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Fatty Acid Composition, Phenolic Compounds, Phytosterols, and Lipid Oxidation of Single- and Double-Fractionated Olein of Safflower Oil Produced by Low-Temperature Crystallization

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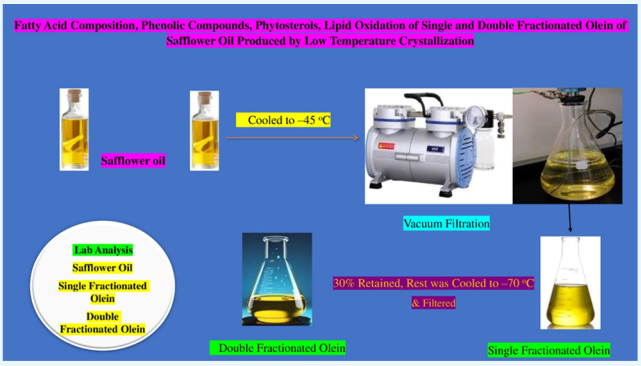
ABSTRACT: By dry crystallization, concentrations of unsaturated fatty acids and bioactive compounds can be increased in olein and super-olein fractions in vegetable oils. Among all sources of vegetable oils, safflower oil (SO) possesses the maximum linoleic acid content. To boost the industrial applications of SO, two variants were produced by single- and two-stage crystallization. This study aimed to determine the fatty acid compositions, phenolic compounds, phytosterols, and oxidative stability of fractionated olein (OF) and double-fractionated olein (DFO) produced by dry crystallization. For this, SO was cooled to −45 °C and filtered, the filtrate was denoted as single-fractionated olein (OF), and 40% of this section was taken for analytical purposes, while the remaining 60% was again cooled to −70 °C and filtered, and the filtrate was denoted as double-fractionated olein (DFO). Unfractionated safflower (SO) was used as a control, filled in amber glass bottles, and stored at 20−25 °C for 90 days. Fatty acid compositions and phytosterols were determined by gas chromatography-mass spectrometry (GC-MS). Phenolic compounds and induction periods were determined by high-performance liquid chromatography (HPLC) and Rancimat. GC-MS analysis revealed that the C18:2 contents of SO, OF, and DFO were 77.63 ± 0.82, 81.57 ± 0.44, and 89.26 ± 0.48 mg/100 g ($p < 0.05$), respectively. The C18:1 contents of SO, OF, and DFO were 6.38 ± 0.19, 7.36 ± 0.24, and 9.74 ± 0.32 mg/100 g ($p < 0.05$), respectively. HPLC analysis showed that phenolic compounds were concentrated in the low-melting-point fractions. In DFO, concentrations of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were 57.36 ± 0.12, 129.45 ± 0.38, 165.11 ± 0.55, 183.61 ± 0.15, 65.94 ± 0.11, and 221.75 ± 0.29 mg/100 g, respectively. In SO, concentrations of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were 24.79 ± 0.08, 78.93 ± 0.25, 115.67 ± 0.41, 34.89 ± 0.51, and 137.26 ± 0.08 mg/100 g, respectively. In OF, concentrations of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were 35.96 ± 0.20, 98.69 ± 0.64, 149.14 ± 0.13, 57.53 ± 0.74, and 188.28 ± 0.82 mg/100 g, respectively. The highest concentrations of brassicasterol, campesterol, stigmasterol, β-sitosterol, avenasterol, stigmastenol, and avenasterol were noted in DFO followed by OF and SO. The total antioxidant capacities of SO, OF, and DFO were 54.78 ± 0.12, 71.36 ± 0.58, and 86.44 ± 0.28%, respectively. After the end of the storage time, the peroxide values (POVs) of SO, OF, and DFO stored for 3 months were 0.68, 0.85, and 1.16 mequiv O_2/kg, respectively, with no difference in the free fatty acid content.



1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an oilseed crop that possesses a huge potential for the production of edible oil and is a source of protein. It is abundantly grown in Asian, North America, and South America. Because of its lower water requirements and drought and flood resistance, the area under safflower production worldwide is increasing at the rate of approximately 4.9%/year.[1] The average yield of safflower seeds ranges from 805 to 827 kg/ha, with a global production of 867,659 tons.[2] The oil content of safflower seeds is about 38−48%, with 15−22% protein; this oil content is significantly higher than those of soybean, sunflower, corn, and cottonseed. Gas chromatographic analysis showed that the most abundant fatty acid in safflower oil is linolenic acid (about 70%), followed by oleic acid (14%), palmitic acid (4.25%), and stearic acid (3.15%). Three different varieties of safflower oil

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were tested for fatty acid composition. Gas chromatographic analysis showed that linoleic acid, oleic acid, stearic acid, and palmitic acid constituted about 97% of the total fatty acids, while smaller portions of behenic, ecosenoic, and lignoceric acids were also detected.[3] Because of a reasonable amount of oleic acid, the oxidative stability of safflower oil is superb, and it does not give a smoky smell during frying.[4] The balanced fatty acid profile of safflower decreased fat accumulated in the body of rats as compared to a tallow-based diet.[5] The fatty acid composition of safflower oil offers a great deal of opportunities and potential avenues for its application in the food industry.[6] The triglyceride profile of safflower showed that stearate, oleate, PLO-palmitate, and LL-Linoleate constituted about 80% of the triglycerides.[7] Phospholipids of safflower oil possess hypocholesterolemic properties, and the consumption of safflower oil phospholipids reduces the lipid level in the liver and increases the HDL level.[6] The phospholipid content was significantly influenced by technological methods applied during oil extraction.[8] Triglycerides and fatty acid profiles of oils and fat can be modified to a great extent by technological methods or fat modification strategies such as chemical/enzymatic interesterification, transesterification, blending, and low-temperature crystallization/winterization/fractionation. Among the listed techniques, low-temperature crystallization/fractionation is one of the most commonly used methods as versions of oils and fat modified by this method can be used in the production of traditional foods and the development of functional foods.[8] Modification of fats can be achieved via solvent and low-temperature crystallization; however, the latter is more suited for the food industry as the fats and oils modified by the former method may have residues of hazardous solvents used for fractionation.[9] Hussain et al.[10] produced olein and super-olein fractions of date seed oil by the dry crystallization method. The fatty acid profile, antioxidant capacity, sterol content, and induction period of both fractions derived from date seed oil were significantly different from the original version of the date seed oil with no difference in color (Lovibond tintometer scale). Palm olein is commercially produced from palm oil by low-temperature crystallization. The chemical characterization of safflower oil showed that the oil was edible without any processing, and it had a slightly yellow color, quite similar to refined versions of commercially available cooking oils. To convert commercial oils to edible form, massive energy and inputs are required. According to the published data, to refine, bleach, and deodorize one metric ton of edible oil, about two metric tons of saturated steam is required during the processing of oils.[11] Fractions of buffalo milk fat were formed by gradually cooling and holding at 10, 15, and 25 °C. The triglyceride content, fatty acid composition, cholesterol, and slip melting point of all fractions were different from those of the unmodified parent fat and from each other. Scientific evidence has shown that harshly processed/ultra-processed foods should be avoided, and minimally processed or unprocessed foods should be included in the daily diet regime to prevent metabolic disorders. Safflower oil may be used in the crude form. However, its application in new avenues in the food industry should be explored in detail. From the economic point of view, the production of OF and DFO is sustainable as the recovery of low-melting-point fractions in date seed oil was 25−41%.[10] Modifying the chemistry of fats also alters their oxidative stability. Olein and stearin fractions of chia oil were prepared and stored at ambient temperature for a duration of 6 months. In the entire storage phase, stearin had better oxidative stability than olein.[12] When chemically modified oils and fats are produced, their storage stability should also be taken into consideration. The generation of lipid oxidation products in food systems is harmful to consumers. Scientists believe that the consumption of oxidized fats is even worse than the intake of bad fats.[13] To obtain scientific information pertaining to the food industry and domestic applications, fractionation of safflower oil should be performed, and the chemistry of different fractions should be studied in detail as no previous study is reported on these aspects of safflower oil. Presently, the application of safflower oil is limited. To increase industrial applications and provide choices to the food industry among safflower oil, OF, and DFO according to the demand of consumers for the development of functional foods, two variants of safflower oil were produced. This study aimed to determine the fatty acid compositions, phenolic compounds, phytosterols, and oxidative stabilities of OF and DFO produced by dry crystallization.

2. MATERIALS AND METHODS

2.1. Raw Materials. Safflower seeds were procured from an agricultural research station (15 day old seeds; last week of April, 2022). Oil was extracted by the cold extraction method at 20−25 °C using a screw press. HPLC-grade chemicals were supplied by Sigma-Aldrich.

2.2. Low-Temperature Crystallization/Fractionation. Safflower oil was gradually cooled to −45 °C in 24 h, held at this temperature for a further 16 h, and filtered using a vacuum (600 mmHg)-assisted filtration assembly; the filtrate was denoted as single-fractionated olein (OF), and 40% of this section was used for analytical purposes. The remaining 60% was again cooled to −70 °C in 24 h, held at this temperature for a further 16 h, and filtered in the same fashion; the filtrate was denoted as double-fractionated olein (DFO). Fractionation was repeated at least six times in a completely random design (CRD). Unmodified safflower oil (SO) was treated as the control.[10] The control, OF, and DFO were filled in amber glass bottles and stored at 20−25 °C for 90 days and analyzed at 0, 45, and 90 day intervals.

2.3. Chemical Testing of SO, OF, and DFO. Free fatty acids (FFAs) were determined by titrating the samples in neutral ethanol with 0.1 N NaOH and expressed in terms of oleic acid. The moisture contents were determined by the oven-drying method until a constant weight and saponification value were determined by reacting the sample with alcoholic KOH for 30 min, followed by titration with 0.5 N HCl (values were reported in mg KOH/g). Unsaponifiable matter (UM) was determined by saponifying the oil samples with alcoholic KOH, and unsaponified fractions were collected from separating funnels and expressed in percentage. The refractive index was determined at 40 °C on a digital refractometer, and the iodine value (IV) was analyzed by the Wijs method. Samples were reacted with 25 mL of Wijs solution and then titrated against a standard sodium thiosulfate solution; the peroxide values were analyzed in samples by reacting 5 g of the sample with 30 mL of solution (3 parts glacial acetic acid and 2 parts chloroform) using starch as an indicator.[14] The colors of SO, OF, and DFO were measured on the Lovibond tintometer (Salisbury, UK) in a 5.25 in. quartz cell following the guidelines of the manufacturer.

2.4. Fatty Acid Composition. The fatty acid compositions of SO, OF, and DFO were analyzed by GC-MS (7890-B, Agilent Technologies) using an SP-2560 column (100 m ×

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0.25 mm id; 0.20 μm) and an FID. For ester preparation, 50 mg of the sample was reacted with 2 mL of HCl in C_2H_5OH (15%) at 100 °C/60 min in a heating block, then cooled to 20−25 °C, followed by the addition of 2 mL each of *n*-hexane (99.99%) and deionized H_2O; test tubes were then vortexed (1500 rpm/1 min) after 15 min; the upper layer was extracted and dried over Na_2SO_4 and put in GC vials for injection (1 μL) by ALS at a 1:50 split ratio. The temperatures of the oven, injector, and FID were 225, 250, and 260 °C, and He, O_2, and hydrogen flow rates were 2, 4, and 40 mL/min, respectively. FAME-37 was used as the identification and quantification (Supelco) standard.[15]

2.5. Analysis of Phenolic Compounds by HPLC. Phenolic compounds of SO, OF, and DFO were characterized on HPLC (Shimadzu) using an ODS-SP column (460 mm × 250 mm × 5 μm). Phenolic compounds from SO, OF, and DFO (0.1 g of sample) were extracted with 4 mL of aqueous methanol (50:50) for 30 min; samples were then centrifuged at 5000 g/10 min, followed by drying under N_2 at 35−40 °C and centrifuging at 10,000*g*/5 min; the upper layer was placed in an HPLC vial, and 10 μL was injected using TFA as the mobile phase (0.2%) at 280 nm. Standards of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were used to identify and enumerate phenolic compounds in the tested samples.[10]

2.6. Phytosterols. Samples (0.3 g) of FO, OF, and DFO were taken in a test tube. 5α-Cholestane 0.5 mg/mL (10 μL) and 3 mL (2 M) of a solution of ethanolic NaOH were added, vortexed for 1 min (1500 rpm), and heated in a 90 °C water bath for 15 min. *n*-Hexane and deionized H_2O (2 mL each) were added, followed by centrifugation at 5000 g for 10 min. Hexane was evaporated under N_2, and the rest was treated with Tri-Sil (10 μL) for 30 min, again dissolved in hexane, and placed in GC vials for automatic injection by an Auto Liquid Sampler into the GC-MS (7890-B, Agilent Technologies) DB-5HP fused silica capillary column (30 m × 0.32 mm × 0.1 μm). The temperature of the injector and FID was 260 °C; the oven temperature was increased from 50 to 315 °C at 40 °C/min. He, H_2, and O_2 were circulated in the system at 2, 4, and 40 mL/min, respectively. Sterols were identified by internal standards.[16]

2.7. Total Antioxidant Capacity (TAC). 1 mL each of Na_2SO_4 solution (28 mM), $(NH_4)_2MoO_4$ (4 mM), and H_2SO_4 (6 M) were blended; the samples (100 μL) were treated at 95 °C/15 min and cooled to room temperature. Five standards of ascorbic acid were prepared, and the absorbance was recorded at 695 nm on a double-beam spectrophotometer. TAC was reported in terms of percentage.[17]

2.8. 1,1-Diphenyl-2-picrylhydrazyl (DPPH). The DPPH solution (20 mg/L) was prepared in C_2H_5OH. 1.5 mL of the DPPH solution was blended with 750 μL of the sample, and the tubes were kept in the dark for 30 min. The absorbance was recorded at 517 nm on a double-beam spectrophotometer, and the DPPH value was reported in percentage.[17]

2.9. Oxidative Stability. FFAs and POVs of SO, FO, and DFO were determined at regular intervals of 0, 45, and 90 days.[14] For the measurement of the induction period, the samples (2.5 g) were weighed in reaction vessels and oxidized in an accelerated manner at 120 °C using 20 L/h dried O_2. All measurements were electronically recorded using Stab Net software.

2.10. Statistical Analysis. The data collected in CRD were analyzed by one-way and two-way ANOVA to determine the impact of fractionation by low-temperature crystallization and storage. For screening the significant values of fractionation and storage of SO, OF, and SOF, the Duncan multiple range test was applied in SAS 9.4 software, and means were considered significant at a *p*-value of <0.05.[18]

3. RESULTS AND DISCUSSION

Increasing food insecurity in developing countries and the rapidly increasing human population has forced researchers to find new sources of oils and fats or modify their physical, chemical, and functional characteristics to decrease food insecurity. Nature has blessed mankind with more than 5000 edible-oil-producing plants; however, only a few are capitalized for large-scale human usage.[11] SO is among the most neglected edible oils; its physical and chemical characteristics and fatty acid profile show that it is edible. After this study, edible oil processors will have OF and DFO to consider production at a commercial level. Edible oil processors prefer to use lighter-colored, low-FFA, low-moisture-content oils. In the current investigation, the chemical and physical characteristics of SO, OF, and DFO were studied to determine their suitability for large-scale processing. The colors of SO, OF, and DFO were 23, 23, and 21, respectively (red + blue by a Lovibond tintometer). In the crude form, the colors of SO, OF, and DFO were pale yellow, which is similar to those of fully processed edible oils. This is highly encouraging for underdeveloped nations as they are facing an acute shortage of energy resources, and sustainable food-processing strategies can be helpful for these nations, as SO and its fractions can be used in the crude form without applying any commercial operation. The FFA contents of SO, OF, and DFO were 0.14 ± 0.01, 0.13 ± 0.02, and 0.140.02% (oleic acid), respectively, which indicated that fractionation did not affect the FFA content, as per the guidelines of EU. The FFA content in foods should be less than 0.2% (Table 1). The FFA contents of SO and its fractions were considerably lower than those of soybean, canola, sunflower, and corn oils. The FFA contents of crude versions of the above-mentioned oils were 0.86, 0.58, 0.62, and 0.73%, respectively.[19] However, fractionation significantly increased the UM and IV. From a nutritional viewpoint, an increase in the UM and IV is highly significant for health-

Table 1. Chemical Characteristics of Single- and Double-Fractionated Olein of Safflower Oil

parameter	SO	OF	DFO
FFA (%, oleic acid)	0.14 ± 0.01^{A}	0.13 ± 0.02^{A}	0.14 ± 0.02^{A}
moisture (%)	0.19 ± 0.03^{A}	0.18 ± 0.01^{A}	0.20 ± 0.02^{A}
saponification value (mg KOH/g)	194 ± 0.54^{A}	191 ± 0.33^{A}	192 ± 0.27^{A}
unsaponifiable matter (%)	1.22 ± 0.05^{C}	1.48 ± 0.06^{B}	1.79 ± 0.03^{A}
refractive index (@40 °C)	1.472 ± 0.01^{C}	1.479 ± 0.02^{B}	1.1491 ± 0.01^{A}
iodine value (Wijs method, Cg/100 g)	148 ± 0.22^{C}	165 ± 0.76^{B}	182 ± 0.48^{A}
peroxide value (mequiv O_2/kg)	0.25 ± 0.04^{A}	0.29 ± 0.02^{A}	0.26 ± 0.01^{A}
color Lovibond tintometer scale (red + yellow)	23 ± 0.07^{A}	24 ± 0.05^{A}	21 ± 0.02^{A}

In a single row of this table, means with dissimilar letters show a significant difference ($p < 0.05$). SO = safflower oil; OF = single-fractionated olein: DFO = double-fractionated olein.

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Table 2. Fatty Acid Profile of Single- and Double-Fractionated Olein of Safflower Oil

	SO		OF		DFO	
fatty acid	0 day	90 days	0 day	90 days	0 day	90 days
C14:0	0.48 ± 0.02^{A}	0.46 ± 0.01^{A}	0.32 ± 0.05^{B}	0.31 ± 0.01^{B}	0.23 ± 0.02^{C}	0.21 ± 0.03^{C}
C16:0	4.12 ± 0.15^{A}	4.07 ± 0.03^{A}	2.41 ± 0.05^{B}	2.35 ± 0.09^{B}	1.29 ± 0.02^{C}	1.22 ± 0.01^{C}
C18:0	2.54 ± 0.06^{A}	2.51 ± 0.01^{A}	1.47 ± 0.04^{B}	1.44 ± 0.07^{B}	0.89 ± 0.03^{C}	0.85 ± 0.02^{C}
C18:1	6.38 ± 0.19^{E}	5.72 ± 0.13^{F}	7.36 ± 0.24^{C}	6.34 ± 0.17^{D}	9.74 ± 0.32^{A}	8.16 ± 0.18^{B}
C18:2	77.63 ± 0.82^{E}	74.48 ± 0.36^{F}	81.57 ± 0.44^{C}	78.24 ± 0.91^{D}	89.26 ± 0.56^{A}	87.17 ± 0.48^{B}
C20:4	0.72 ± 0.02^{E}	0.61 ± 0.01^{F}	1.12 ± 0.04^{C}	1.03 ± 0.02^{D}	1.42 ± 0.02^{A}	1.33 ± 0.07^{B}
C20:1	0.64 ± 0.02^{E}	0.54 ± 0.03^{F}	0.83 ± 0.05^{C}	0.75 ± 0.02^{D}	1.17 ± 0.04^{A}	0.98 ± 0.02^{B}

In a single row of this table, means with dissimilar letters show a significant difference ($p < 0.05$). SO = safflower oil; OF = single-fractionated olein: DFO = double-fractionated olein.

conscious consumers and food applications. UM contains a large number of functional compounds, such as tocopherols, carotenoids, phenolic compounds, and sterols. The increase in the UM in OF and DFO was due to the intensification of unsaponifiable substances due to their affiliation with low-melting TAGs.[20] The IVs of SO, OF, and DFO were 148 ± 0.22, 165 ± 0.48, and 182 ± 0.76 cg/100 g, respectively. The IV of oils and fats is dependent on the degree of unsaturation in fatty acids. Fractionation significantly increased the extent of unsaturation in OF and DFO, which was the reason for the IV of OF and DFO being higher than that of the parent SO. To enhance omega-3 fatty acids, chia oil was fractionated at −30 °C. The FFA content, moisture, and color of the parent chia oil and its OF were almost the same ($p > 0.05$). However, the UM and IV of OF were higher than those of chia oil due to the accumulation and increase in unsaponifiable substances and unsaturated fatty acids.[12] Azeem et al.[9] reported that the UM and IV of OF of cottonseed oil were greater than those of the unfractionated oil with no variation in the POV. From industrial, nutritional, and storage stability viewpoints, it is evident that SO, OF, and DFO have huge potential to be used as sources of commercial oils.

3.1. Fatty Acid Composition. In this trial, SO was cooled to −45 °C to produce OF, which was further cooled to −70 °C to obtain DFO. Crystallization of fatty acids at two different temperatures in two different substrates gave rise to major changes in the fatty acid composition. SO, OF, and DFO had enormously different fatty acid compositions from each other ($p < 0.05$). The slip melting points of individual fatty acids are different from each other. This difference in the melting point can be capitalized to tailor the fat using the dry crystallization technique. For example, the melting points of C14:0 (44.5 °C), C16:0 (63.5 °C), C18:1 (−4.9 °C), and C18:2 (−16.5 °C) were used to enhance C18:2 and C18:1 in OF and DFO. During dry crystallization, the fatty acid composition of successive fractions depends on the crystallization temperature and melting point of fatty acids. The difference in the melting point of fatty acids is a key factor to modify the fatty acid composition of oils and fats.[21] GC-MS analysis of SO showed that C14:0, C16:0, C18:0, C18:1, C18:2, C20:4, and C20:1 were detected, and C18:2 (77.63 ± 0.82 mg/100 g), C18:1 (6.38 ± 0.19 mg/100 g), and C16:0 (4.12 ± 0.15 mg/100 g) were the most abundant fatty acids (Table 2). Dry crystallization of SO to produce OF and DFO significantly altered the fatty acid composition. The difference in the fatty acid compositions of OF and DFO was recorded in both saturated and unsaturated fatty acids. C14:0, C16:0, and C18:0 were significantly lower in OF and DFO compared to SO. C18:1 and C18:2 were increased in OF and DFO and exhibited higher concentrations than SO ($p < 0.05$). The C18:2 contents of SO, OF, and DFO were 77.63 ± 0.82, 81.57 ± 0.48, and 89.26 ± 0.56 mg/100 g, respectively ($p < 0.05$). The C18:1 contents of SO, OF, and DFO were 6.38 ± 0.19, 7.36 ± 0.24, and 9.74 ± 0.32 mg/100 g, respectively ($p < 0.05$). The fatty acid composition of OF produced from milk fat has a higher content of C18:1 and C18:2 than milk fat.[22] DFO possesses the highest concentration of 18:2 among the known dietary sources of edible fats. C18:2 can be used for the production of conjugated linoleic acid, a fatty acid that possesses a large number of therapeutic properties. The anticarcinogenic, anti-inflammatory, antidiabetic, antiobesity, and cardioprotective effects of conjugated linoleic acid have been extensively documented in the literature. Safflower oil and four commercial vegetable oils were used as substrates for the conversion of C18:2 to conjugated linoleic acid. The conversion was largely dependent on the C18:2 content in the substrate. Safflower oil had the highest C18:2 content and yielded the highest number of isomers of conjugated linoleic acid.[19] In another study, CLA produced from SO was used to increase the CLA in cheddar cheese. CLA isomers survived a long ripening phase of 3 months.[23] OF and DFO can be used to produce health-friendly bakery products, table margarine, and ice cream, for blending with vegetable oils, and for the production of calcium salts of fatty acids for dairy and poultry feed. PUFA-enriched oils and fats have inferior storage stability. OF and DFO may be safeguarded from lipid oxidation using nanotechnology, microencapsulation, and natural antioxidants. The status of lipid oxidation can also be adjudged by monitoring the fatty acid composition in the storage phase.[24] At the termination of the storage phase, the loss of C18:2 in SO, OF, and DFO were 4.05, 5.32, and 5.71%, respectively. The tremendous storage stabilities of OF and DFO can be justified by the intensification of phytochemicals and antioxidant compounds due to their affiliation with low-melting TAGs. Hussain et al.[10] produced OF and DFO from date seed oil, and fractions were stored for 3 months. The fatty acid profile of 3 month old OF and DFO was slightly different from that of unfractionated date seed oil.

3.2. Phenolic Compounds of SO, OF, and DFO. The effects of low-temperature crystallization on the concentrations of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid in SO, OF, and DFO were determined by HPLC. Low-temperature crystallization significantly affected the contents of phenolic compounds in OF and DFO. The highest contents of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were present in DFO, followed by OF and SO. In DFO, concentrations of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were 57.36 ± 0.12, 129.45 ± 0.38, 165.11 ± 0.55, 183.61 ± 0.15, 65.94 ±

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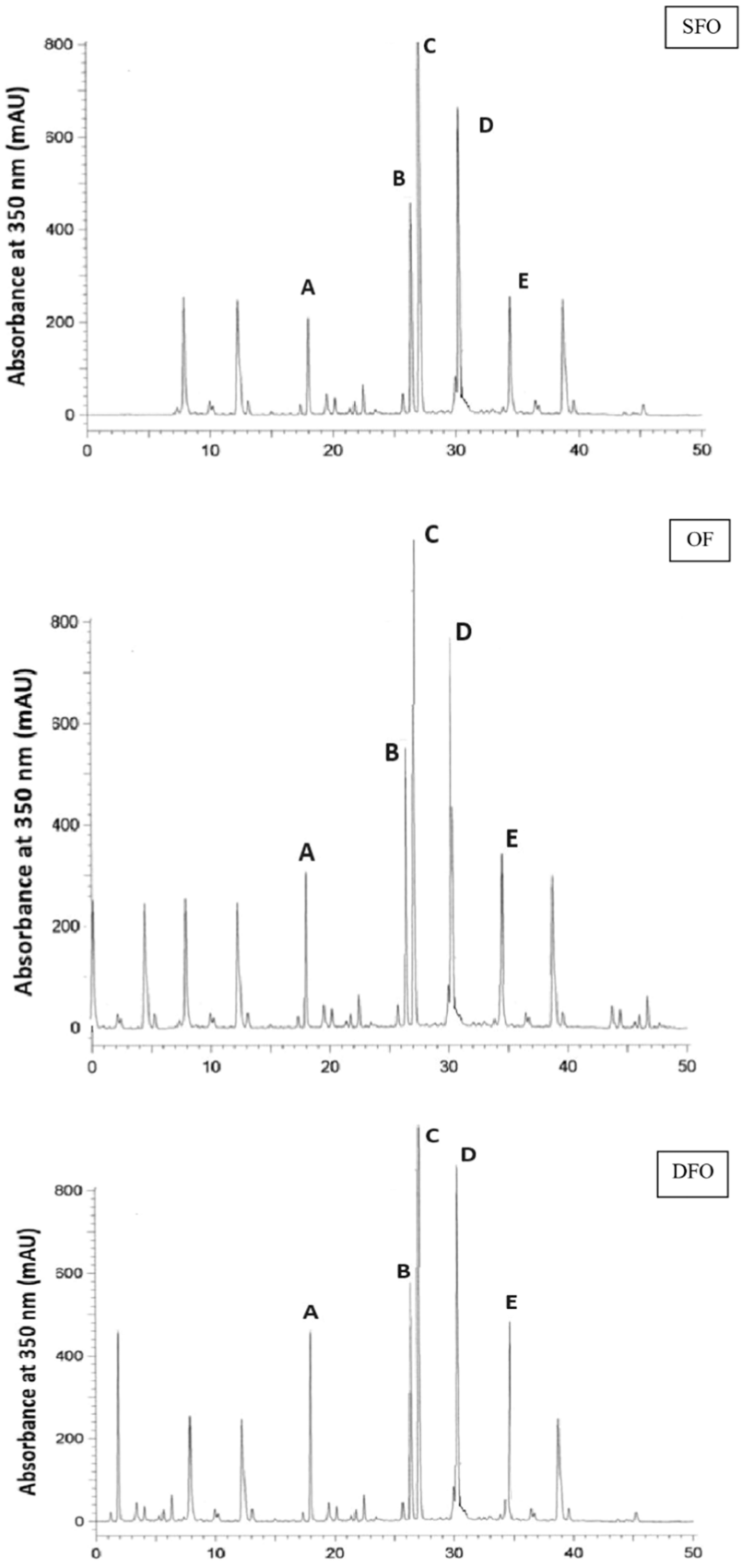

Figure 1. Phenolic Compounds of SFO, OF, and DFO.

0.11, and 221.75 ± 0.29 mg/100 g, respectively. In SO, concentrations of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were 24.79 ± 0.08, 78.93 ± 0.25, 115.67 ± 0.41, 34.89 ± 0.51, and 137.26 ± 0.08 mg/100 g, respectively. In

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OF, concentrations of tyrosol, rutin, vanillin, ferulic acid, and sinapic acid were 35.96 ± 0.20, 98.69 ± 0.64, 149.14 ± 0.13, 57.53 ± 0.74, and 188.28 ± 0.82 mg/100 g, respectively (Figure 1). The intensification of phenolic compounds in DFO and OF was due to their affiliation with low-melting TAGs. Successive low-temperature crystallization of palm oil led to an enhancement of phenolic compounds in OF and DFO of palm oil.[25] UM and phenolic compounds of DFO and OF were strongly correlated (R^2 = 0.9874 and 0.9913). Phenolic compounds of four varieties of safflower oil were analyzed by HPLC; 13 phenolic compounds were identified, and these were 2−2 hydroxybenzoic acids, naringin, tyrosol, rutin, vanillin, pinoresinol, trans-chalcone, ferulic acid, sinapic acid, and cinnamic acid.[7] Phenolic compounds of safflower oil have not been extensively reported in the literature; phenolic contents of safflower oil ranged from 2616 to 4079 mgGAE/100 g.[26] Phenolic contents of safflower oil recorded in this study were tremendously higher than those of *Moringa oleifera* oil, palm oil, soybean oil, and sunflower oil.[11] The distribution of phenolic compounds in different varieties of safflower oil needs to be studied in detail.

3.3. Phytosterols. Sterols belong to the unsaponifiable fraction of dietary lipids, and they constitute a substantial portion of the unsaponifiable matter. Sterols are rightly considered shelf-life extenders by acting as antioxidants, which thus protect oils from auto-oxidation. During the frying of foods, campesterol protects oils from oxidative and thermal deterioration.[27] Kinds of sterols and impacts of low-temperature crystallization on the concentrations of sterols in SO, OF, and DFO are shown in Table 3. Brassicasterol, campesterol, stigmasterol, β-sitosterol, avenasterol, stigmastenol, and avenasterol were detected in SFO, OF, and DFO by GC-MS. The highest concentrations of brassicasterol, campesterol, stigmasterol, β-sitosterol, avenasterol, stigmastenol, and avenasterol were noted in DFO, followed by OF and SO. Because of the affiliation of brassicasterol, campesterol, stigmasterol, β-sitosterol, avenasterol, stigmastenol, and avenasterol with low-melting TAGs, they were intensified in DFO and OF. Cholesterol was not detected in SO, OF, and DFO, and the contents of brassicasterol in SO, OF, and DFO were 14.25 ± 0.16, 24.19 ± 0.10, and 31.36 ± 0.17 mg/100 g, respectively. The concentrations of stigmasterol in SO, OF, and DFO were 11.45 ± 0.09, 19.48 ± 0.13, and 35.67 ± 0.23 mg/100 g, respectively. The concentrations of β-sitosterol in SO, OF, and DFO were 65.46 ± 0.18, 91.87 ± 91.87, and 176.81 ± 1.16 mg/100 g, respectively. The concentrations of stigmastenol in SO, OF, and DFO were 19.88 ± 0.21, 29.12 ± 0.17, and 54.42 ± 0.22 mg/100 g, respectively. Phytosterols are significant constituents of food due to their perceived cardioprotective effects; structurally, these are similar to cholesterol and provide health benefits when present in foods.[28] The total sterol content of safflower oil may vary from 1248 to 2976 mg/kg; however, it largely depends upon genetics, varieties, agronomic practices, and soil conditions.[29] The ripening stage also had a major effect on the sterol composition of safflower oil. β-Sitosterol was the dominant sterol in the later stages of ripening, while free and esterified sterols were present in reasonable amounts in the early stages of ripening. Safflower produced in Japan had more β-sitosterol as compared to those produced in other countries.[30] The phytosterol contents of palm oil and its OF and DFO were tested by GC-MS, and total sterol contents were in the order of DFO > OF > palm oil.[31] The high-oleic-acid fraction of *M. oleifera* oil was produced using low-temperature crystallization; the extent of sterols in the high-oleic-acid fraction was significantly higher than that in *M. oleifera* oil.[32] Cholesterol is mainly found in fats of animal origin; however, certain vegetable oils such as tomato seed oil may contain up to 20% USM.[33]

Table 3. Phytosterols (mg/100 g) of Single- and Double-Fractionated Olein of Safflower Oil

sterol	SO	OF	DFO
cholesterol	not found	not found	not found
brassicasterol	0.52 ± 0.04^{C}	0.95 ± 0.05^{B}	1.77 ± 0.08^{A}
brassicasterol	14.25 ± 0.16^{C}	24.19 ± 0.10^{B}	31.36 ± 0.17^{A}
stigmasterol	11.45 ± 0.09^{C}	19.48 ± 0.13^{B}	35.67 ± 0.23^{A}
β-sitosterol	65.46 ± 0.18^{C}	91.87 ± 0.14^{B}	176.81 ± 1.16^{A}
avenasterol	1.14 ± 0.02^{C}	2,11 ± 0.02^{B}	4.11 ± 0.03^{A}
stigmastenol	19.88 ± 0.21^{C}	29.12 ± 0.17^{B}	54.42 ± 0.22^{A}
avenasterol	3.49 ± 0.06^{C}	5.17 ± 0.09^{B}	8.27 ± 0.16^{A}

In a single row of this table, means with dissimilar letters show a significant difference (p < 0.05). SO: safflower oil; OF: single-fractionated olein: DFO: double-fractionated olein.

3.4. Antioxidant Capacity. The physiological effects of flavonoids and lignans of safflower oil are similar to those of phytoestrogens. They exhibit strong antioxidant activities and anticarcinogenic and cholesterol regulation properties.[34] Lignans, flavones, and serotonin derivatives were isolated from safflower oil and exhibited strong antioxidant activity *in vitro*.[35] The antioxidant activity and inhibition of lipid peroxidation by serotonin derivatives were significantly higher than those of α-tocopherol and acacetin.[36] Seven antioxidant serotonin derivatives were isolated from safflower oil, and all showed strong antioxidant activity in DPPH and ferric thiocyanate assays.[37] Tocopherols are natural antioxidants that usually exist in vegetable oils and play a pivotal role in safeguarding them from lipid oxidation. As an antioxidant, tocopherol is extensively used in food, feed, frying oil, margarine, fried foods, and pharmaceutical products.[38] Moumen et al.[7] reported four types of carotenoids in safflower oil; the total carotenoid content of safflower oil was 1.14−1.34 mg/kg. β-Carotene is a useful antioxidant for the prevention of photo-oxidation in oils and fats. Chemists normally use TAC to measure the oxidation resistance of foods; food matrixes showing higher TAC are perceived to have better radical-scavenging ability.[39] To test the antioxidant potential of naturally existing antioxidants in food systems, the DPPH assay is normally performed. TAC and DPPH values of SO, OF, and DFO are shown in Table 4. The TAC values of OF and DFO were significantly higher than those of unmodified SO. The TAC values of SO, OF, and DFO were 54.78 ± 0.12, 71.36 ± 0.58, and 86.41 ± 0.57% (at 0 day), respectively. DPPH values of SO, OF, and DFO were 41.32 ± 0.15, 55.97 ± 0.33, and 68.44 ± 0.28% (at 0 day), respectively. The higher TAC and DPPH values of DFO and OF than those of SO can be attributed to the migration of phenolic compounds, tocopherols, carotenoids, and other antioxidant substances from SO to DFO and OF. TAC and DPPH values of SO, OF, and DFO remained unchanged until 45 days of storage (p > 0.05). Testing of the TAC and DPPH values after the termination of the storage phase indicated a significant decline. TAC values of SO, OF, and DFO were 48.62 ± 0.63, 64.24 ± 0.31, and 82.47 ± 0.73% (at 90 days), respectively. DPPH values of SO, OF,

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Table 4. Antioxidant Capacity of Single- and Double-Fractionated Olein of Safflower Oil

treatments	days in storage	TAC (%)	DPPH (%)
SO	0	54.78 ± 0.12^{E}	41.32 ± 0.15^{E}
	45	53.91 ± 0.28^{E}	40.95 ± 0.49^{E}
	90	48.62 ± 0.63^{F}	35.80 ± 0.71^{F}
OF	0	71.36 ± 0.58^{C}	55.97 ± 0.33^{C}
	45	70.49 ± 0.37^{C}	55.28 ± 0.47^{C}
	90	64.24 ± 0.31^{D}	51.21 ± 0.39^{D}
DFO	0	86.41 ± 0.57^{A}	68.44 ± 0.28^{A}
	45	85.78 ± 0.81^{A}	67.73 ± 0.15^{A}
	90	82.47 ± 0.73^{B}	60.59 ±. 083^{B}

In a single column of this table, means with dissimilar letters show a significant difference ($p < 0.05$). SO: safflower oil; OF: single-fractionated olein: DFO: double-fractionated olein; TAC: total antioxidant capacity; DPPH: 1,1-diphenyl-2-picrylhydrazyl.

and DFO were 35.80 ± 0.71, 51.21 ± 0.39, and 60.59 ± 0.83% (at 90 days), respectively. Ullah et al.[40] produced OF from chia oil by the low-temperature crystallization method. Antioxidant characterization showed that TAC and DPPH values of OF were higher than those of unmodified chia oil. Total phenolic contents of safflower oil were between 2616 and 4079 mgGAE/100 g.[26] TAC and DPPH values of OF and DFO of date seed oil were significantly higher than those of the parent date seed oil.[10] TAC and DPPH values of OF and DFO of flaxseed oil decreased in the storage phase.[8]

3.5. Lipid Oxidation. Results presented in Table 5 show that low-temperature crystallization did not cause a major difference in the FFA contents of SO, OF, and DFO ($p > 0.05$). The FFA contents of SO, OF, and DFO were 0.10, 0.11, and 0.11% ($p > 0.05$), respectively. The FFA contents of cold-extracted SO, OF, and DFO were similar to those of commercially processed vegetable oils. Lower FFA contents of SO, OF, and DFO provide an opportunity for the food industry to apply SO and its fractions in the crude form for the development of functional foods and increase the functional value and functionality of traditional foods. In the scenario of diminishing energy resources and the alarming situation of food insecurity in third-world countries, it is highly encouraging for the food industry to reduce energy consumption, decrease production costs, and develop minimally processed foods. Crude oils usually have higher FFA contents; a reduction in the FFA content is normally achieved via alkali or physical refining, both of which lead to a considerable loss of neutral oil, chemicals, and water as well as energy consumption. The price of edible oils is normally determined on the basis of the FFA content and color. A reduction in the FFA content is mandatory to produce health-friendly, shelf-stable oil products and to meet the regulatory requirements. EU guidelines recommend an FFA content lesser than 0.2%. The generation of FFA in the storage phase of crude and processed edible oils is a natural process. The hydrolysis of TAGs occurs due to lipases, moisture, metal ions, and storage conditions.[41] The FFA contents of SO, OF, and DFO stored for 90 days were 0.16 ± 0.01, 0.15 ± 0.02, and 0.17 ± 0.02% ($p > 0.05$), respectively. Successive fractionation of SO increased the degree of unsaturation; no connection was established between the FFA content and the degree of unsaturation. The hydrolysis of TAGs results in an increase in the FFA content; unsaturated sites do not exhibit any prohydrolysis or catalytic effects to increase the FFA content during storage.[42] No connection was found between the degree of unsaturation and a storage phase of 3 months.[43] The increase in the FFA content should be kept under control in the storage phase as greater extents of FFA can lead to serious flavor defects and have also been implicated in catalyzing the auto-oxidation process.[44] By testing the POV in fresh and stored samples of oils and fats, their expected keeping quality and oxidative conditions can be measured. In fresh samples, a POV greater than 0.4 (mequiv O_2/kg) indicates poor storage stability.[11] From the POV, the age/storage conditions can be adjudged: if the POV is higher than 2.5 (mequiv O_2/kg), it indicates that either the samples are older or stored in inappropriate conditions.[11] In this study, the POVs of freshly prepared SO, OF, and DFO were 0.25 ± 0.01, 0.24 ± 0.01, and 0.27 ± 0.27 (mequiv O_2/kg), respectively. After the end of the storge time, the POVs of SO, OF, and DFO stored for 3 months were 0.68 ± 0.05, 0.85 ± 0.07, and 1.16 ± 0.08 (mequiv O_2/kg), respectively. Naturally existing phenolic compounds, tocopherols, carotenoids, and other antioxidant compounds in SO, OF, and DFO protected unsaturated sites from oxidative deterioration. Oxidation in oils and fats can be determined in several ways; the initial stages of auto-oxidation (peroxides) can be tested by the POV. Anwar et al.[45] monitored the POVs of three vegetable oils for 6 months. In all kinds of oils, the POV increased to different extents, and C18:2- and C18:3-rich oils showed higher POVs.

Table 5. Lipid Oxidation of Single- and Double-Fractionated Olein of Safflower Oil

treatments	days of storage	FFA (%, oleic acid)	POV (mequiv O_2/kg)	induction period (h)
SO	0	0.10 ± 0.01^{B}	0.25 ± 0.03^{D}	4.28 ± 0.05^{A}
	45	0.11 ± 0.02^{B}	0.29 ± 0.02^{D}	4.19 ± 0.04^{A}
	90	0.16 ± 0.01^{A}	0.68 ± 0.05^{C}	3.52 ± 0.13^{B}
OF	0	0.11 ± 0.01^{B}	0.24 ± 0.01^{D}	3.17 ± 0.10^{C}
	45	0.10 ± 0.01^{B}	0.30 ± 0.02^{D}	3.11 ± 0.14^{C}
	90	0.15 ± 0.02^{A}	0.85 ± 0.07^{B}	2.47 ± 0.04^{D}
DFO	0	0.11 ± 0.01^{B}	0.27 ± 0.02^{D}	1.89 ± 0.12^{E}
	45	0.11 ± 0.01^{B}	0.32 ± 0.04^{D}	1.83 ± 0.02^{E}
	90	0.17 ± 0.02^{A}	1.16 ± 0.08^{A}	1.24 ± 0.11^{F}

In a single column of this table, means with dissimilar letters show a significant difference ($p < 0.05$). SO: safflower oil; OF: single-fractionated olein: DFO: double-fractionated olein; FFA: free fatty acids; POV: peroxide value.

3.6. Induction Period. In determining the suitability of oils and fats for food applications, their oxidative stability is a very important qualitative parameter. However, measuring the oxidative stability is time-consuming in storage conditions. Therefore, in many studies, the correlation between the oil composition and oxidative stability is determined. Most of these investigations were performed using the Rancimat method, a superior method for measuring oxidative stability in accelerated oxidation. Initially, the Rancimat method was used only to determine the oxidative stability of oils and fats, but its effectiveness in measuring the oxidative stability of milk and dairy products has been reported in the literature.[46] Oxidative stability of oils and fats is governed by the fatty acid content, TAG composition, antioxidants (tocopherols, β-carotenes, etc.), and exposure to oxygen, light, temperature, and metal ions such as iron and copper. To determine the oxidative stability of oils and fats and food matrixes containing their products, research and development sectors of the

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industries have to wait for a longer period of time to ascertain the shelf stability/oxidative stability. With the advancement of analytical equipment, accelerated oxidation techniques such as Oxipres and Rancimat have been developed to shorten the product development time and to obtain the desired information on the oxidative stability of oils and fats in the shortest possible time. In this study, an inverse relationship was found between the induction period and the extent of PUFAs. Samples having higher PUFAs had a lower induction period. The induction periods for SO (C18:2 = 77.63 mg/100 g), OF (C18:2 = 81.57 mg/100 g), and DFO (C18:2 = 89.26 mg/100 g) were 4.28 ± 0.05, 3.17 ± 0.10, and 1.89 ± 0.12 h, respectively, at day 0 ($p < 0.05$). The coefficient of correlation (R^2) values between C18:2 and the induction periods of SO, OF, and DFO were 0.9834, 0.9918, and 0.9872, respectively. Khan et al.[19] compared the induction period of unmodified vegetable oils with their winterized versions (using low-temperature crystallization) and observed that winterized versions had more PUFAs and lower induction periods than unmodified vegetable oils. Nadeem et al.[21] reported that olein fractions of fats produced by low-temperature crystallization had higher extents of PUFAs with a lower induction period. The induction periods of date seed oil and its olein and super-olein fractions were in the order of date seed oil > olein fraction > super-olein fraction.[10] However, a detailed investigation of the oxidative stabilities of OF and DFO should be performed for increasing their applications in foods.

4. CONCLUSIONS

Single- and double-fractionated oleins were produced for the first time from safflower oil by dry crystallization at −45 and −70 °C. The measurement of the fatty acid composition, phytosterols, and phenolic compounds by GC-MS showed that unsaturated fatty acid, phenolic compound, and phytosterol contents were increased in double-fractionated olein. The linoleic acid content in double-fractionated olein was 89.26%, the highest compared to any known dietary lipid. The sensory features of olein and double-fractionated olein were similar to those of safflower oil. After 3 months of storage at ambient conditions, the peroxide values of safflower oil, single-fractionated olein, and double-fractionated olein were considerably lower than the allowable limit of the European Union. Single- and double-fractionated oleins of safflower oil may be used for the production of value-added bakery and dairy products and the supplementation of edible oils and ghee.

■ ASSOCIATED CONTENT

Data Availability Statement

The data set supporting the conclusions of this article is included within the article.

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A.K.: methods and investigations; M.N., R.U., and F.A.-A.: conceptualization, funding acquisition, and supervision of the analysis; M.N., M.I., N.G., M.A.R., I.H., and E.Z.: support for original drafting of the manuscript; R.S.A.: writing—original draft preparation; M.T.: writing—review and editing; M.T. and R.S.A.: visualizations. All authors agree to the publication of this manuscript.

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REFERENCES

(1) Mihaela, P.; Josef, R.; Monica, N.; Rudolf, Z. Perspectives of safflower oil as biodiesel source for South Eastern Europe (comparative study: Safflower, soybean and rapeseed). *Fuel* **2013**, *111*, 114−119, DOI: 10.1016/j.fuel.2013.04.012.

(2) FAOSTAT. FAOSTAT Online Database 2015 http://faostat3.fao.org/saffower.

(3) Ben, M. A.; Mansouri, F.; Zraibi, L.; Abid, M.; Nabloussi, A.; Fauconnier, M. L. In *Comparative Study of Four Safflower Oils (Carthamus tinctorius) Varieties Grown in Eastern of Morocco*, Inside Food Symposium, 2013.

(4) Han, X.; Cheng, L.; Zhang, R.; Bi, J. Extraction of safflower seed oil by supercritical CO_2. *J. Food Eng.* **2009**, *92* (4), 370−376, DOI: 10.1016/j.jfoodeng.2008.12.002.

(5) Norris, L. E.; Collene, A. L.; Asp, M. L.; Hsu, J. C.; Liu, L. F.; Richardson, J. R.; et al. Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. *Am. J. Clin. Nutr.* **2009**, *90* (3), 468−476, DOI: 10.3945/ajcn.2008.27371.

(6) Kim, E. O.; Kim, K. S.; Lee, W. J.; Choi, S. W. Proliferative and differentiative effects of trachelogenin isolated from germinated safflower (*Carthamus tinctorius*) seeds on calvarial bone cells. *Food Sci. Biotechnol.* **2009**, *18* (3), 689−693.

(7) Moumen, A. B.; Mansouri, F.; Richard, G.; Abid, M.; Fauconnier, M. L.; Sindic, M.; Serghini, C. H. Biochemical characterisation of the seed oils of four safflower (*Carthamus tinctorius*) varieties grown in north-eastern of Morocco. *Int. J. Food Sci. Technol.* **2015**, *50* (3), 804−810, DOI: 10.1111/ijfs.12714.

(8) Azad, M.; Muhammad, N.; Nabila, G.; Muhammad, I. Impact of fractionation on fatty acids composition, phenolic compounds, antioxidant characteristics of olein and super olein fractions of flaxseed oil. *J. Food Process. Preserv.* **2021**, *45*, No. e15369.

(9) Azeem, W.; Muhammad, N.; Shakeel, A. Stabilization of winterized cottonseed oil with chia (*Salvia hispanica* L.) seed extract at ambient temperature. *J. Food Sci. Technol.* **2015**, *52*, 7191−7199.

(10) Hussain, A.; Tusneem, K.; Sawera, S.; Ayesha, S.; Abdul, A. A.; Muhammad, A. J.; Saima, N.; Ayesha, R.; Khansa, I.; Jawed, A.; Muhammad, Y. Q.; Muhammad, A. M.; Mehwish, Z. Utilization of pumpkin, pumpkin powders, extracts, isolates, purified bioactives and pumpkin based functional food products: A key strategy to improve health in current post COVID 19 period: An updated review. *Appl. Food Res.* **2022**, *2* (2), 100241.

(11) Nadeem, M.; Muhammad, I. Promising features of *Moringa oleifera* oil: recent updates and perspectives. *Lipids Health Dis.* **2016**, *15*, 212.

(12) Ullah, R.; Muhammad, N.; Muhammad, A.; et al. Fractionation of chia oil for enrichment of omega 3 and 6 fatty acids and oxidative stability of fractions. *Food Sci. Biotechnol.* **2016**, *25*, 41−47.

(13) Arif, A. M.; Imran, J.; Muhammad, A.; Muhammad, I.; Athar, M.; Muhammad, A. Chemical characteristics of mango (*Mangifera indica* L.) kernel oil and palm oil blends for probable use as vanaspati. *J. Oil Palm Res.* **2016**, *28* (3), 344−352.

(14) *Official Method Cd 19-90. Sampling and Analysis of Commercial Fats and Oil*, AOCS1995.

(15) Qian, M. *Gas Chromatography, Food Analysis, Laboratory Manual*; Kluwer Academic Publisher, 2003.

(16) Azadmard-Damirchi, S. Review of the use of phytosterols as a detection tool for adulteration of olive oil with hazelnut oil. *Food Addit. Contam.* **2010**, *27* (1), 1−10.

(17) Wolfe, K.; Wu, X.; Liu, R. H. Antioxidant activity of apple peels. *J. Agric. Food Chem.* **2003**, *51*, 609−614.

(18) Larmond, E. *Laboratory Methods for Sensory Evaluation of Foods*; Ottawa Publications, 1987.

(19) Khan, I. T.; Muhammad, N.; Rahman, U.; Muhammad, I. Triglyceride, fatty acid profile, antioxidant characteristics, vitamins, lipid oxidation and induction period of ice cream produced from cow and Buffalo Milk. *J. Food Process. Preserv.* **2021**, *46*, No. e17278, DOI: 10.1111/jfpp.17278.

(20) Shahidi, F. *Baileys' Industrial Edible Oil and Fat Products*, 6th ed.; John Willey and Sons, 2005.

(21) Nadeem, M.; Chen, S.; Muhammad, A. Effect of Olein Fractions of Milk Fat on Oxidative Stability of Ice Cream. *Int. J. Food Prop.* **2015**, *18* (4), 735−745.

(22) Yella Reddy, S. Improving plasticity of milk fat for use in baking by fractionation. *J. Am. Oil Chem. Soc.* **2010**, *87*, 493−497.

(23) Khan, I. T.; Muhammad, N.; Muhammad, I.; Rahman, U.; Muhammad, A.; Muhammad, H. J. Antioxidant Properties of Milk and Dairy Products: A Comprehensive Review of the Current Knowledge. *Lipids Health Dis.* **2019**, *18*, 41.

(24) Khan, I. T.; Muhammad, N.; Muhammad, I.; et al. Triglyceride, fatty acid profile and antioxidant characteristics of low melting point fractions of Buffalo Milk fat. *Lipids Health Dis.* **2019**, *18*, 59.

(25) Prasanth Kumar, P. K.; Gopala Krishna, A. G. Physico-chemical characteristics and nutraceutical distribution of crude palm oil and its fractions. *Grasas Aceites* **2014**, *65* (2), 18−35, DOI: 10.3989/gya.097413.

(26) Aydeniz, B.; Guneş, O.; Yılmaz, E. Physico-chemical, sensory and aromatic properties of cold press produced safflower oil. *J. Am. Oil Chem. Soc.* **2014**, *91* (1), 99−110, DOI: 10.1007/s11746-013-2355-4.

(27) Jin, J.; Jie, L.; Zheng, L.; Cheng, M.; Xie, D.; Jin, Q.; Wang, X. Characteristics of palm mid-fractions produced from different fractionation paths and their potential usages. *Int. J. Food Prop.* **2018**, *21* (1), 58−69.

(28) Nestola, M.; Schmidt, T. C. Fully automated determination of the sterol composition and total content in edible oils and fats by online liquid chromatography, gas chromatography flame ionization detection. *J. Chromatogr. A* **2016**, *1463*, 136−143, DOI: 10.1016/j.chroma.2016.08.019.

(29) Vosoughkia, M.; Ghavamib, M.; Gharachorloo, M.; Sharrifmoghaddasi, M.; Omidi, A. H. Lipid composition and oxidative stability of oils in safflower (*Carthamus tinctorius* L.) seed varieties grown in Iran. *Adv. Environ. Biol.* **2011**, *5*, 897−903.

(30) Vosoughkia, M.; Hossainchi, G. L.; Ghavami, M.; Gharachorloo, M.; Delkhosh, B. Evaluation of oil content and fatty acid composition in seeds of different genotypes of safflower. *Int. J. Agr. Sci. Res.* **2012**, *2* (1), 59−66.

(31) Ok, S. Fast screening of Turkish olive oil by NMR spectroscopy for geographical determination and discrimination purposes. *Grasas Aceites* **2014**, *65* (2), No. e024.

(32) Nadeem, M.; Muhammad, A.; Imtiaz, H. Improvement of the oxidative stability of butter oil by blending with Moringa oleifera oil. *J. Food Process. Preserv.* **2013**, *38*, 1491−1500, DOI: 10.1111/jfpp.12108.

(33) Hamm, W.; Hamilton, R. J.; Calliauw, G. *Edible Oil Processing*; Wiley, 2013.

(34) Draper, C. R.; Edel, M. J.; Dick, I. M.; Randall, A. G.; Martin, G. B.; Prince, R. L. Phytoestrogens reduce bone loss and bone resorption in oophorectomized rats. *J. Nutr.* **1997**, *127* (9), 1795−1799.

(35) Kim, E. O.; Oh, J. H.; Lee, S. K.; Lee, J. Y.; Choi, S. W. Antioxidant properties and quantification of phenolic compounds from safflower (*Carthamus tinctorius* L.) seeds. *Food Sci. Biotechnol.* **2007**, *16*, 71−77.

(36) Kang, G. H.; Chang, E. J.; Park, S. W. Antioxidative activity of phenolic compounds in roasted safflower (*Carthamus tinctorius* L.) seeds. *Prev. Nutr. Food Sci.* **1999**, *4* (4), 221−225.

(37) Zhang, H. L.; Nagatsu, A.; Watanabe, T.; Okuyama, H. Antioxidative compounds isolated from safflower (*Cartthamus tinctorius* L.) oil cake. *Chem. Pharm. Bull.* **1997**, *45* (12), 1910−1914, DOI: 10.1248/cpb.45.1910.

(38) Mailer, R.; Potter, T.; Redden, R.; Ayton, J. In *Quality Evaluation of Safflower (Carthamus tinctorius L.) Cultivars*, 7th International Safflower Conference, New South Wales, 2008.

(39) Sies, H. Total antioxidant capacity: Appraisal of a concept. *J. Nutr.* **2007**, *137* (6), 1493−1495.

https://doi.org/10.1021/acsomega.3c08099
ACS Omega 2024, 9, 6787−6796

(40) Ullah, R.; Muhammad, N.; Muhammad, I.; et al. Omega Fatty Acids, Phenolic Compounds and Lipolysis of Cheddar Cheese Supplemented with Chia (*Salvia hispanica* L.) Oil. *J. Food Process. Preserv.* **2018**, *42* (4), No. e13566, DOI: 10.1111/jfpp.13566.
(41) O'Brien, R. D. *Fats and Oils: Formulating and Processing for Application*, 3rd ed.; CRC Press, 2008.
(42) Zheng, L.; Sun, D. W. Innovative applications of power ultrasound during food freezing processes-a review. *Trends Food Sci. Technol.* **2006**, *17* (1), 16−23.
(43) Nadeem, M.; Chen, S.; Muhammad, A. Effect of olein fraction of milk fat on oxidative stability of ice cream. *Int. J. Food Prop.* **2015**, *18* (4), 735−745, DOI: 10.1080/10942912.2013.814666.
(44) Frega, N.; Mozzon, M.; Lercker, G. Effects of free fatty acids on oxidative stability of vegetable oil. *J. Am. Oil Chem. Soc.* **1999**, *76* (3), 325−329.
(45) Anwar, F.; Latif, S.; Ashraf, M.; Gilanim, A. H. Moringa oleifera: a food plant with multiple medicinal uses. *Phytother. Res.* **2007**, *21* (1), 17−25.
(46) Ajmal, M.; Nadeem, M.; Imran, M.; Junaid, M. Lipid compositional changes and oxidation status of ultra-high temperature treated milk. *Lipids Health Dis.* **2018**, *17*, 227.

https://doi.org/10.1021/acsomega.3c08099
ACS Omega 2024, 9, 6787−6796