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Separation of Unsaturated and Saturated Fatty Acids from Sunflower Oil Via Low-Temperature Methanol Crystallization Method

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Sunflower oil (SFO) is rich in unsaturated fatty acids which is more than 80%, making it suitable for diverse applications. The primary aim of this study was to separate the sunflower oil unsaturated fatty acids (SFOUFA) and sunflower oil saturated fatty acids (SFOSFA) from the mixture of sunflower oil fatty acids (SFOFA) using the low-temperature methanol crystallization method. This separation serves as a preparatory step for producing a biolubricant base stock, leveraging SFOUFA as the starting material. The separation process was executed with a fatty acid to methanol molar ratio of 1:15 (w/v), at a temperature of -15 °C for 24 hours. Gas chromatography-mass spectrometry (GC-MS) was employed to determine the fatty acids composition of SFOUFA and SFOSFA, while Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) were utilized to characterize the separated compounds. The highest percentage of SFOUFA exceeded 98%, achieving a percentage yield of 85.72%, while the highest percentage of SFOSFA surpassed 99%, yielding 8.32%. The utilization of methanol as a solvent in low-temperature solvent crystallization was due to its notable attributes, including high efficiency, cost-effectiveness, stability, ready availability and ease of recovery.

Keywords: Fatty acids; low-energy separation; methanol crystallization; sunflower oil; separation technique

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The sunflower plant (*Helianthus annuus*) originally came from the temperate climates of North America, where the temperature typically ranges between 20 and 25 °C. In the sixteenth century, the sunflower plant was brought to Europe by Spanish explorers [1]. The term Helianthus is a Greek word that comes from two separate words which are helios (the sun) and anthos (a flower). "Sunflower" is a common term used to refer to the plant species named Helianthus in English [2]. At present, sunflower seeds are cultivated in various regions across the globe. The production of sunflowers as an oilseed crop has significantly increased worldwide, owing to their adaptability to diverse soil and climatic conditions [3]. Sunflower oil (SFO) is considered the fourth most significant oilseed crop globally in terms of economic profitability, following soybeans, rapeseed, and safflower. The largest producers of SFO are Ukraine and Russia, which collectively produced around 16 million tons of SFO in 2017 [3,4]. In Malaysia, according to 6Wresearch, India is the primary exporter of SFO followed by Turkey and China. SFO can be extracted through different types of methods such as mechanical pressing, cold pressing and solvent extraction [5]. SFO is widely used in the food, cosmetics and pharmaceutical industries.

SFO is predominantly composed of unsaturated fatty acids, which make up about 85% of the oil's total fatty acid content. Within the unsaturated fatty acids, there are two main types: monounsaturated fatty acids, such as oleic acid, which make up about 14-43% of the total fatty acids and polyunsaturated fatty acids, such as linoleic acid, which make up about 44-75% of the total fatty acids. The remaining 15% of the fatty acids in sunflower oil are saturated and are mainly composed of palmitic acid with a small amount of stearic acid [6,7]. Saturated fatty acids are solid at room temperature because they lack double bonds, allowing the molecules to align neatly and form a compact, organized structure. This tight packing results in a higher melting point. On the other hand, unsaturated fatty acids remain liquid due to the

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presence of double bonds, which introduce kinks in the hydrocarbon chain. These kinks disrupt close packing, creating a less organized structure and lowering the melting point.

There are several techniques for the separation of fatty acid, as established in prior studies, including urea complexation [8], enzymatic splitting [9], adsorption chromatography [10], molecular distillation [11] and low-temperature solvent crystallization [12, 13]. A simple yet efficient method for the separation of fatty acids into saturated and unsaturated forms is low-temperature solvent crystallization. By cooling the solvent to a low temperature, the solubility of fatty acids decreases, leading to selective crystallization. Fatty acids with higher polarity and melting points such as saturated fatty acids, crystallize first due to the polarity and melting characteristic which favor crystallization at lower temperatures. As the polarity of a fatty acid decrease, its solubility in the solvent also decreases, which means that the more polar fatty acids remain soluble at low temperature [12-14]. This well-established technique facilitates the distinct separation of mono- and polyunsaturated fatty acids from their saturated counterparts. Notably, the cost-effectiveness of this approach is underscored by the economical nature of the solvent, typically methanol, which not only renders the process economically feasible but also permits solvent reusability [14].

In previous studies, low-temperature solvent crystallization has been employed by various researchers for the separation of fatty acids mixture into saturated and unsaturated fatty acids. Jumaah et al. [12] conducted a study on the separation of saturated and unsaturated fatty acids in palm fatty acid distillate, utilizing a fatty acid to methanol ratio of 1:15 (w/v) at a temperature of -15 °C for 24 hours. The outcomes revealed that the saturated fatty acids constituted more than 95% of the product, yielding 52%, whereas the unsaturated fatty acids exceeded 93% with a yield of 48%. In a separate study, Fadzel et al. [13] focused on the purification of fatty acids from palm stearin, aiming to separate saturated and unsaturated components within the palm stearin fatty acids mixture. Employing a fatty acid to methanol ratio of 1:9 (w/v) at a temperature of 20 °C for 24 hours, the results demonstrated a remarkably high separation yield of approximately 98%. Additionally, the iodine value for unsaturated fatty acids exhibited a notable increase to 98, compared to 56 for the initial palm stearin fatty acids mixture. Japir et al. [14] worked on the separation of saturated fatty acids from a crude palm oil fatty acids mixture, employing a fatty acid to methanol ratio of 1:15 (w/v) at -15 °C for 24 hours. The results indicated that the separated saturated fatty acids accounted for 89% of the product, with a corresponding yield of 48.9%.

This study focuses on the separation of sunflower oil fatty acids (SFOFA) into sunflower saturated fatty acids (SFOSFA) and sunflower oil unsaturated fatty acids (SFOUFA) through the application of a low-temperature methanol crystallization separation technique. Until now, there have been no studies employing this technique for the separation of fatty acids from SFO. The choice of the low-temperature methanol crystallization technique is motivated by its notable attributes, including high efficiency, cost-effectiveness, stability, accessibility, and ease of recovery. The separated component, SFOUFA, resulting from this process, will serve as a starting material in the synthesis of biolubricants. SFOUFA are preferred for the production of bolubricant due to its ability to remain in liquid form across a board temperature range.

EXPERIMENTAL

Chemicals and Materials

Sunflower oil (SFO) was obtained from Melbelle Natural, Selangor, Malaysia. All chemicals were purchased from Systerm and R&M Chemicals, characterized as analytical reagent grade and employed without additional purification.

Physicochemical Properties of Sunflower Oil

A preliminary physicochemical characterization of sunflower oil (SFO) was conducted to determine the properties of oil as a raw material. The characterization tests applied to SFO encompassed determinations of iodine value, acid value, free fatty acids value, saponification value, moisture content, pour point and flash point. The determination of the iodine value for sunflower oil (SFO) was conducted using the Wijs method outlined in the American Oil Chemists' Society (AOCS) Cd 1-25 standard [13]. The acid value, representing the quantity of potassium hydroxide (KOH) required to neutralize free acids in 1 g of fat, was assessed to ascertain the quality of the oil, following the AOCS Cd 3d-63 method [15]. Saponification value, denoting the milligrams of KOH needed to saponify 1 g of fat, was determined following British Standard BS 6842.6:1977 [15]. The moisture content was determined using the Karl-Fisher method [15]. The pour point of SFO was determined utilizing the American Society for Testing and Materials (ASTM) D-97-17b (2016) method, with a modification involving the use of a U-tube as a sample container. The U-tube, filled with SFO, was placed in a freezer for 24 hours at the lowest temperature (-30 °C), and the temperature at which the sample began to flow was recorded as the pour point [13,15]. The flash point of SFO was assessed following the ASTM D 92-05a method (2010).

Hydrolysis of Sunflower Oil

SFOFA was produced from SFO via hydrolysis. According to the method from Khairuddin et al. [16], 12.5 g of SFO was weighted and mixed with 75 mL 2.3M alkaline ethanol into a 250 mL two-neck round bottom flask equipped with a reflux condenser,

thermometer and mechanical stirrer. The mixture was heated at 50.1 °C for 0.97 hour and continuously stirred at 300 rpm using a magnetic stirrer. After that, 25 mL of hydrochloric acid was added, and the washing continued by adding 50 mL of distilled water and 25 mL of n-hexane. After washing, anhydrous sodium sulphate was added and left overnight and the product was filtered using the Whatman No. 1 filter paper. The solvent (hexane) was removed by using a rotary evaporator.

Separation of SFOFA into SFOUFA and SFOSFA via Low-Temperature Methanol Crystallization

The separation of SFOUFA and SFOSFA from SFOFA was conducted using the low-temperature solvent crystallization method, where the solvent used was methanol. According to Japir et al. [14], the fatty acid mixture (SFOFA) was subjected to crystallization, with a fatty acid to methanol ratio of 1:15 (w/v), at a temperature of -15 °C, and left to crystallize for 24 hours. After 24 hours, two distinct layers of fatty acids formed. The upper layer was expected to contain the SFOUFA in combination with methanol, while the lower layer contained SFOSFA. The solidified mixture underwent filtration using a borosilicate glass filter funnel connected to a vacuum pump to ensure a uniform and expedited filtration process. The solid SFOSFA was rinsed with cold methanol to dissolve any remaining traces of SFOUFA. This rinsing process was repeated three times to ensure complete separation. To recover the SFOUFA from methanol, a rotary evaporator was employed.

Analysis of Fatty Acids Composition

Preparation of Fatty Acids Methyl Ester (FAME)

Fatty acid methyl esters (FAME) were prepared using two distinct methods: base-catalyzed for SFO and acid-catalyzed for SFOFA, SFOUFA and SFOSFA according to Nor et al. [17]. For the base-catalyzed method, a mixture was created by combining 0.1 mL of SFO with 1 mL of hexane. Subsequently, 1 mL of sodium methoxide solution (prepared by dissolving 1.55 grams of sodium hydroxide in 50 mL of methanol) was introduced to the oil solution. The mixture was vigorously stirred using a Vortex stirrer for 10 seconds. After allowing the solution to stand for 10 minutes, the clear FAME layer separated from the cloudy aqueous layer was collected and injected into a gas chromatograph (GC-MS) for analysis.

For the acid-catalyzed method, 1 gram of fatty acids (SFOFA, SFOUFA, and SFOSFA) were mixed with 3.75 mL of methanol. Subsequently, a 0.75 mL reagent mixture (prepared by mixing 5 mL of methanol and 1.25 mL of concentrated hydrochloric acid (37%)) was added, followed by 0.75 mL of toluene. The mixture was heated at 65 °C for 1.5 hours. The mixture was then transferred to a separation funnel and 7.5 mL of hexane and 5 mL of distilled water were added. After allowing the mixture to stand to allow the separation of the two layers, the upper layer was carefully collected. It was then dried using anhydrous sodium sulphate overnight. The sample was filtered and subsequently injected into a GC-MS for analysis.

GC-MS Analysis

The composition of fatty acids in SFO, SFOFA, SFOUFA and SFOSFA were determined using gas chromatography mass spectrometry (GC-MS) analysis using Agilent technologies 7890A gas chromatograph coupled to Agilent Technologies 5975 mass spectrometer and equipped with HP-88 fused silica capillary column (100 m x 250 μm x 0.25 μm i.d; film thickness 0.25 μm; Agilent, USA). The operating conditions for GC-MS were from Abdullah et al. [18] with slight modifications as follows, injection volume 1μL, split ratio of 90:1 with helium as a carrier gas with a flow rate of 25 mL/min. The oven temperature was maintained at 150 °C for 5 min and increased to 240 °C and held for 20 min at a rate of 4 °C/min. The temperature of the injector and detector were maintained at 250 °C and 260 °C, respectively.

Structural Analysis

Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) were used to determine the functional groups and identify the structure of SFO, SFOFA, SFOUFA and SFOSFA, respectively. For FTIR, the Perkin Elmer Infrared spectrometer was used at a range of 650 to 4000 cm^{-1}. For NMR, JOEL-ECP 400 spectrometer was used at 400 MHz for ^{1}H NMR and 100.61 MHz for ^{13}C NMR and deuterated chloroform ($CDCl_3$) was used as a solvent.

RESULTS AND DISCUSSION

Physicochemical Properties of Sunflower Oil

The physicochemical properties of SFO were tabulated in **Table 1** by comparing with previous studies reported by Aboki et al.[19] and Karmakar et al. [20]. The iodine value of SFO was determined to be 118.01, exhibiting a marginal decrease in comparison to the referenced value. The acid value for SFO was recorded at 0.14, indicating a lower acidity level than the reference (0.953), while the free fatty acids content was measured at 0.072%, signifying an elevated value compared to the reference value of 0.042%. The saponification value for SFO was noted at 190.37, surpassing the reference value of 182.23. The moisture content in SFO was determined to be 0.091%. The flash point and pour point of SFO were -13 °C and 280 °C, respectively, demonstrating minimal deviation from the reference values.

Table 1. Physicochemical properties of sunflower oil.

Physicochemical Properties	Experimental	Reference*
Iodine value (g I_2/100g)	118.01	119.92[a]
Acid value (mg KOH/g)	0.14	0.953[a]
Free fatty acids value (%)	0.072	0.042[a]
Saponification value	190.37	182.23[a]
Moisture content (%)	0.091	NA
Pour point (°C)	-13	-15[b]
Flash point (°C)	280	274[b]

*Sources: [a][19]; [b][20]

Hydrolysis and Separation of SFOUFA into SFOUFA and SFOSFA

The hydrolysis of sunflower oil (SFO) resulted in the production of sunflower oil fatty acids (SFOFA) and glycerol as byproducts, with a yield percentage of 96%. Subsequently, the obtained SFOFA was subjected to a separation process into saturated fatty acids (SFOSFA) and unsaturated fatty acids (SFOUFA) using the low-temperature solvent crystallization (LTSC) method, employing methanol as the solvent. This choice of solvent is based on its ability to crystallize fatty acids, with the key determinant being the solubility of the different fatty acid components [14].

In the composition of sunflower oil fatty acids, approximately 20% was comprised of saturated fatty acids (SFOSFA), while the remaining 80% consisted of unsaturated fatty acids (SFOUFA). Among the saturated fatty acids, those with longer carbon chains, such as palmitic and stearic acids (C16 and above), are categorized as non-polar compounds. Conversely, shorter-chain saturated fatty acids like myristic and lauric acids (C14 and below) exhibit a slightly higher degree of polarity in comparison to their longer-chain counterparts. The polarity of saturated fatty acids diminishes as the carbon chain length increases, resulting in increased non-polarity. In contrast, unsaturated fatty acids, which encompass both monounsaturated and polyunsaturated varieties, exhibit higher levels of polarity when compared to saturated fatty acids [12]. This increased polarity is a crucial factor in their enhanced solubility in methanol during the low-temperature crystallization process.

In this study, the methanol crystallization method was used to separate saturated from unsaturated fatty acids, offering a simpler approach compared to the urea complexation method. Urea complexation, as described by Salimon et al.[8], is typically used to isolate polyunsaturated fatty acids from saturated and monounsaturated fatty acids. However, other research, such as Yong et al. [21], applied urea encapsulation to separate fatty acids without specifically focusing on polyunsaturated ones. In contrast, the methanol crystallization method employed here achieves a broad separation of saturated and unsaturated fatty acids and requires only methanol as a solvent, whereas urea complexation requires both urea and ethanol, making the crystallization method more straightforward and accessible.

The choice of methanol as the solvent in the low-temperature solvent crystallization (LTSC) process is also driven by its propensity to selectively dissolve unsaturated fatty acids (SFOUFA) over saturated fatty acids (SFOSFA), owing to its inherent polar characteristics. As the temperature decreases during the crystallization procedure, the solubility of less polar saturated fatty acids decreases, resulting in their precipitation as crystalline solids. In contrast, more polar unsaturated fatty acids remain in solution due to their elevated solubility at lower temperatures [12,13].

The solvent choice adheres to the "like dissolves like" principle, which stipulates that polar solvents are predisposed to dissolve polar compounds, while non-polar solvents favor their non-polar counterparts. Methanol's polar nature aligns with this principle, thus promoting the enhanced solubility of unsaturated fatty acids, which exhibit greater polarity when compared to their less polar saturated counterparts [14].

The solubility of the fatty acids in the mixed SFOFA compounds in methanol adheres to a specific order from the most to least polar, with linolenic acid, linoleic acid, oleic acid, paullinic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, tricosanoic acid and lignoceric acid, illustrating varying degrees of solubility. This solubility trend is attributed to the polarity and hydrogen bonding characteristics inherent in the compounds present within the mixture, underscoring the importance of these properties in dictating their interaction with the methanol solvent.

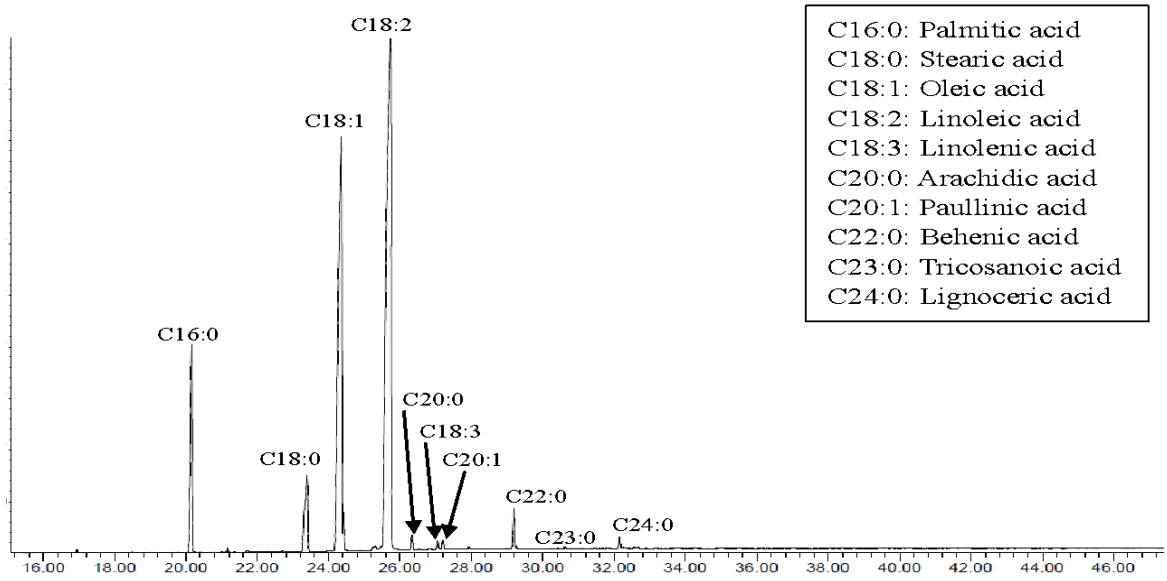

Figure 1. GC-MS chromatogram of SFO.

The separation of saturated fatty acids (SFOSFA) and unsaturated fatty acids (SFOUFA) from the mixture of sunflower oil fatty acids (SFOFA) yielded 8.32% for SFOSFA and 85.72% for SFOUFA. This successful separation can be attributed to the differential solubility of individual fatty acids in methanol and the solvent used in the low-temperature solvent crystallization (LTSC) process. The high solubility of linolenic, linoleic, oleic and paullinic acids in methanol enables them to remain in solution, contributing to the higher yield of SFOUFA. In contrast, the lower solubility of palmitic, stearic, arachidic, behenic, tricosanoic and lignoceric acids in methanol causes them to preferentially crystallize out during the LTSC process, leading to the enrichment of SFOSFA. The solubility of fatty acids is closely related to their melting points, which in turn, are influenced by the number of carbon atoms and the degree of unsaturation in the fatty acid molecules [12, 14]. These factors make the separation of SFOFA into SFOSFA and SFOUFA feasible. Notably, the solubility of fatty acids increases with rising temperature. This behavior is evident in the LTSC process, where high melting point fatty acids display lower solubility in methanol compared to low melting point fatty acids. Consequently, at lower temperatures during crystallization, the less soluble fatty acids with higher melting points tend to precipitate, allowing the selective separation of SFOSFA from SFOUFA.

Fatty Acids Composition

The fatty acids composition in SFO, SFOFA (before separation), SFOSFA and SFOUFA (after separation) were shown in Figures 1, 2, 3 and 4, respectively. **Table 2** shows the comparison in the composition of fatty acids in SFO, SFOFA, SFOUFA and SFOSFA. The percentage of fatty acids in SFO as shown in **Figure 1** consists of 15.23% saturated fatty acids and 84.77% unsaturated fatty acids. The saturated fatty acids mainly consisted of palmitic acid (8.12%), stearic acid (5.06%), arachidic acid (0.40%), behenic acid (1.23%), tricosanoic acid (0.04%) and lignoceric acid (0.38%), while oleic acid (33.11%), linoleic acid (51.18%), linolenic acid (0.25%) and paullinic acid (0.23%) were the main component of unsaturated fatty acids.

The fatty acid composition in SFOFA (after hydrolysis) was shown in **Figure 2**. The fatty acids composition of SFO and SFOFA were slightly different. In SFOFA, the saturated fatty acids were 13.27% and unsaturated fatty acids were 86.73%. The saturated fatty acids mainly consisted of palmitic acid (7.44%), stearic acid (4.29%), arachidic acid (0.30%), behenic acid (0.91%), tricosanoic acid (0.02%) and lignoceric acid (0.31%). The unsaturated fatty acids consisted of oleic acid (32.33%), linoleic acid (54.01%), linolenic acid (0.21%) and paullinic acid (0.18%).

The fatty acids composition after separation for SFOSFA and SFOUFA were shown in **Figures 3** and **4**, respectively. For SFOSFA after the separation, the fatty acids composition consisted of only saturated fatty acids such as palmitic acid (29.28%), stearic acid (41.24%), arachidic acid (04.55%), behenic acid (17.48%), tricosanoic acid (0.72%) and lignoceric acid (6.73%). For SFOUFA, the composition of unsaturated fatty acids mainly consisted of oleic acid (38.35%), linoleic acid (59.91%), linolenic acid (0.34%) and paullinic acid (0.29%) with a small amount of palmitic acid (1.11%).

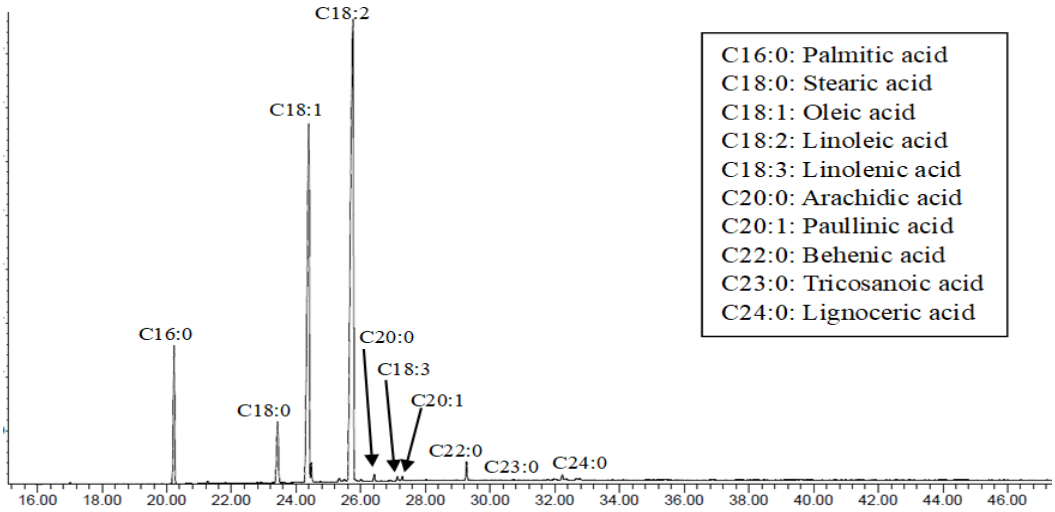

Figure 2. GC-MS chromatogram of SFOFA.

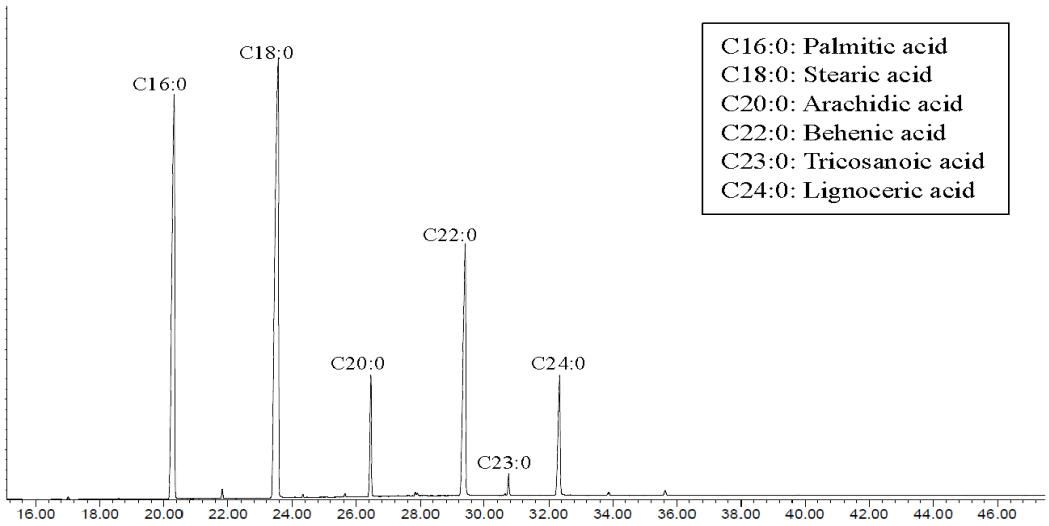

Figure 3. GC-MS chromatogram of SFOSFA.

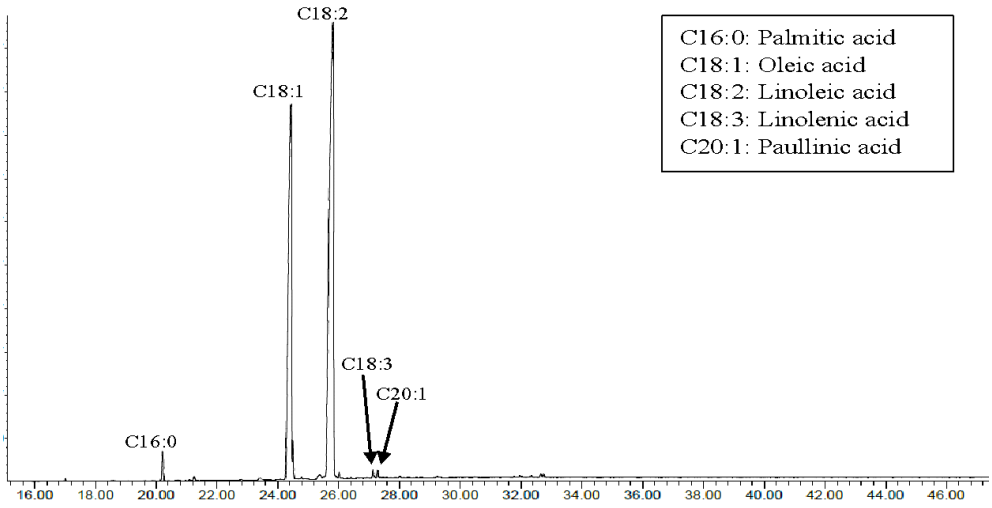

Figure 4. GC-MS chromatogram of SFOUFA.

Table 2. Fatty acids composition of SGO, SFOFA, SFOSFA and SFOUFA.

Fatty acids	Structure	Percentage fatty acids composition (%)			
		SFO	SFOFA	SFOSFA	SFOUFA
Palmitic acid	C16:0	8.12	7.44	29.28	1.11
Stearic acid	C18:0	5.06	4.29	41.24	-
Oleic acid	C18:1	33.11	32.33	-	38.35
Linoleic acid	C18:2	51.18	54.01	-	59.91
Linolenic acid	C18:3	0.25	0.21	-	0.34
Arachidic acid	C20:0	0.40	0.30	4.55	-
Paullinic acid	C20:1	0.23	0.18	-	0.29
Behenic acid	C22:0	1.23	0.91	17.48	-
Tricosanoic acid	C23:0	0.04	0.02	0.72	-
Lignoceric acid	C24:0	0.38	0.31	6.73	-
Saturated fatty acids		15.23	13.27	100	1.11
Unsaturated fatty acids		84.77	86.73	-	98.89

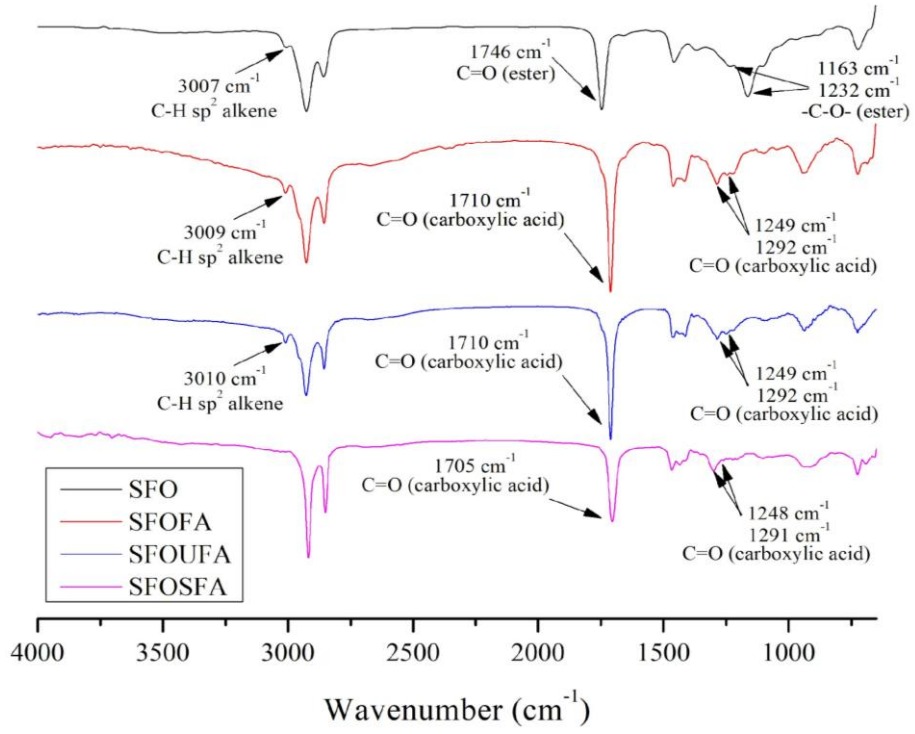

Figure 5. FTIR spectra for SFO, SFOFA, SFOSFA and SFOUFA.

Structural Analysis

Fourier-Transform Infrared (FTIR)

The Perkin Elmer Model 100 spectrometer, utilizing attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy, was employed to analyze the functional groups present in sunflower oil (SFO), sunflower oil fatty acids (SFOFA), sunflower oil unsaturated fatty acids (SFOUFA) and sunflower oil saturated fatty acids (SFOSFA). The FTIR spectra of these samples, depicted in **Figure 5**, exhibit distinct peaks and bands indicative of specific functional groups. In the spectrum of SFO, a sharp peak at 1746

cm^{-1} signifies the presence of C=O ester, while peaks at 1163 cm^{-1} and 1232 cm^{-1} correspond to C-O ester. Following hydrolysis (SFOFA), a sharp peak at 1710 cm^{-1} represents C=O carboxylic acid, and peaks at 1249 cm^{-1} and 1292 cm^{-1} indicate C-O carboxylic acid. After separation, the FTIR spectra for SFOUFA and SFOSFA showed peaks at 1710 cm^{-1} and 1705 cm^{-1}, respectively, indicating the presence of C=O for carboxylic acid. Additionally, a broad peak spanning 2400 to 3400 cm^{-1} in SFOFA, SFOUFA, and SFOSFA signify the presence of hydroxyl groups (OH) associated with carboxylic acid (COOH) functional groups inherent in the fatty acids. The successful separation of the SFOFA mixture into SFOSFA was evidenced by the absence of peaks at 3009 cm^{-1} in the SFOSFA spectrum, typically associated with the stretching vibrations of sp^2 carbon-hydrogen (C-H) bonds commonly found in alkenes. This notable absence of alkene peaks provides compelling evidence of the efficacy of the separation process. **Table 3** shows the functional group and wavenumber for FTIR spectra of SFO, SFOFA, SFOUFA and SFOSFA.

Table 3. Functional group and wavenumber for FTIR spectra of SFO, SFOFA, SFOUFA and SFOSFA.

Functional group	Wavenumber (cm^{-1})			
	SFO	SFOFA	SFOUFA	SFOSFA
OH stretching (carboxylic acid)	-	2400-3400	2400-3400	2400-3400
C-H sp^2 stretching (aliphatic)	3007	3009	3010	-
C-H sp^3 stretching (aliphatic)	2926, 2857	2926, 2856	2926, 2856	2926, 2857
C=O stretching (ester)	1746	-	-	-
C=O stretching (carboxylic acid)	-	1710	1710	1705
-C-O- stretching (ester)	1232, 1163	-	-	-
-C-O- stretching (carboxylic acid)	-	1292, 1249	1292, 1249	1291, 1248

Table 4. The chemical shift for ^1H NMR spectra of SFO, SFOFA, SFOUFA and SFOSFA.

	Type of proton	Chemical shift (ppm)			
		SFO	SFOFA	SFOUFA	SFOSFA
H_1	$-CH_3$	0.88-0.92	0.89-0.93	0.89-0.93	0.90-0.92
H_2	$-CH_2-$	1.29-1.41	1.29-1.40	1.28-1.39	1.28-1.34
H_3	$O=C-CH_2-CH_2-$	1.61-1.63	1.64-1.68	1.62-1.67	1.62-1.69
H_4	$-CH=CH-CH_2-$	2.00-2.09	2.03-2.10	2.03-2.10	-
H_5	$O=C-CH_2-$	2.30-2.35	2.35-2.38	2.34-2.38	2.34
H_6	$=CH-CH_2-CH=$	2.77-2.80	2.78-2.81	2.78-2.81	-
H_7	$-CH_2-COOR-$ (glycerol)	4.14-4.18	-	-	-
H_8	-CH-COOR (glycerol)	4.29-4.33	-	-	-
H_9	-CH=CH-	5.28-5.41	5.33-5.41	5.33-5.41	-

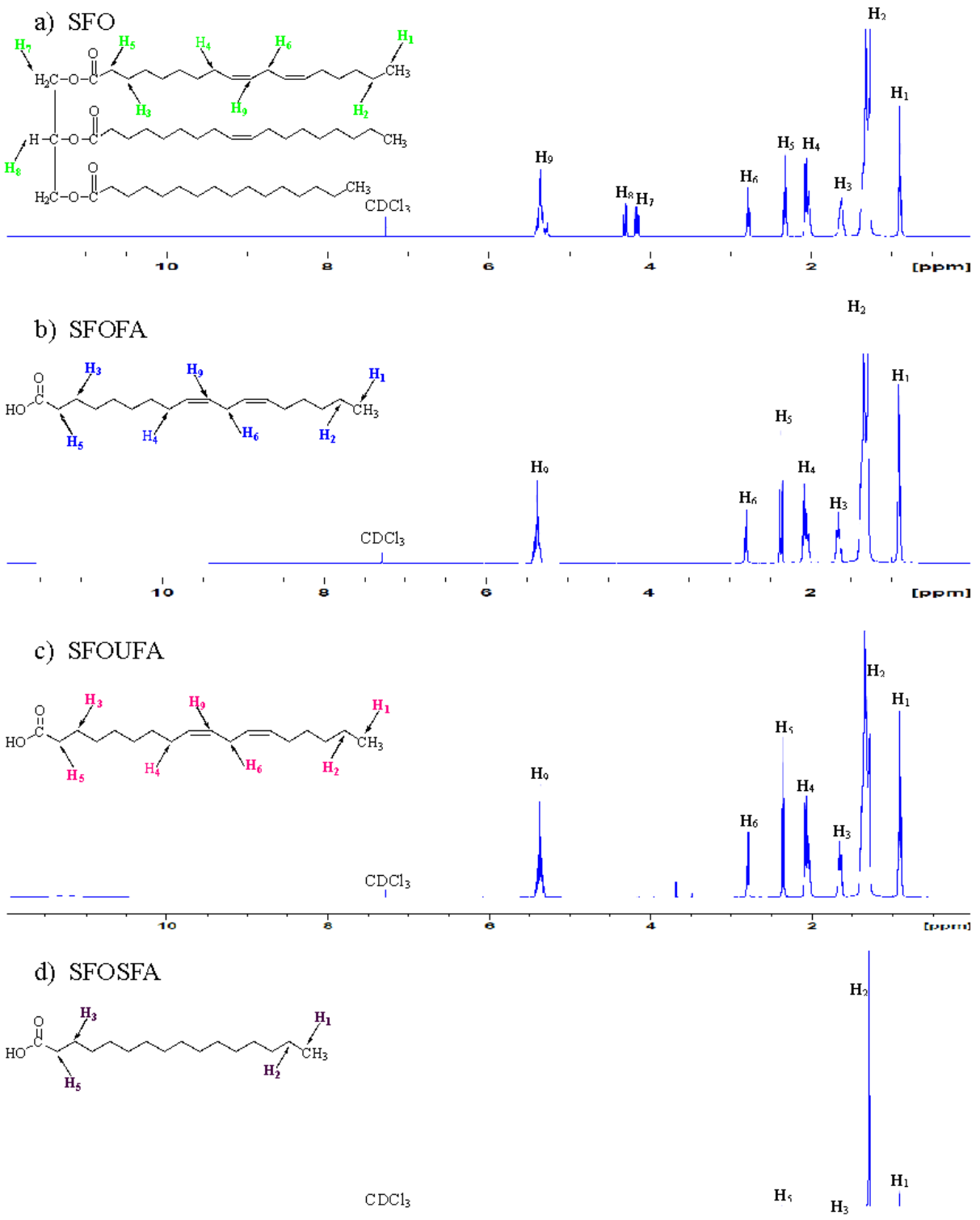

Figure 6. ^{1}H NMR spectra of SFO, SFOFA, SFOUFA and SFOSFA.

Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance spectroscopy (NMR) was utilized to determine the chemical structures of SFO, SFOFA, SFOSFA and SFOUFA. Pavia et al. [22] was used as reference for the NMR. **Figure 6** and **Table 4** present the ^{1}H NMR spectra of SFO, SFOFA, SFOUFA and SFOSFA, along with their respective chemical shifts. Before the hydrolysis, a chemical shift appeared at range 4.14-4.18 for -C**H**$_2$-COOR- and 4.29-4.33 for -C**H**-COOR, which come from the glycerol backbone. After the hydrolysis (SFOFA),

these two chemical shifts disappeared. The successful separation of SFOFA into these two components was confirmed by observing specific signals in the ^{1}H NMR spectra. At ^{1}H NMR spectra of SFOUFA, a distinctive signal was observed in the chemical shift range of 2.03 to 2.10 ppm, indicating the presence of a specific chemical group (-CH=CH-C**H**$_2$-). This signal was absent in the ^{1}H NMR spectrum of SFOSFA. Another signal appeared in the chemical shift range of 2.78 to 2.81 ppm in the ^{1}H NMR spectrum of SFOUFA, representing the presence of another chemical group (=CH-C**H**$_2$-CH=), which was not present in the ^{1}H NMR spectrum of SFOSFA. Additionally, a distinct signal was identified at the chemical shift range of 5.33 to 5.41 ppm in the ^{1}H NMR spectrum of SFOUFA, corresponding to yet another chemical group (-C**H**=CH-). This particular signal was not observed in the ^{1}H NMR spectrum of SFOSFA. The absence of these signals in the ^{1}H NMR spectrum of SFOSFA serves as a confirmation of the successful separation and differentiation between the unsaturated and saturated fatty acids. Both SFOSFA and SFOUFA samples were dissolved in deuterated chloroform ($CDCl_3$) for NMR analysis, which introduced a reference signal at 7.28 ppm originating from the solvent.

^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy was employed to assess the successfulness of hydrolysis and separation of sunflower oil fatty acids (SFOFA) into saturated fatty acids (SFOSFA) and unsaturated fatty acids (SFOUFA). **Figure 7** and **Table 5** present the ^{13}C NMR spectra of SFO, SFOFA, SFOUFA and SFOSFA, along with their respective chemical shifts. Before hydrolysis, the ^{13}C NMR spectrum (SFO) exhibited distinctive peaks at chemical shifts of 62.09 ppm, 68.90 ppm, and 172.79-173.21 ppm, corresponding to -**C**H$_2$-O, -**C**H-O, and O=**C**-OR (ester), respectively. Post-hydrolysis, these peaks disappeared, and a new peak emerged at a chemical shift of 180.45 ppm, indicative of O=**C**-OH (carboxylic acid) in SFOFA spectrum. A peak in the chemical shift range of 127.90 to 130.54 ppm, denoting the -**C**H=CH- group, appeared in the ^{13}C NMR spectrum. This peak was present in the SFOUFA spectrum but absent in the SFOSFA spectrum, providing clear evidence of the successful separation between the two fatty acid types. Furthermore, the ^{13}C NMR spectra revealed the presence of carboxylic acid (**C**OOH) functional groups in both SFOSFA and SFOUFA, with peaks at chemical shifts of 179.71 ppm and 180.33 ppm, respectively. The solvent peak corresponding to deuterated chloroform ($CDCl_3$) in the ^{13}C NMR spectrum was identified at a chemical shift range of 76.71 to 77.36 ppm. The observed chemical shifts in both ^{1}H NMR and ^{13}C NMR spectra aligned well with theoretical values, reinforcing the accuracy of the NMR analysis.

Table 5. The chemical shift for ^{13}C NMR spectra of SFO, SFOFA, SFOUFA and SFOSFA.

	Type of carbon	Chemical shift (ppm)			
		SFO	SFOFA	SFOUFA	SFOSFA
C_1	-**C**H$_3$	14.05-14.09	14.02	14.11	14.03-14.07
C_2	-**C**H$_2$-	22.56-34.18	22.65-34.10	22.39-29.77	22.69-29.67
C_3	-**C**H$_2$-O (glycerol)	62.09	-	-	-
C_4	-**C**H-O (glycerol)	68.90	-	-	-
C_5	-**C**=C-	127.9-130.2	127.9-130.2	127.9-130.5	-
C_6	O=**C**-OR (ester)	172.8-173.2	-	-	-
C_7	O=**C**-OH (carboxylic acid)	-	180.45	180.33	179.71

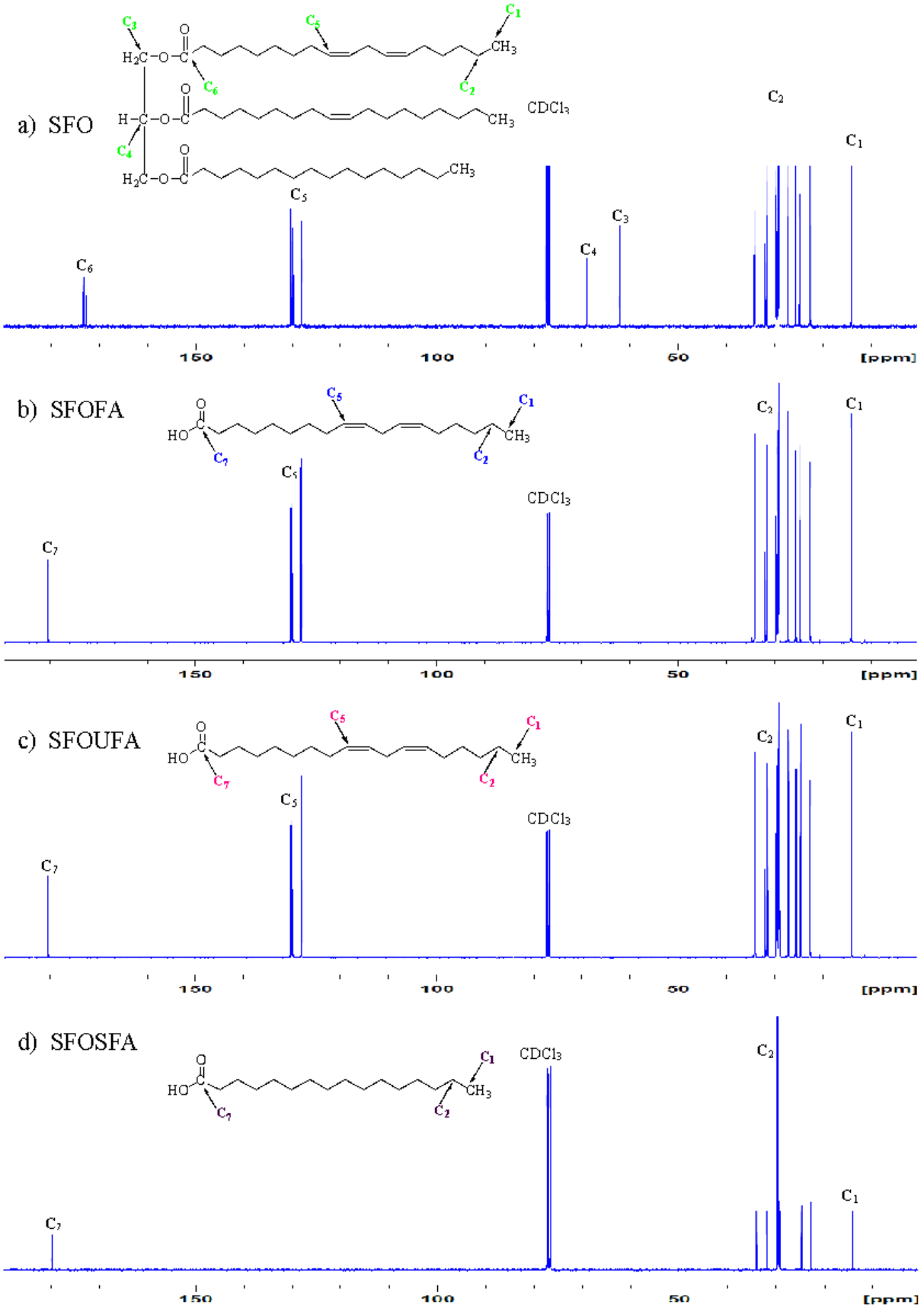

Figure 7. ^{13}C NMR spectra of SFO, SFOFA, SFOUFA and SFOSFA.

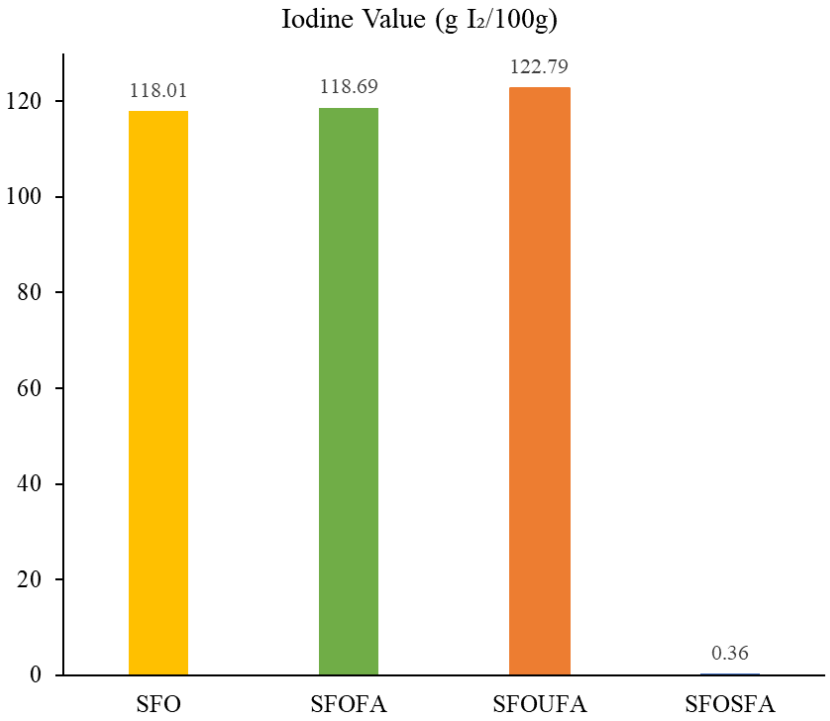

Figure 8. Iodine value before and after separation.

Iodine Value

The iodine value test serves as a quantitative measure to evaluate the level of unsaturation in different components, including SFO, SFOFA, SFOUFA and SFOSFA. **Figure 8** illustrates the iodine values obtained both before (SFO and SFOFA) and after (SFOSFA and SFOUFA) the separation process achieved through methanol crystallization. Initially, the iodine value of SFO was determined to be 118.01 g I_2/100 g, indicative of the oil's degree of unsaturation. Following the hydrolysis process, which produced SFOFA, the iodine value slightly increased to 118.69 g I_2/100 g. The separation of SFOFA into SFOSFA and SFOUFA had a pronounced effect on the iodine values. The iodine value of SFOUFA notably rose to 122.79 g I_2/100 g, underscoring the prevalence of unsaturated fatty acids in this fraction. In contrast, the iodine value of SFOSFA exhibited a substantial decrease, plummeting to 0.36 g I_2/100 g, indicating the almost complete removal of unsaturated fatty acids from this component. Japir et al. [14] stated that decrease in iodine value for the saturated fatty acids indicates the successful separation of the fatty acids.

CONCLUSION

The sunflower oil fatty acids (SFOFA) were successfully separated into sunflower oil unsaturated fatty acids (SFOUFA) and sunflower oil saturated fatty acids (SFOSFA) using the separation technique low-temperature methanol crystallization method. The percentage of SFOUFA exceeded 98%, achieving a percentage yield of 85.72%, while the highest percentage of SFOSFA surpassed 99%, yielding 8.32%. The fatty acids composition in SFOUFA consisted of 38.35% oleic acid, 59.91% linoleic acid, 0.34% linolenic acid and 0.29% paullinic acid with small amount of palmitic acid (1.11%) while the fatty acids composition of SFOSFA consisted of 29.28% palmitic acid, 41.24% stearic acid, 4.55% arachidic acid, 17.48% behenic acid, 0.72% tricosanoic acid and 6.73% lignoceric acid. FTIR and NMR analyses further confirmed this separation, as the disappearance of characteristic peaks at 3009 cm^{-1} in the FTIR spectrum, 2.03–2.10, 2.78–2.89, and 5.23–5.41 ppm in the ^1H NMR spectrum, and 127.9–130.2 ppm in the ^{13}C NMR spectrum for SFOSFA indicated the absence of double bonds, consistent with a highly saturated profile. The utilization of methanol as a solvent in low-temperature solvent crystallization for the separation of saturated and unsaturated fatty acids is recommended due to its notable attributes, including high efficiency, cost-effectiveness, stability, ready availability and ease of recovery.

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