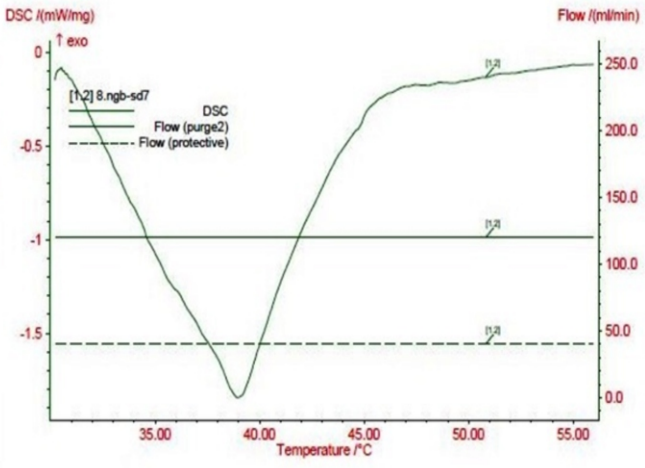
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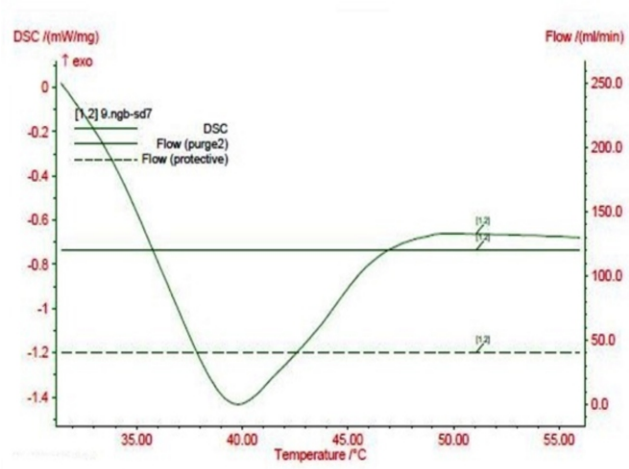
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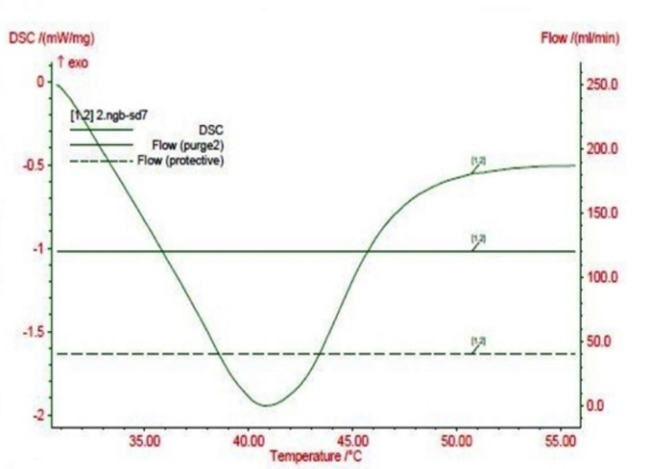
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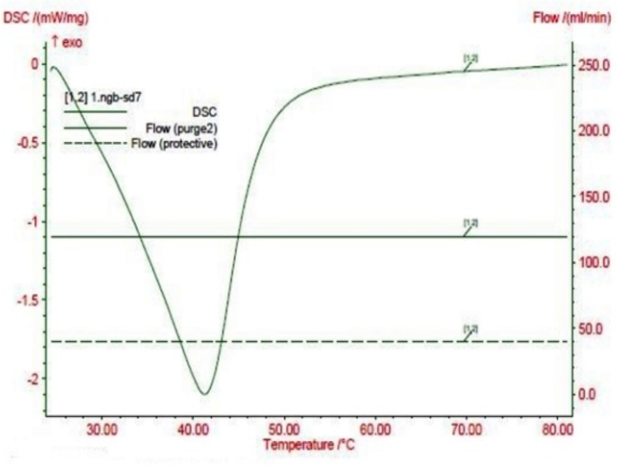
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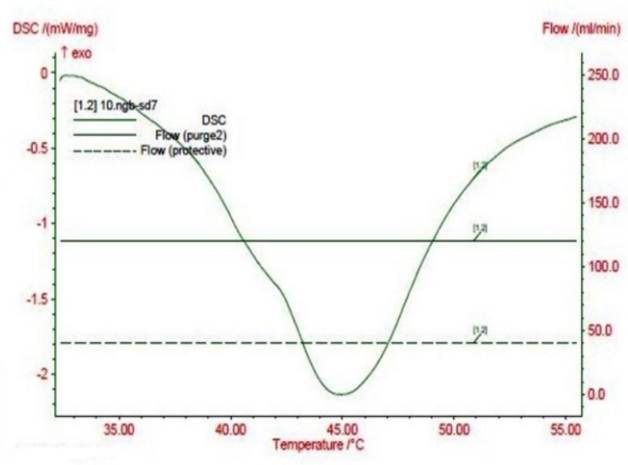
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(CB)

3.5 Determination of Melting behaviour

Different melting profiles of blends were obtained due to difference in the triglyceride composition of both fats (palm mid fraction and kokum butter) used for blending. The changes in the peak melting temperature as a function of blending different amounts of palm mid fraction and kokum butter is shown in Figure 1.

Polymorphism was observed by the triglycerides in the solid state. The six different polymorphic forms are γ, α, β'_2, β'_1, β_2 and β_1. Among these forms, the least stable form is the γ form and the most stable form is the β_1 form (Talhat *et al*., 2015).

4. Conclusion

To produce good quality cocoa butter equivalents (CBEs) palm mid fraction and kokum butter were blended in different ratios and their iodine value and slip melting point were also investigated. The use of kokum butter for the production of cocoa butter equivalents is a novel fat alternative for blending with low cost palm mid fraction. A total of 9 blends were prepared and studied: 70:30 (blend 7), 80:20 (blend 8) and 90:10 (blend 9) blends were found compatible to cocoa butter equivalents. These blends are recommended on the basis of their SMP, iodine value, fatty acid composition, triacylglycerol composition and melting profile.

The blends containing 10-30% palm mid fraction had similar properties, fatty acid composition and triglycerides similar to commercial cocoa butter equivalent. The blending technology is feasible and cheap for production of cocoa butter equivalents.

Acknowledgement

The financial support to Deepali Lehri provided by Department of Science and Technology-Science and Engineering Research Board (DST-SERB), New Delhi; M/S B.L. Agro Industries Ltd., Bareilly, Uttar Pradesh, India; Confederation of Indian Industry (CII), New Delhi under the Prime Minister's Fellowship Scheme for Doctoral Research; and Prof. Aditya Shastri, Vice Chancellor, Banasthali Vidyapith for providing the research facilities are gratefully acknowledged.

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Development of Chromatographic Technique for Identification and Quantification of Oryzanol in Deodorized Distillate Obtained During Refining of Rice Bran Oil

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Keywords: *Oryzanol, Rice Bran Oil Deodorized Distillate, High Performance Liquid Chromatography, Rice Bran Oil, Antioxidant*

Abstract

Oryzanol; a class of compounds found in rice bran oil, is an antioxidant that has various health applications. Most of the oryzanol is lost in the refining process of crude oil and recovered from the waste.

The simplest and widely used technique for the analysis of oryzanol in rice bran oil is UV-visible spectrophotometry. Due to the presence of a high quantity of free fatty acids in deodorized distillate (DD), the analysis of oryzanol is uncertain, leading to inaccurate results. Hence, we have developed a new accurate, and simple method for oryzanol analysis using high-performance liquid chromatography (HPLC) technique to utilize less hazardous solvents. The obtained results were compared with UV-visible spectrophotometry.

The oryzanol content present in crude rice bran oil (CRBO), refined rice bran oil (RRBO), and rice bran oil deodorized distillate (RBODD) was determined by UV-visible spectrophotometry and was found to be 1.29, 0.834, and 0.74 w/w %, respectively. Oryzanol content in CRBO and RRBO determined by the novel HPLC method was 1.01 and 0.76 w/w %, respectively, showing linearity with spectrophotometry. Although the oryzanol content determined in RBODD using HPLC was 0.035 w/w %, which is far less than spectro-photometry, this significant difference was observed in oryzanol content in the case of RBODD, most probably due to higher fatty acid content.

1. Introduction

Rice bran oil (RBO) is popular in various countries like Japan, India, Korea, China, and Indonesia. It is used in cooking and salad dressing due to its high smoke point, delicate flavor, and higher nutritional values. Rice bran oil is extracted using food grade hexane as solvent from rice bran, a by-product obtained from the outer layer (husk) of brown rice kernel during the milling process. Rice bran consists of 17-20 % oil, which is rich in unsaponifiable fraction (4 %), which contains micronutrients like vitamin E complexes, oryzanol, phytosterols, polyphenols, and squalene. In contrast, all other oils have an unsaponifiable matter of less than 1-2 % .

RBO holds good shelf life due to the presence of higher antioxidants in it. Due to its low viscosity, its absorption in food products is lower while cooking, which helps to reduce the overall daily calorie intake. It also has the right balance of mono-unsaturated, poly-unsaturated, and saturated fatty acids . The refining of crude rice bran oil is carried out to remove various impurities to improve the storage stability of the oil. Crude oil contains multiple impurities like phosphatides, carbohydrates, mucilage, proteins, high FFA, waxes, bran fines, pigments, and resins as a gummy substance that imparts color, turbidity, odor, and also leads to high losses during refining processes. These losses can be overcome with careful attention to handling out techniques, start with the rice mill by producing high yields and high-quality RBO .

In most industries, the alkali refining process is used. It results in a lighter color and leads to a higher loss of oil and the nutritional components. This loss can be reduced by using physical refining . The content of oryzanol in the RBO, soapstock, acid oil, and deodorized distillate is in the range of 1.7-2.1 %, 6.3-6.9 %, 3.3-7.4 %, and 0.79 % respectively . Deodorized distillate is the waste product of the deodorization process, which is a complex mixture of free fatty acids, monoglycerides, diacylglycerols, triacylglycerols, sterols, sterol esters, tocopherols, oryzanol, hydrocarbons, and broken down products of fatty acid such as aldehydes, ketones, and oxidized products . Nowadays, the consumption of rice bran oil has increased due to its health benefits. Due to an increase in demand, daily production, and oil refining in India and abroad have increased. This leads to an increased demand for the refining of rice bran oil, which leads to an increase in higher waste generation.

Oryzanol is ferulic acid esters of triterpene alcohols and plant sterols, with several biological effects such as hypocholesterolemic, anti-itching, anti-dandruff, anti-aging, and anti-oxidative . Owing to these properties, it is crucial to recover oryzanol and use it for food/ pharmaceutical/cosmetic and allied applications . Oryzanol content in rice bran oil is analyzed using various methods like UV-visible spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR), Thin Layer Chromatography (TLC) image analysis, and other chromatographic techniques. It should be noted that these methods are only accurate to analyze the oryzanol content in the samples with lower contents of the free fatty acids like rice bran oil and need to saponify or need to carry various pretreatments with solvents to the sample before the analysis . In the previous study, the author quantified the oryzanol by using HPLC with four solvent mixtures for the mobile phase with the gradient condition. Still, it requires more solvent as the run time is more than 30 min, which creates more hazardous waste containing chlorinated compounds . With the change in the solvent, the lambda max of Oryzanol shows a variation . The deodorized distillate obtained from the deodorization during the refining of rice bran oil contains almost 80-85 % of fatty acid and other unsaponifiable matters. It is challenging to separate oryzanol from deodorized distillate due to the higher content of free fatty acids.

In the present work, we have developed a new simple and accurate chromatographic technique to identify and quantify the concentration of oryzanol present in rice bran oil deodorized distillate (RBODD) without saponification or pretreatment to remove FFA and the mobile phase used, which has a lower impact on the environment. We also studied the oryzanol content in the CRBO, refined rice bran oil (RRBO), and RBODD without any saponification, and the results obtained by high-performance liquid chromatography (HPLC) were compared with UV-visible spectrophotometry to identify the best method.

2. Materials and Methodology

2.1. Materials

Rice bran oil deodorizer distillate, discharged from the deodorization step in the rice oil refining process, was kindly provided by Marico Limited, Baddi (Himachal Pradesh). Crude and refined rice bran oil were provided by Muez-Hest India Pvt. Ltd., Mumbai (Maharashtra). Gamma-oryzanol (99% pure) was procured from Sigma Aldrich, and all the other chemicals used for the extraction was extra pure and HPLC grade and were procured from Thomas Baker (Chemicals) Pvt. Ltd.

2.2. Analysis of Sample

2.2.1. Physical and Chemical Characteristics of RBODD

Acid Value (AV) [Te 1a-64], saponification value (SV) [Cd 3-25], unsaponifiable matter (UM) [Ca 6a-40] and moisture insoluble volatiles (MIV) [Ca 2c-25], were determined using standard methods (AOCS) (Firestone, 2009). The fatty acid composition was determined by the Ce 1a-13

(AOCS) method and using the Thermo fisher scientific (GC1000) system. The esterification of fatty acid to methyl esters was done according to the procedure Ce 2-66 (AOCS). The analysis system was a capillary gas chromatograph equipped with a capillary column (BPX-70; 50 m × 0.22 mm, 0.2 μm film thickness). The oven temperature and detector temperature was 240˚C. Injector temperature: 185˚C, detector temperature: 200˚C; carrier gas: nitrogen; split ratio 1:50; injection volume: 1.0 μL. The qualitative composition was determined by comparing the peak retention time of standard fatty acids.

2.2.2. UV-visible spectrophotometric analysis

Standard solution of γ -oryzanol was prepared at a concentration of 5 mg/mL in solvents and afterwards diluted to obtain a series of daily working standards, ranging from 2-20 ppm to construct a calibration curve. Initially, the UV-visible spectrophotometric measurements of the prepared standard oryzanol samples in different solvents and oleic acid were carried out to identify the maximum wavelength and the difference between them. The absorbance of the serially diluted standards was measured at a fixed wavelength, and the calibration curves were prepared by plotting the absorbance against the concentration. The variability of the method was studied by analyzing different concentrations of standard solutions of γ-oryzanol (2-20 ppm).

The accuracy of the method was determined by varying the dilution solvents (n-hexane, n-heptane, acetone, isopropanol, methanol, ethyl acetate) depending on the solubility of oryzanol and the raw material. The confirmation of the correct analysis method was evaluated by spiking known amounts of the standard oryzanol sample to the CRBO, RRBO, RBODD sample solutions, and oleic acid separately. A homogenized solution of RBODD was prepared by heating it using a water bath at 40˚C for 5 min. The homogenized sample (about 200-500 mg) was dissolved in 10 mL solvent, and the absorbance spectra of samples were detected at an optimum wavelength in 1 cm cell using a UV-visible spectrophotometer (Agilent- 8453), and the oryzanol content in the distilled fatty acid was quantified against the standard curve.

2.2.3. HPLC Analysis

Standard solution of γ -oryzanol was prepared by dissolving 20 mg of oryzanol standard in 100 mL of isopropyl alcohol (IPA): acetonitrile (ACN) [50:50 v/v] solution and afterwards diluted to obtain a series of working standards, ranging from 10-200 ppm to construct a calibration curve. RBODD was heated to 40˚C in a water bath and stirred to make a homogenized mixture as it was in a semi-solid state at room temperature (25 ± 2˚C). A known amount of homogenized DD was diluted in IPA: ACN (50:50 v/v) solvent mixture. The sample was mixed, degassed, and filtered (0.22 μm pore size, Nylon filters) before the analysis.

The estimation of oryzanol was carried out using an HPLC system of Thermo Fisher Scientific, coupled with an injector with a 20 μL sample loop. The sample (10 μL) was injected into an HPLC column for the detection of oryzanol. The column used was a Hypersil Gold C18 (250 mm × 4.6 mm, 5 μm). The mobile phase was ACN: Methanol (MeOH): IPA in the ratio 50:25:25 (v/v/v), respectively, which had a run time of 10 min with a flow rate of 1 mL/min. The oryzanol content was detected at a wavelength of 320 nm using a UV detector, and the column temperature was maintained at 50˚C during the run and quantified based on retention time and peak area/height of a standard sample. The standard stock solution with variable dilution was also analyzed by the same method, and calibration curves were made by plotting the area under the curve against concentration. The variability and the accuracy of the method were studied by analyzing different concentrations of standard solutions and by spiking known amounts of the oryzanol standard compounds to the CRBO, RRBO, RBODD, and oleic acid separately. Minor changes in the mobile phase composition were carried out to determine the robustness of the methods.

3. Results and Discussion

3.1. Physio-chemical Properties of RBODD

The RBODD is a brownish yellow color solid at room temperature, containing 85 % of free fatty acids in it (Table 1) and the fatty acid composition of the RBODD shows that oleic acid is the primary fatty acid, and contains saturated, mono unsaturated, polyunsaturated fatty acid 16.3, 36.2, and 47.8% respectively in the material. The FFA content was calculated from the acid value and the saponification value, where we considered the molecular weight of the sample as 275 g/mol based on the fatty acid composition.

3.2. Analysis of Oryzanol Content Using UV-visible Spectrophotometer

3.2.1. Study of absorption behavior of oryzanol in different solvents by plotting a standard curve

Reputedly, hexane has been used as the extraction solvent for the extraction of rice bran oil due to high recovery. Therefore, other solvents were compared to determine the accuracy in the determination of oryzanol content. Table 2 shows that as the solvent changes, the lambda max (λ_{max}) of the oryzanol changes from 314 to 327 nm in solvents like n-hexane, n-heptane, ethyl acetate, acetone, and methanol as per solubility of oryzanol and material. This method of detection of oryzanol using a UV-visible spectrophotometer is considered as one of the rapid and easiest methods . The maximum wavelengths (λmax) and standard calibration of oryzanol analysis in different solvents have been summarized in Table 2, which are similar to previously published reports .

Figure 1 shows the absorbance of Oleic acid, CRBO, RRBO, and DD at wavelength 315nm, where the initial absorbance of Oleic acid, CRBO, RRBO, & DD is 0.14, 2.0, 2.2, and 0.42 AU, respectively, without the addition of oryzanol standard. To check the absorbance behavior of oryzanol, in all the samples known amount of oryzanol was added, which showed linearity in the absorbance for all samples. Oleic acid was used as the standard fatty acid for the study as it is the primary fatty acid present in RBODD . Oleic acid shows absorbance at 0.144 AU at a concentration of 20 mg/mL, which is negligible compared to oryzanol absorbance of 1.692 AU at just 2 mg/mL of its concentration at 315 nm of wavelength.

3.2.2. Verification of UV-visible spectrophotometric method for the analysis of oryzanol content in the CRBO, RRBO, and RBODD:

Pure oryzanol in heptane gives a distinct λ_{max} at 315 nm (Figure 2(a)). Mixed with DD, however, the peak at 315 nm disappears, and a different absorption spectrum characteristic of the combination of the two components is obtained. In the spectrum of the mixture, oleic acid shows absorbance at 291 nm, and their intensity of which increases with an increase in the concentration. By using the values of the absorbance obtained at a wavelength of 315 nm and values of the concentrations used, we plotted the standard curve and obtained the linear equation $y = 37.533x + 0.012$ ($R^2 = 0.987$), which we used for the calculation of the oryzanol content in the sample. Table 5 shows the actual concentration of the oryzanol present in the CRRBO is 1.29 ± 0.04 %, which reduces to 0.834 ± 0.07 % in the RRBO during the refining process, and the RBODD contains 0.74 ± 0.05 % oryzanol.

3.3. Oryzanol Content by HPLC Method

HPLC is considered one of the widely used techniques for the detection of various compounds; it can accurately and precisely determine the values with a minimal standard deviation of 0.03% . Hence, for the confirmation of the oryzanol present in RBODD, the HPLC method was used and compared with the results obtained by UV-visible spectrophotometer . Table 3 shows the solvent peak at 3.02 min, as we injected only the solvent system, which was used for the preparation of the sample. The standard oleic acid sample showed four peaks at 3.11, 3.20, 3.84, and 4.10 min, whereas the oryzanol standard run showed two peaks at 3.12

min and 5.17 min. From Table 3, we can say that the first peak observed in the oryzanol standard and DD sample was of the solvent, whereas the other peak obtained with the four compounds from 4.8-5.5 min was indicative of the presence of oryzanol (Figure 3).

3.3.1. Verification of the Oryzanol Peak

As observed in the HPLC chromatogram, the fatty acid showed a peak at 5 min retention time, which is the same retention time as that for standard oryzanol. These results correlate with the results obtained by the author with rice bran oil as per the previous study (Joshi et al. 2016). To confirm if the peak was of oryzanol or the fatty acid, we spiked oryzanol standard (0.5 % and 1 %) in the DD sample and analyzed using HPLC. The result showed an increase in the peak height and area under the curve at 5 min retention time. This confirms that the peak at 5 min retention time in the DD was of oryzanol only (Table 4). Verification of the actual peak of the oryzanol at particular retention time in CRBO and RRBO was done by adding a known amount of oryzanol in samples followed by the analysis for their area under the curve. Figure 4 shows that the initial area under the curve of oryzanol present in CRBO and RRBO was 2.93 mAU*min and 2.1 mAU*min, respectively, which increases linearly after adding external oryzanol. These results confirmed that the peak obtained at retention time 5 was of the oryzanol, and this method could be used to analyze the concentration of oryzanol.A known concentration of oryzanol was chosen and analyzed using HPLC to obtain the area of that respective concentration at the respective retention time. By using the values obtained of the area under the curve and the actual concentration, we plotted the standard curve and obtained the linear equation $y = 939.7 x - 2.0514$ ($R^2 = 0.999$), which we used for the calculation of the oryzanol content in the sample. We also used another single point calculation formula to calculate the oryzanol content in DD using the total area. The result obtained is shown in Table 5. The formula mentioned below was used for calculating the percentage of oryzanol present in the material analyzed using HPLC.

By using the standard curve method and the single point formula, we calculated the oryzanol content in the CRBO, RRBO, and RBODD samples (Table 5), which shows the oryzanol content in the DD sample around 0.035-0.042 %. The oryzanol content was found to be 0.74 ± 0.05 % and 0.035 ± 0.002 % using spectrophotometric and HPLC methods, respectively.

1. Conclusion

Owing to the lower amount of free fatty acid, the detection of Oryzanol by UV-visible spectrophotometric method can be considered as the easiest method for oil samples. On the other hand, in case of RBODD, which shows high fatty acid content, the λ_{max} for fatty acids is 291 nm with an impact on the behavior of the oryzanol peak at 315 nm. The oryzanol content in CRBO and RRBO analyzed using UV-visible, and an HPLC method was nearly the same, only the difference in the oryzanol content in the DD sample. The correlation coefficient for spectrophotometry is $R^2 = 0.987$ and for the HPLC its value i.e. $R^2 = 0.999$ shows that the HPLC method is more accurate than UV-Spectrophotometry. Based on the results obtained in this study, we propose that the HPLC method is only the technique which is more sensitive and highly accurate for the analysis of oryzanol present in distilled fatty acid.

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The authors like to thank M/s. Marico Ltd., Baddi, and M/s. Muez-Hest India Ltd., Mumbai, for their generosity for providing the rice bran oil and deodorized distillate samples. The authors would like to thank M/s. Marico Ltd. and Prime Minister's Fellowship Scheme for Doctoral Research, a public-private partnership between the Science & Engineering Research Board (SERB), Department of Science & Technology (DST), Government of India (GOI), and Confederation of Indian Industry (CII).

List of Tables:

Table 1: Physio-chemical properties of DD

Parameter	**Value**
Physical Appearance	Brownish yellow color, solid at RT
Acid Value (mg KoH/g)	170 ± 1.4
Free Fatty Acid (%)	85.2 ± 0.7
Saponification Value (mg KoH/g)	223 ± 2.0
Unsaponifiable Matter (%)	3.67 ± 0.1
MIV (%)	1.80 ± 0.2

Table 2: Maximum wavelength (λ_{max}) and standard calibration curve of oryzanol in different solvents

Solvents	**Experimental λ_{max} (nm)**	**Calibration Curves**	
		Regression Equations*	R^2
n-hexane	314	y = 43.1x + 0.00987	0.999
n-heptane	315	y = 37.5x + 0.0117	0.987
Acetone	327	y = 42.6x + 0.0217	0.999
Ethyl Acetate	321	y = 45.6x – 0.0431	0.999
Methanol	325	y = 42.5x – 0.0235	0.997

*y = absorbance, x= concentration (mg/mL)

Table 3:HPLC analysis for dilution solvent, oleic acid and DD sample

Sample Name	**Concentration (mg/mL)**	**Retention Time (min)**	**Area (mAU*min)**
Solvent (IPA:ACN; 50:50 v/v)	-	3.02	1.17
Oleic Acid	20	3.11	0.990
		3.20	0.491
		3.84	0.534
		4.10	0.465
Oryzanol Standard	2	3.12	1.41
		5.17	16.1
RBODD	2	3.12	15.4
		5.31	17.0
		7.21	5.06
		7.60	3.02

Table 4: Retention time and the area under curve of DD with or without added oryzanol

DD Concentration (mg/mL)	**Oryzanol Added (%)**	**Retention Time (min)**	**Area Under Curve (mAU*min)**
2	0	5.18	16.8
2	0.5	5.11	77.2
2	1.0	5.12	150

Table 5: Percent Oryzanol content in the CRBO, RRBO and RBODD

Sample name	**Oryzanol content (%)**		
	UV-visible Spectrophotometer	**HPLC**	
		Standard curve equation	Single point formula
CRBO	1.29 ± 0.04	1.1 ± 0.2	1.01 ± 0.02
RRBO	0.83 ± 0.07	0.9 ± 0.2	0.76 ± 0.06
RBODD	0.74 ± 0.05	0.0424 ± 0.003	0.035 ± 0.002

List of Figures:

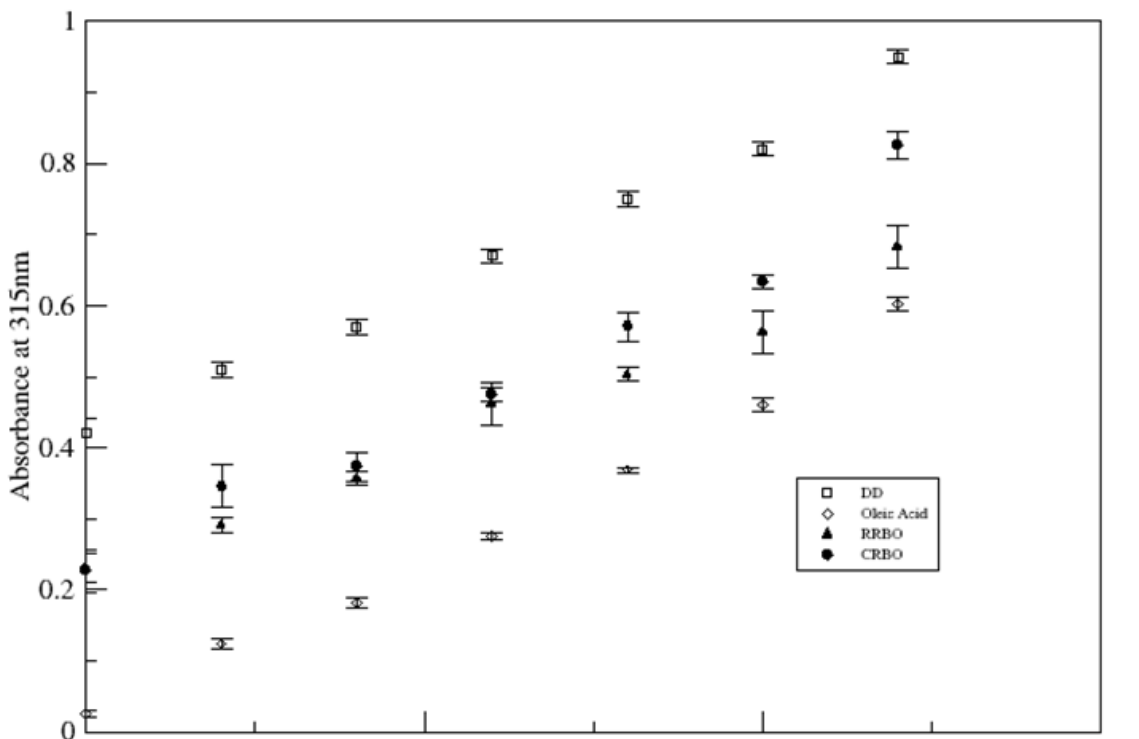

Figure 1: Absorbance of DD, Oleic acid, CRBO and RRBO with or without added oryzanol

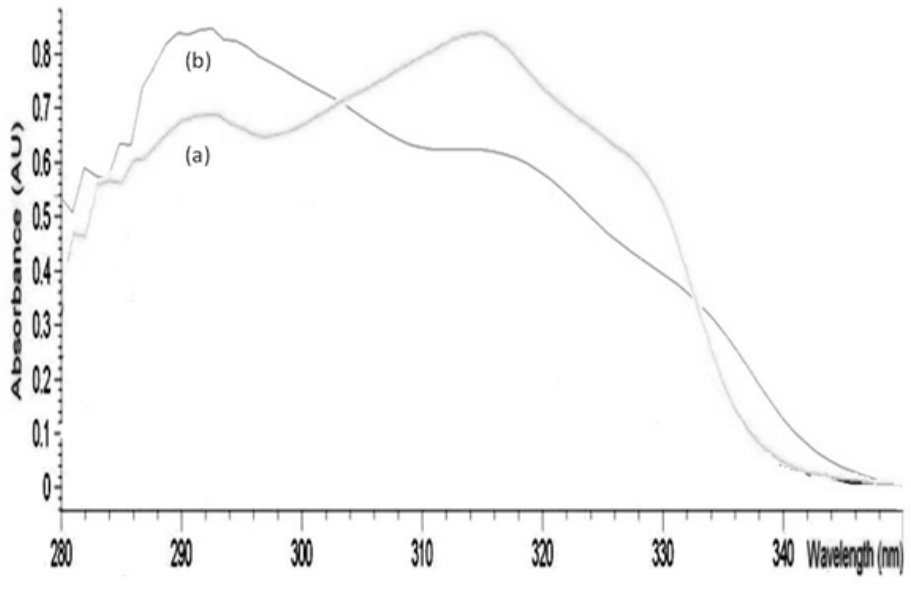

Figure 2: UV peaks for (a) oryzanol standard and (b) DD sample in n-heptane at 315nm

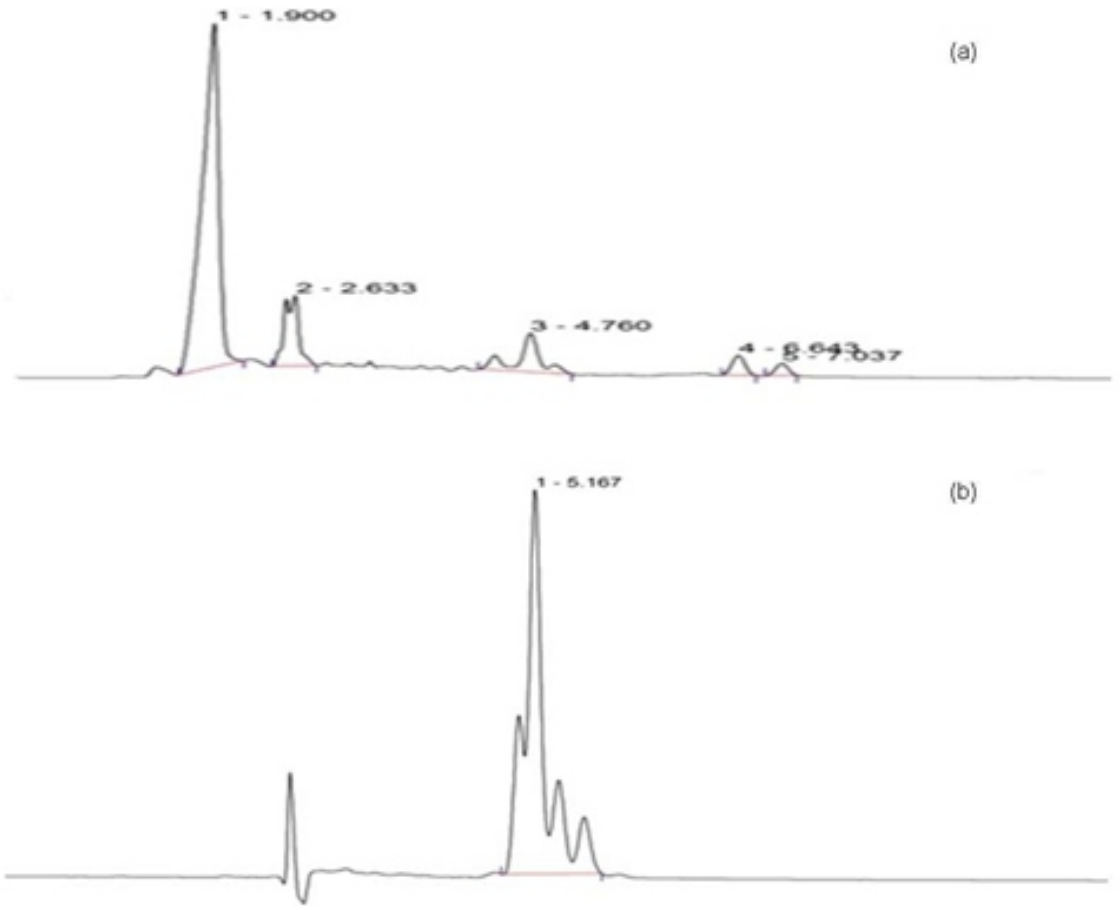

Figure 3: HPLC peak for (a) DD sample and (b) oryzanol standard

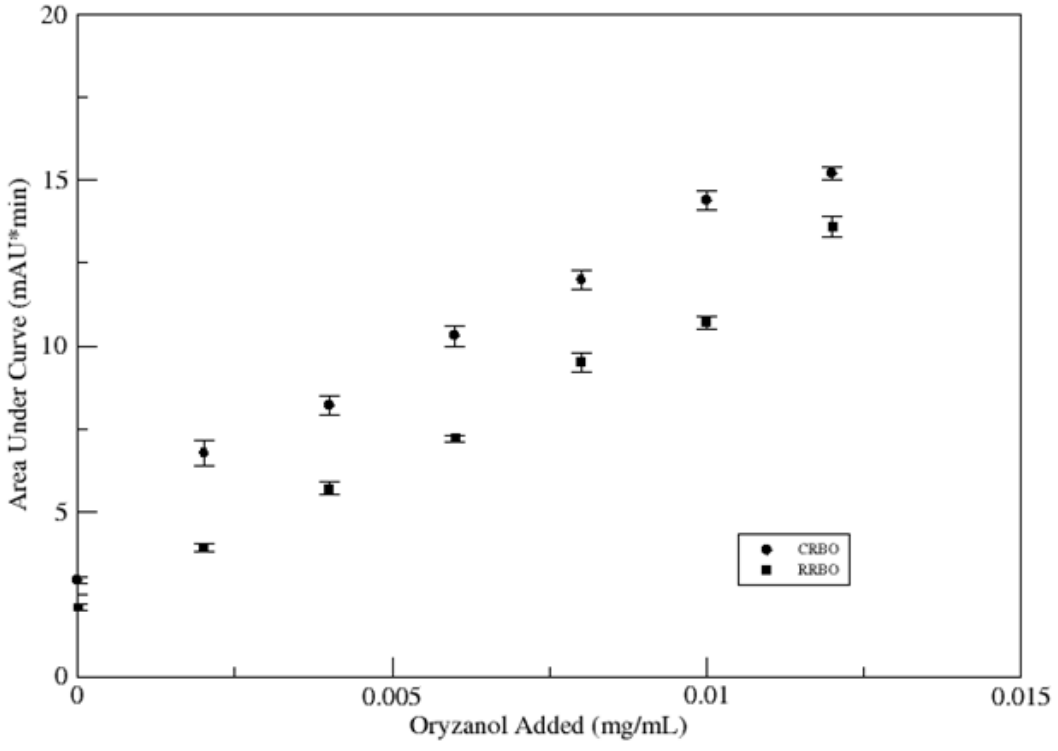

Figure 4: Area under curve for CRBO and RRBO with or without added oryzanol

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Thermal properties of Castor oil for the production of chemicals

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Keywords: *Castor seed, Fatty acid composition, Ricinoleic acid, Thermal sability*

Abstract

Commercially important crop of castor beans grown mainly in western India especially in Gujarat state for the production of lubricants, coating materials, and soaps. Replacing petroleum based raw material makes castor oil more valuable. For characterization, the castor seeds are collected from different locations of India, some are the wild collection. Oils are isolated from these seeds using soxhlet apparatus. The fatty acid profiles and thermal stability studies have been carried out. The chemical compositions of fatty acids are quite different and Gujarat variety is superior with a high percentage of ricinoleic acid (86.9%). The Castor oil was de volatilised at 400 C, whereas the vegetable oil Winged bean showed the degradation at 450C. Due to low volatilization temperature with high percentage ricinoleic acid is favourable for the production of chemicals such as sebacic acid and capyl alcohol, etc. Further, this oil is used for the preparation of free fatty acids (FFA), which can be used for different commercial applications. Utilization of these high percentage of ricinoleic in various industries is the main focus.

1. Introduction

Castor bean is an alternative and renewable source of raw material for industrial applications. Castor seed (*Ricinus Communis*) is one of the major non-edible oil seeds cultivated in arid and semi-arid regions. Oil content in castor seed varies from 45% to 50%, and the major compounds in this oil is ricinoleic acid (80-85%). Castor oil and its derivatives (sebacic acid,capryl alcohol, etc) have wide ranging applications in the manufacturing of soaps, lubricants, hydraulic and brake fluids, paints, dyes, coatings, inks, cold resistant plastics, nylon, pharmaceuticals and perfumes. Total castor cultivation in India is 7,70,000 ha and produced 11,27,000 ton of castor seed in the year 2018-19. Total castor oil export stands 5,39,962 ton of oil (Value Rs 5,709.83 crore) in the year 2019-2020, and 5,71,985 ton of oil (Value Rs 5561.60 crore) in the year 2018-2019 [Anon, 2021]. India occupied the first position in castor seed production with 90% world's demand and exports the castor oil and meal to China, the US and European countries. Whereas China and Brazil produce only 50,000 MT and 45,000 MT of seeds and occupied second and third positions in the World map. The major castor seeds producer states are Gujarat (9,35,000 Ton), Rajasthan (1,45,000 Ton) and Andhra Pradesh & Telangana (25,000 MT) in the year 2018-19, respectively. The castor meal produced 5,05,194 MT in the year 2019-20 (average price is Rs 120 per kg). Though, it is a commercial vegetable oil of Indian origin, so that the value addition is very important for getting a better economic return to the farmers and entrepreneurs.

Castor oil is composed of about 85% of ricinoleic acid (12-hydroxy-9*Z*-octadecenoic acid). Due to an unique structure, this compound is easily derived to

high value chemicals. Though India has a monopoly for the production of castor oil, but in value addition, China produced over 20,000 MT sebacic acid through alkali pyrolysis of ricinoleic acid [Pavaskar M, Kshirsagar A, 2013 & Ogunniyi DS], representing 90% of the global trade. The front-line Chinese companies produced this chemical are Hebei Kaide Biomaterial Co. Ltd., Hebei Hengshui Jinghua Chemical Co. Ltd., Casda Biomaterials Co. Ltd, etc. In the year 2015, the highest consumed derivatives of castor oil are sebacic acid, which produced by crackingof caustic (alkaline) ricinoleic acid methyl ester in the presence of phenol, which is used as a thinning agent at 280 C [Yu S, Cui J et. al.]. Sebacic acid is an open chain dicarboxylic compound (1.10 - decanedioic acid) with wide commercial potential. It is an industrial feedstock used for the preparation of lubricants, cosmetics, plastics, etc. Recently, Jeon et al. developed a novel engineered yeast strain of *Candida tropicalis* for producing an exceptionally high percentage(46%) of sebacic acid using 50 L bio-fermenter set-up [Jeon W-Y, Jang M-J et. al.]. This bio-fermentation approach is greener and it can avoid the alkali pyrolysis process practiced by the industry.

Though castor contributes around 0.15% of the vegetable oil produced globally, but it is the only source of hydroxylated fatty acids. This crop is Globally important because of high oil content (480 g/kg of seed), ricinoleic acid content (900 g/kg of oil) and higher yield (1250-2500 L/ha) [Severino LS, Auld DL et. al.]. It is highly drought and salinity resistance crop. This is the reason in the present research objective to collect the sample of castor seeds from various geographical areas of India.Compared their quality and thermal stability for further possible value additions. Further, the free fatty acids (FFA) has been isolated including the ricinoleic acid for industrial applications.

2. Materials and Methods

2.1. Seeds collection and extraction of fatty oils

The seeds of different geographical locations such as Gujarat, Rajasthan, Andhra Pradesh and Uttar Pradesh (Basti, Lucknow; Varanasi) were collected. Then dried and ground the seed using domestic grinder and this power was used for the isolation of fatty oils. About 200 g of seed-powder was loaded in Soxhlet apparatus and then extracted with hexane for 12 h. After extraction, filtered the solution and evaporated the solvent in a rotary evaporator under 150 mbar vacuum. These oils were stored in a refrigerator for further analysis.

2.2 GC-FID and GC/MS analysis

Qualitative GC analyses of FAMEs were evaluated using Agilent 4890D Gas Chromatograph furnished with a flame ionization detector (FID). The column used for this analysis was carried out in a Carbowax column of 60 m x 0.32 mm x 0.25 μm film thickness The carrier gas was hydrogen with 20 psi column head pressure. Sample (0.1 l) was taken in a micro-syringe and applied into the GC injection port with 40:1split ratio. The FID and injector temperatures were kept at 250 C with column temperature was programmed as follows: 160-240 C (2 C/min), with initial and final hold times of 1 and 10 min, respectively. Further, GC/MS was carried out using Perkin Elmer auto system XL GC interfaced with a Turbomass Quadrupole mass spectrometer followed the above oven temperature program. The source, transfer line and injector temperatures were set at 250 C; electron ionization energy 70 eV; Hegas at 10 psi constant pressure; and mass scan range of 50-550 amu. The compound characterization was accomplished on the basis of retention time, elution order, mass spectra and calculated relative retention index.

2.3 Thermo-gravimetric analysis

TGA evaluations of oil were analysed using Mettler-Toledo TGA/DSC 1 Star system. For this, about 0.7-8.5 mg oils were taken in the silica crucible and run the analysis in the following temperature program of 50-700 C (10C/min), under a nitrogen flow rate of 40 ml/min as a purge gas. The acquired TGA results were further evaluated after

taking the first derivative (DTG) for clear interpretation.ThisTGA profile was compared with an edible vegetable oil such as winged bean oil. Further, their first derivative (DTG) was taken for determining the devolatilization temperature.

2.4 Isolation of Free Fatty Acids (FFA)

Firstly, for the isolation process, 20 g of castor oil was reflux with alcoholic KOH for 1 h, after removal of alcohol, 96 ml of acidified (HCl) was added to maintain the pH 1 for hydrolysis of triglycerides to FFAs. Further, the FFAs were isolated in ethyl acetate and dried the ethyl acetate solution over $MgSO_4$, and thereafter removed the solvent to obtain FFAs. The composition of FFAs was determined as per the above GC-FID procedure.

3. Results and Discussion

The seeds are looking dissimilar and there is a clear distinction in between the commercial seeds and wild collections (Fig 1a). It is also observed that the commercial oils are faint yellowish colour, whereas the wild collection is very dark-raddish colour (Fig. 1b). From the above GC-FID and GC/MS analysis indicated that Gujarat and Rajasthan seeds were accumulated a higher percentage of oil with 46.1% and 45.9%, respectively. Besides, the ricinoleic acid was 86.9% and 84.7% in Gujarat and Rajasthan seed-oil, respectively. The comparative chemical compositions of different locations are presented in Table 1. In terms of yield and ricinoleic acid content, the Basti (Lucknow) and Varanasi varieties were inferior in quality. Comparing the yield of oil, the cultivated seeds were contained significant percentage of oil such as Gujarat (46.1%) followed by Rajasthan (45.9%) and Telangana (40.2%). The seeds of wild varieties collected from Basti (Lucknow) and Varanasi of Uttar Pradesh gave poor oil yield such as Basti (34%), while Varanasi (24%).

The lipid profile showed the highest percentage of ricinoleic was reported in the Gujarat seed (86.9%) followed by the Rajasthan sample (84.7%). Whereas, Telangana state sample was contained 83.5% of ricinoleic acid. Patel et al., also reported comparable but slightly high ricinoleic acid (90%) [7]. The highest production of castor seed, approximately 86% is contributed from the state of Gujarat only. So, the superior percentage of ricinoleic acid containing castor seeds is available in the large fraction of the total production of castor seed in India [7].

The fatty acids like linoleic acid (18:2) ranges from 5 to 6.9%, and Patel et. al. reported 4% of this fatty acid. Similarly, the present analysis claimed 4.9-7.5% of oleic (18:1) as compared to 3% by the earlier reported value [7]. But, the stearic acid is almost comparable with the above literature of around 1%, and wild varieties contained slightly higher amounts (1.6%). Overall unsaturated fatty acid percentage was found to be highest in the Gujarat state sample (96.8%), while others also have a good percentage of unsaturated fatty acids ranges from 94.8-91.3%. The saturated fatty acid percentage ranges between 2.9-7.1%.

Thermo-gravimetric analysis of castor oil in comparison with winged bean oil is presented in Fig. 2a and 2b. The fatty oils of Gujarat, Rajasthan, Hyderabad and Basti (Lucknow) are very close to their de-volatilization behaviours. It is noticed that the wild seed collected for Varanasi is very poor quality and it de-volatilized at lower temperature (Fig 2a).

Whereas the ricinoleic acid content was poor in Uttar Pradesh collected wild samples (76.9-80.0%). It is evident from this figure that the degradation temperature of castor oil is around 400 C, and it is nearly 50 C less as compared to winged bean vegetable oil (Fig. 2b). Winged bean is well known vegetable oil of soybean family. Therefore, castor oil has an advantage for the production of chemicals through thermo-pyretic route as compared to other vegetable oils. Further, the preparation of fatty acid from crude oil is efficient and it is almost close to the theoretical yield [8]. This fatty acids might be used as feedstocks for the chemical, bio-lubricant, and cosmetics industries.

4. Conclusions

The wild seeds were clearly distinguish from the commercial varieties by their colour. The chemical analysis of these oils suggested that Gujarat origin seed variety displayed superiority in terms of oil yield (46.1%) and ricinoleic acid content. Gujarat seed sample contained 86.9% of ricinoleic acid followed by Rajasthan (84.7%). The wild collected varieties of Uttar Pradesh were inferior in quality in terms of yield as well as percentage of ricinoleic acid. This could also be because of soil quality and environmental conditions available in that state.

The GC analysis proves the lipid profile of castor oil is suitable as feedstock to the chemical industries. It also has the flexibility to introduce saturation or unsaturation among the fatty acid composition according to the requirement of the industries. From thermo-stability study, it was found that castor oil more suitable for the production of chemicals through pyrolysis route as compared to the common vegetable oils (winged bean).

In the present research, a protocol for the isolation of ricinoleic acid from the castor oil has been developed. This improve ricinoleic acid is further used for the preparation of commercially important derivatives. Ricinoleic acid is highly demanded in precious deodorants and fine cosmetic applications.

Acknowledgements

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Fig 1a: Seed of different origin; A: Gujarat, B: Rajasthan, C: Varanasi, D: Basti (Lucknow)

Fig 1b: Seed oil colour A: Varanasi, B: Basti (Lucknow), C: Rajasthan, D: Gujarat

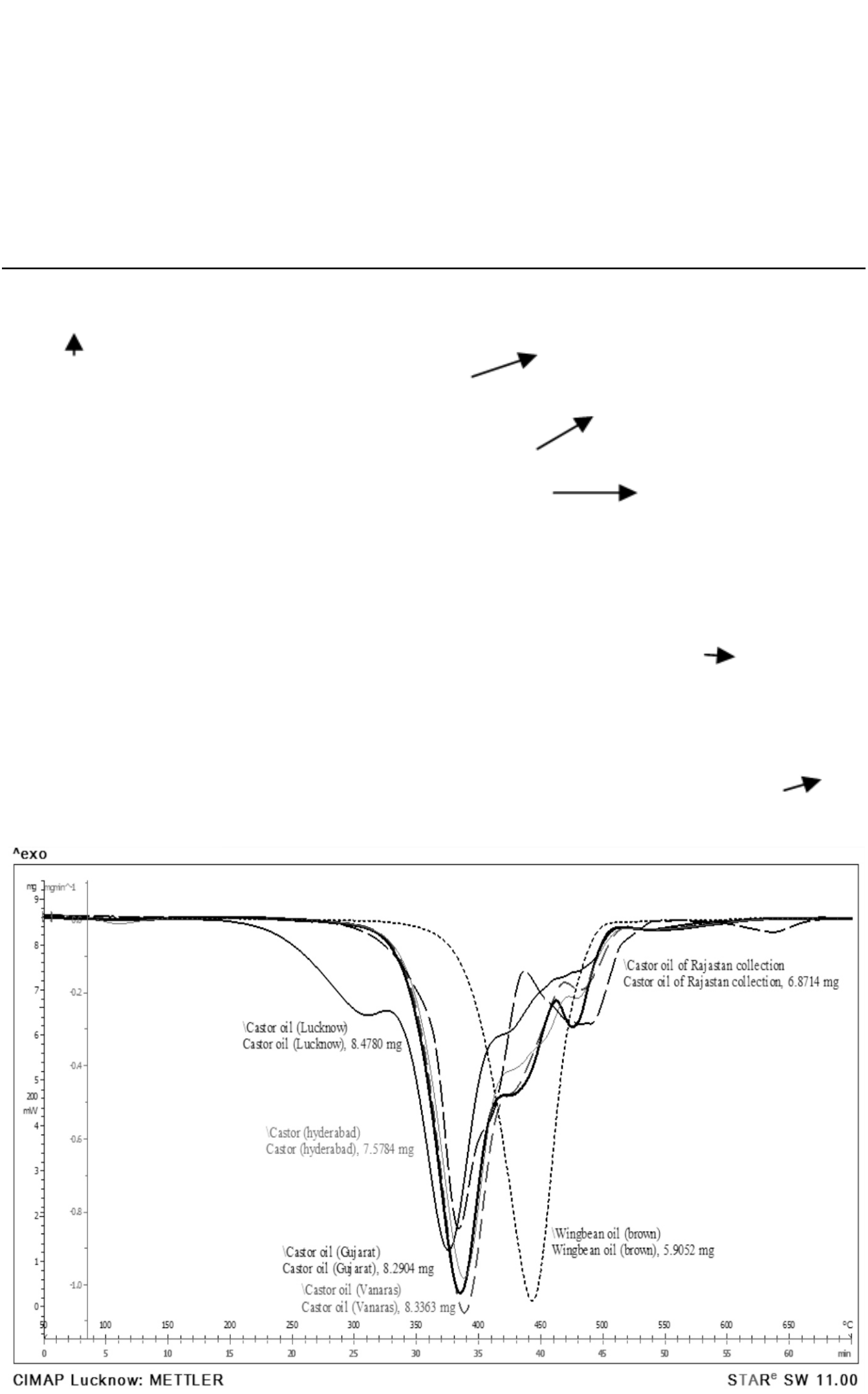

Fig 2b: TGA analysis of castor oil in compared to winged bean oil

Table 1: Composition of Castor oil collected from different locations

Composition	Gujarat	Rajasthan	Telengana	Varanasi (UP)	Basti (UP)
Yield of the oil (%)	**46.1**	**45.9**	**40.2**	**24**	**34**
Palmitic acid (16:0)	1.1	1.2	1.5	1.7	1.6
Linoeic acid (18:2)	5.0	5.1	5.8	6.3	6.9
Oleic acid (18:1)	4.9	5.0	5.3	7.0	7.5
Stearic acid (18:0)	1.2	1.3	1.4	1.6	1.5
Arachidic acid (20:0)	0.6	1.9	1.9	2.7	4.0
Ricinoleic acid (18:1 OH)	86.9	84.7	83.5	80.0	76.9
Saturated fatty acids	2.9	4.4	4.8	6.0	7.1
Unsaturated fatty acids	96.8	94.8	94.6	93.3	91.3
Total	**99.6**	**99.2**	**99.4**	**99.3**	**98.4**

Research Roundup July-September 2020

Estimating Oil and Protein Content of Sesame Seeds Using Image Processing and Artificial Neural Network

Image processing has many applications in different fields of agriculture. The present study by Parsaeian et al., is aimed to use image processing techniques and artificial neural networks (ANN) to estimate oil and protein contents of sesame genotypes without the use of time consuming and costly laboratory methods. The proposed method accurately estimates the parameters in sesame seeds without destructing the genetically valuable material. In this study, a set of 138 morphological features were extracted from the digital image of 125 sesame seed genotypes. A multilayer perceptron (MLP) ANN was then employed to estimate oil and protein contents and determine the relationship between estimated values and laboratory measured values. The efficiency of this model was compared to radial bases function (RBF), extended RBF (ERBF), GRNN, M5□Rule, M5 Tree, support vector machine regression, and linear regression models [J. Amer. Oil Chem. Soc. 97 (7), 409-423(2020)]. Results showed that MLP performed better in estimating qualitative parameters of seeds in the sesame germplasm. The model estimated oil content with an root mean square error (RMSE) of 2.13% (the accuracy of 97.87%) and an R^2 of 0.93. Protein content was estimated by an RMSE of 0.378% (the accuracy of 99.62%) and an R^2 of 0.96.

Oxidative Stability of Chia (*Salvia hispanica* L.) and Sesame (*Sesamum indicum* L.) Oil Blends

Chia and sesame oils are important sources of essential fatty acids; however, their ω-3:ω-6 proportions do not comply with nutritional recommendation. A feasible approach to improve the ratio is to blend different oils, but only after understanding physical and chemical changes of the new matrix. Objective of the investigation by Rodriguez was to determine the physico□chemical characteristics and the oxidative stability index (OSI), using the Rancimat method, of chia-sesame oil blends. The four ω-3:ω-6 blends tested (1:4, 1:6, 1:8, and 1:10) were exposed to temperatures of 110, 120, and 130 °C. The OSI values of the mixtures varied between 6.24–8.08, 3.07–4.00, and 1.62–2.01-hours for each temperature, respectively. In addition, their mean activation energy, enthalpy, entropy, and Q_{10} were 88.4 kJ/mol, 85.2 kJ/mol, −41.1 J/mol-K, and 2.0[J. Amer. Oil Chem. Soc. 97 (7), 729-735(2020)]. Finally, a shelf life prediction performed at 25°C indicated stability times between 80 and 123days. Therefore, combining chia and sesame oils produced blends with a good balance of essential fatty acids.

Properties of Coated Controlled Release Diammonium Phosphate Fertilizers Prepared with the Use of Bio-based Amino OilIn an effort to enhance the efficiency of fertilizer use and minimize their negative impact on the environment, a novel biomass based, functional controlled-release fertilizer was used by Sofyane et al., to improve nutrient use efficiency and increase crop production systems for more sustainable agriculture practices. Here, bio-based amino-oil (Priamine) mixtures were proposed as an outer coating with different layers for the control of phosphorus release from diammonium Phosphate (DAP).

These hydrophobic coatings conferred excellent barrier properties and flexibility to coatings.

The morphological characterization of the coated fertilizer was performed by scanning electronic microscopy (SEM), electronic diffraction X-ray (EDX) and mapping, and revealed the formation of a cohesive film and a good adhesion between DAP fertilizer and coating film. The release rate of nutrients (phosphate) in water was investigated by UV–Vis spectrophotometer. The effect of coating thickness was investigated on release time and diffusion coefficient of phosphor release in distilled water. Release time increased with the coating thickness. The diffusion coefficient of nutrient release decreased with the coating thickness. Compared with uncoated granule which is totally solubilized after less than 2 h, the *P* release profiles of the coated granules reached the equilibrium stage approximately after 98 and 126- h when the DAP is coated with only Priamine single-layer (1L) and double-layer (2L), respectively [J. Amer. Oil Chem. Soc. 97 (7), 751-763(2020)]. Moreover, the strategy adopted has successfully provided a very slow release and long-term availability of nutrient sources with bio-based coating oil compared

to uncoated fertilizer (DAP) and therefore exhibited promising application for sustainable development of modern agriculture and circular economic.

Optimization of the Degumming Process for Aqueous-Extracted Wild Almond Oil

Wild almond (*Amygdalus scoparia*) oil is rich in oleic acid and, considering both nutritional and stability points of view, it can be utilized for future food applications. In the current study by Nekoue et al., acid degumming was investigated based on a method by response surface methodology using four degumming parameters, namely the amount of phosphoric acid (0.0–0.2%, w/w), the amount of water (1.0–5.0%, w/w), degumming temperature (30–70 C), and degumming time (10–50-min). Optimum conditions for the minimum phosphorus level in the oil were found to be 0.15% phosphoric acid, 3.0% water, 40 C degumming temperature, and 28-min degumming time, resulting in an almost complete removal of phosphorus.

The final degummed wild almond oil had less than 1-mg-kg^{-1} phosphorus (reduced from an original value of 206-mg-kg^{-1}). The experimental value of phosphorus reduction at optimum conditions agreed well with that predicted by the model.

Peroxide value, anisidine value, iron, copper, and lead contents, phytosterols, unsaponifiable matter, and color of the oil decreased significantly during the degumming process; however, the fatty acid composition did not change [J. Amer. Oil Chem. Soc. 97 (7), 765-778(2020)]. Also, degumming did not significantly impact the free fatty acid level, refractive index, density, iodine value, and the saponification value of the oil. However, tocopherols and the oxidative stability of the oil increased during degumming.

Crude wild almond oil contained a trace level of amygdalin, which was completely eliminated during the degumming process.

Isothermal Crystallization of Palm Oil-Based Fats with and without the Addition of Essential Oils

The objective of study by Chikhoune et al.,was to evaluate the crystallization behavior of palm oil-based fats processed with and without the addition of essential oils (5% w/w) obtained from the flowers (EsOF) and stems (EsOS) of *Pituranthos scoparius*. Palm oil (PO) and a mixture of PO, soybean oil, and sunflower oil (Mix) were tested. The addition of the essential oils did not change the melting points of the fats but affected their crystallization behavior. A delay in crystallization was observed, evidenced by lower crystallization rates, and lower solid fat contents. This delay was comparable in the samples crystallized with EsOF and EsOS for the PO samples but EsOF was more efficient at delaying crystallization in the Mix sample. EsOF generated a less organized crystalline network in both samples (lower enthalpy values) while EsOS generated a more organized crystalline network (high enthalpy values) when used in the Mix sample. The addition of EsO also affected the crystal microstructure in some cases [J. Amer. Oil Chem. Soc. 97 (8), 861-878(2020)]. While a slight increase in crystal size was observed for some PO samples crystallized with EsOF, no change or a decrease in crystal size was observed for the samples crystallized with EsOS. A slight decrease in crystal size was observed for Mix samples crystallized with EsOF while no effect was observed for these samples crystallized with EsOS. Results from this study show that these essential oils can be used as natural additives to modify the crystallization of fats for food applications and therefore widen their functional properties.

Effective pyrolysis of waste cooking oils into hydrocarbon rich biofuel on novel mesoporous catalyst with acid and alkali coexisting

The high value usage of waste biomass-based resources is highly demanded. Here Yu et al., synthesized an acid and base based on SBA-15 bifunctional catalyst, SBA-15@MgO@Zn, for the catalytic pyrolysis of waste cooking oils (WCO) into hydrocarbon rich biofuel with deoxygenation. First, C-C bond broke at Lewis acid sites to form hydrocarbons and short-chain fatty acids, and then these short-chain fatty acids deoxidized at the basic sites to form hydrocarbon compounds [Ind. Crops Prod., 150, 112362 (2020)]. Therefore, the acid and base coexist bifunctional catalyst overcomes not only the shortcomings for the acidic catalysts that easily produce coke, but also for the basic catalysts that induces

saponification during the catalytic pyrolysis of high acid value WCO. At the same time, this kind of high efficient catalyst makes deoxidation in the process of the pyrolysis, which not only improves the quality of biofuel, but also simplifies the production process.

Simulating oilseed fatty acid composition through a stochastic modelling approach

A simulation model was developed by Fila et al., to study the accumulation dynamics of seed oil fatty acids (FAs) in response to environmental stimuli. The Petri Net (PN) formalism was used to encode a virtual analogue of the FA biosynthetic pathway, where the process is governed by rules targeting enzymatic regulatory mechanisms. Four alternative types of rules were conceived: i) constant enzymatic activity throughout seed development; ii) enzymatic activity regulated by temperature; iii) enzymatic activity regulated by time-dependent gene expression; iv) combination of temperature- and time-dependent regulatory mechanisms. A set of 10 PN-based candidate models were built upon these rules to be evaluated against real oil composition data gathered during seed development.*Camelina sativa* (L.) Crantz was chosen as a case-study crop to test model performance. Published experimental datasets from Italy, Spain and the USA were used to calibrate and validate the models through a cross-validation procedure.Models incorporating endogenous time-based regulation showed higher predictive power than those built upon temperature-based rules, but the best results were obtained when combinations of both types of rules were used[Ind. Crops Prod., 150, 112381 (2020)].The highest scoring model used the beta function to model endogenously regulated time-dependency of enzymatic activity, and the response to temperature. The fitted model correctly reproduced time-course FA accumulation. In order to assess its behaviour under climate change, sample simulations were run under two artificial scenarios, one characterized by increased air temperature, and the other by imposed restriction to photosynthetic carbon supply to the seed. The latter scenario was intended to mimic drought stress in particular. At higher temperatures the model predicted an increase in saturated and monounsaturated FAs, with a concomitant decrease of the polyunsaturated fraction.Under simulated carbon restrictions the prevailing behaviour was a lowering of mono-unsaturated FAs whereas the polyunsaturated fraction markedly increased. In both case-studies the predicted responses were found to be consistent with previous literature reports.

Chemometric methods for fatty acid compositions of fenugreek (*Trigonella foenum-graecum* L.) and black cumin (*Nigella sativa* L.) seeds at different maturity stages

This study was conducted to determine the effects of seed maturity stages depending on different harvest dates on fatty acid compositions of fenugreek (*Trigonella foenum-graecum* L.) and black cumin (*Nigella sativa* L.) with the aid of chemometric methods. Principal component analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) analysis were used by Beyzi et al., to assess and compare fatty acid compositions of fenugreek and black cumin seeds. Experiments for fenugreek and black cumin plants were separately conducted side-by side. For both plant species, 9 different harvest dates at 3 seed maturity stages (immature, premature, and full mature) were experimented. Following the harvests, plant height, single plant weight, crude oil content and fatty acid compositions were determined. Plant heights varied between 45.10-63.37 cm for fenugreek and between 34.57-43.67 cm for black cumin; crude oil contents varied between 4.01-4.95% for fenugreek and between 18.42-40.35% black cumin. Present findings revealed that immature stage had positive effects on crude oil content of fenugreek seeds and premature stage on crude oil contents of black cumin seeds. Also, 9 fatty acid components were identified in fenugreek and 7 in black cumin seeds. In both plant species, linoleic acid (C18:2) was the major component. With the greatest UFA (total unsaturated fatty acids) and the lowest SFA (total saturated fatty acids) ratios, F8 (19 July 2019) harvest date could be recommended for fenugreek plants and B4 (17 July 2019) for black cumin plants [Ind. Crops Prod., 151, 112488 (2020)]. PCA and AHC analysis revealed that with regard to fatty acid composition, F3 (08 July 2019) harvest date was different from the others in

fenugreek and B1 (10 July 2019) in black cumin plants. It was concluded based on present findings that seed maturity stages had significant effects on fatty acid composition of fenugreek and black cumin seeds.

Physicochemical characteristics of *Act-inostemma lobatum* Maxim. kernel oil by supercritical fluid extraction and conventional methods

There are limited studies available regarding the characteristics of *Actinostemma lobatum* Maxim. kernels. In the present studyby Zheng et al., , the kernels were analyzed for their nutritional composition, especially their oil characteristics. Expressed on the dry weight basis, the kernel oil accounted for 47 % of the kernel, whereas the content of protein represented 26 %. The major unsaturated fatty acids of the kernel oils were linoleic acid and oleic acid (43%–44% and 37%–38%, respectively). The kernel oils were found to be rich in squalene, tocopherols, and phytosterols (2327–2975, 676–759, and 504–676-mg/kg, respectively) [Ind. Crops Prod., 152, 112516 (2020)]. More specifically, δ-tocopherol was the most abundant tocopherol and Δ^5-avenasterol was the major sterol in the kernel oils. Furthermore, three different oil extraction methods (supercritical CO_2 extraction, Soxhlet extraction, and cold pressing) were applied and the physicochemical properties of the extracted oils were compared. No significant differences (P->-0.05) were found in tocopherol and sterol contents for the three extraction methods. Higher oil yield and oil recovery were obtained by supercritical CO_2 extraction. Overall, *A. lobatum* kernel is a promising valuable source of bioactive substances and essential fatty acids for future applications in food, pharmaceutical, and cosmetic industries.

Optimization of supercritical-CO_2 extra-ction of *Iris lactea* seed oil: Component

analysis and antioxidant activity of the oil

Iris lactea Pall. var. *chinensis* (Fisch.) Koidz. is a widely distributed species and the

seeds of this species have been used as medicine. However, the chemical composition and biological activities of the *I. lactea* seed oil (ILSO) have not been studied. In this present workby JieLuan et al., ,extraction time, temperature, and pressure were considered as variables and response surface methodology based on the Box-Behnken design was utilized to identify the optimal conditions for supercritical fluid extraction of the ILSO. Furthermore, the main components (including fatty acids, unsaponifiables, and volatile components) in ILSO extracted with the above optimal conditions were identified by gas chromatography-mass spectrometry. It was found that major fatty acids present in ILSO were linoleic acid (41.31 %), oleic acid (34.74 %), and docosahexaenoic acid (3.18 %). The total content of sterols reached as high as the level of 87.44 % in unsaponifiable matter and sterols with a content over 5% included β-sitosterol (27.14 %) stigmasterol (18.38 %), delta5-avenasterol (15.36 %), campesterol (13.21 %), and betulinicaldehyde (5.80 %)[Ind. Crops Prod., 152, 112553]. The physicochemical properties of ILSO were analyzed according to Chinese national standards. Total polyphenol content (TPC) and total tocopherol content of ILSO were also determined. These predominant compounds were known by their high biological activities and confirm the potential sources of bioactive compounds existing in ILSO. Based on these identified ingredients, the antioxidant activity of the ILSO was evaluated by two different methods.

The antioxidant capacity of ILSO determined by FRAP method was equivalent to 0.4759 m mol/L $FeSO_4 \cdot 7H_2O$ and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity *in vitro* with IC_{50} value of 3.27 mg/mL. These results provide a rich chemical database for the development of a new oil resource, as well as the possibility of using a byproduct in the production of pharmaceuticals and a rich resource of ornamental plants.

Characterization of Trime thylolpropane Based Biolubricant

The oxidative stability index (OSI) of fatty acid methyl esters (FAME) and trimethylolpropane (TMP) esters or TMPE produced from five vegetable oils (*Brassica rapa* L.*, Linum usitatissimum* L.*, Zea mays* L.*, Brassica napus* L.*, Camelina sativa* L.) are compared in this work by Nie et al. The highest stability is observed in vegetable oils while the processed products are less stable.

The major causes in loss of OSI are attributed to excess FAME in the crude product and the loss of natural antioxidants due to refinement with silica and celite. The low-temperature flow properties of TMPE produced from four different vegetable oils (*B. juncea* L., *L. usitatissimum* L., *B. rapa* L., and *C. sativa* L.) are investigated by proton nuclear magnetic resonance (^{1}H-NMR).

The T2 relaxations of different TMPE are measured to observe how the mobility of oil changed as temperature decreased. Increased oil mobility (represented by T2) is correlated with rising temperature. The Gaussian widths of the singlet in ^{1}H-NMR spectra of each oil demonstrated increased molecular mobility as temperature increased. Extrapolation of the relation of T2 signals of these four oils indicates that T2 approached zero between 232 K and 239 K, suggesting the molecular motion leading to a T2 relaxation has largely ceased [Eur. J. Lipid Sci. Technol. 122 (7), 202000025 (2020)].

Erythritol-Based Medium-Chain Sugar Amphiphile: Synthesis and Gelling Capability in Edible Oils

The production of organogels from vegetable oils requires low-molecular-weight gelators. Herein, a novel small-molecule sugar ester gelator is synthesized from erythritol and octanoic acid.

The purified reaction product is identified as erythritol 1,6-dioctanoate by Pang et al., using high performance liquid chromatography (HPLC) with evaporative light-scattering detection and nuclear magnetic resonance (NMR).

This low-molecular-weight erythritol ester exhibits self-assembly behavior in vegetable oils with a minimum gelation concentration of 4 wt%. Microscopy reveals needle-like crystals that became slender as the gelator concentration increases. X-Ray Diffraction (XRD) analyses indicate that the organogels are semicrystalline with both crystalline and amorphous domains. Fourier Transform Infrared (FTIR) spectroscopy reveals that the gel structure is maintained by non-hydrogen-bonding interactions.

Furthermore, rheological tests show that the mechanical strength of the gel is poor, but as the gelator concentration is increased, the mechanical strength and hardness of the gel also increases [Eur. J. Lipid Sci. Technol. 122 (9), 201900412 (2020)].Thus, this erythritol ester effectively promotes the gelation of edible vegetable oils and increases the variety of available organogelators.

Comprehensive Triacylglycerol Characterization of Oils and Butters of 15 Amazonian Oleaginous Species by ESI-HRMS/MS and Comparison with Common Edible Oils and Fats

The Amazon rain forest encompasses an extraordinary source of vegetable oils with many applications, especially for food, pharmaceutical and cosmetics industries. In this work by Fasciotti the main composition of fifteen Amazonian oils and butters are investigated via gas chromatography mass spectrometry (GC-MS) and electrospray ionization high resolution mass spectrometry (ESI-HRMS). Triacylglycerols (TAG) are characterized by their fragmentation spectra and comparison with the LIPID MAPS database, resulting in a detailed compendium of TAG composition of these samples.

Over 70 different TAG are putatively annotated per sample and the occurrence of isomers is remarkable, showing that TAG complexity in these samples is considerably higher than ever reported. The TAG composition of the Amazonian samples are also statistically evaluated using principal component analysis (PCA) for comparison to common edible oils such as soybean, corn, coconut, and olive oil [Eur. J. Lipid Sci. Technol. 122 (9), 202000019 (2020)].

Some tendencies of grouping are observed: butters with medium chain fatty acids (FA); butters with high oleic FA; and oils with high oleic and high linoleic FA contents. This study provided profiles that ensure Amazonian oils and butters authenticity, quality and also aids in understanding their properties and the best applications for each.

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