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Fatty Acid Evaluation of Seeds and Nuts by Spectroscopy and Chromatography

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Abstract The study aimed to determine the oil content and identify fatty acid methyl esters such as stearate, palmitate, linolenate, linoleate, and oleate in seeds and nuts like candlenut, peanut, sesame, sunflower, and sacha inchi. Oil extraction was carried out using Soxtec 8000^{TM} and n-hexane solvent. The samples were dried at 50^{o}C. The extraction conditions optimized were temperature 135^{o}C, n-hexane 50 mL, boiling time 40 min, and total extraction time 115 min. Identification of fatty acids was carried out using Attenuated Total Reflection (ATR), Nuclear Magnetic Resonance (NMR), and Gas Chromatography-Flames Ionization Detector (GC-FID). The oil percentage detected in each sample was candlenut (59.95%), peanut (40.08%), sesame (57.06%), sunflower (43.97%), and sacha inchi (51.71). The ATR results show that the flour of nuts and seeds has a strong vibrational frequency of the O−H molecule. Linolenate was detected at a chemical shift of 0.975 ppm in NMR spectra and was found in sacha inchi and candlenut. The ATR, NMR, and GC-FID results showed that all samples contained unsaturated fatty acids. Candlenut, peanut, sesame, and sacha inchi were rich in linoleate (ω-6) as much as 25.68%, 20.15%, 26.38%, and 20.73%, respectively. Oleate was abundant in sesame (21.87%) and sunflower (16.78%). Linolenate was found only in sacha inchi (22.88%). The maximum percentage of stearate was found in sunflower (4.02%) followed by sesame (2.96%), candlenut (1.81%), sacha inchi (1.52%), and peanut (0.71%). This research provides useful information on fatty acid profiles beneficial for health, especially stearic acid, which can substitute trans fatty acids harmful to health.

Keywords Fatty Acid, Soxtec, Spectroscopy, Chromatography

1. Introduction

Seeds and nuts rich in fatty acids have their prominence in the food and pharmaceutical industries. Consumption of nuts can reduce the potential for cardiovascular disease and reduce diabetes and prostate disease [1]. Depending on their degree of saturation/unsaturation in the carbon chain, fatty acid methyl esters can be divided into three classes; saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). Fatty acid methyl esters commonly found in vegetable oils are palmitate, stearate, oleate, linolenate, and linoleate [2]. Among all fatty acid methyl esters, linolenate is the main in vegetable oils [3]. Linolenate and linoleate are essential PUFAs [4].

Fatty acid methyl esters needed by the industry are linolenate (ω-3), linoleate (ω-6) [5], oleate (ω-9) [6], and stearate [6–8]. Stearic acid or stearate is an SFA found in palm oil and sunflower [6, 9], *Nymphaea pubescens* seeds [10], *Ocimum basilicum* [5], candlenut [11, 12], sesame [13], *Cactus opuntia dillenii* [14], sacha inchi [15] and almond seeds [16]. *Nymphaea pubescens* seeds contain stearic acid as palm oil and peanut oil [10]. Most of the stearic acid is obtained from the sunflower growing at low-temperature conditions [17]. Besides, it also depends on plant breeding and industrial processing methods [4]. Stearic acid can replace trans fatty acids that can trigger cardiovascular disease [18], allergies, cancer, obesity, and even death [4]. Compared to stearate, linolenate has high reactivity and is less stable [19].

Attenuated Total Reflectance (ATR) is a vibration spectrophotometer for determining the structure of organic molecules. It is commonly performed at wavenumbers 670 - 4000 cm^{-1} [20, 21]. The ATR can be carried out on liquid or solid samples. Solid samples are ground into flour. The sample preparation is simple and short. Spectra ATR is similar to Fourier-transform infrared spectroscopy (FTIR). Both can identify the vibrational frequency of molecule bonds at the same wavenumber, but the intensity is relatively different [21]. The application of vibration spectrophotometers in fatty acids has been carried out on vegetable oils [22–26].

Chromatography can be used for the separation, identification, and determination of constituents in the sample. The sample is dissolved in either gas, solid, or supercritical fluid in the stationary phase. After that, sample is distributed in two phases, namely the stationary phase and the mobile phase. The constituents having a long retention time move slowly. The difference migration speed is read as peaks that can be analyzed quantitatively and qualitatively. Identification of samples using chromatography is based on retention times [27]. Gas chromatography performs with a partition between gas and liquid. The detector responds to separate constituents. The analyte concentration can be calculated from the peak area ratio of the constituent to the total peak area. A flame ionization detector's advantage changes in flow rate have little effect on the detector's response [28]. Fatty acid analysis in oils using GC has been carried out [22, 23, 25, 26, 29–33].

Stearic acid or stearate as SFA in seeds and nuts helps in eliminating the potential cardiovascular disease. Also, unsaturated fatty acids linolenate, linoleate, and oleate are good for health. Therefore, researchers have an interest in the evaluation of fatty acids in seeds and nuts using spectroscopy and chromatography. The present study's objectives are (a) optimization of oil extraction method (b) identification of fatty acids in seeds and nuts using ATR, NMR, and GC-FID. It is hoped that the study provides useful information on sources of beneficial fatty acid methyl esters, especially stearate in seeds and nuts.

2. Materials and Methods

Seeds and nuts (candlenut, peanut, sacha inchi, sesame, sunflower) were collected from Taiwan. Chemicals and equipment used were n-hexane high grade (CAS 110543), acetone (CAS 67-64-1), methanol 99.5% (Merck), sodium hydroxide (CAS 1310732), sodium chloride (CAS 764145), hydrochloric acid (CAS 110563), distilled water. d-chloroform (865496), tetramethylsilane (TMS) 99.9% (CIL), distilled water, drying oven (DOS45), Soxtec^{TM} 8000 extraction unit (FOSS), analytical balance (AND GR-200), moisture analyzer (AND MX-50 JASCO), ATR model PRO450-S, NMR Bruker AVANCE 500 MHz, GC-FID (Agilent Technologies 7890B), CP-Sil 88 column 100 m diameter 0.25 mm, temperature limits 50^0C - 225^0C.

1) Oil extraction procedure

The sample was made into flour and then dried at 50^oC until constant weight. Also, the effect of drying time on moisture values was carried out. The time variation range used was 0 - 96 hours. The samples were stored in a closed glass bottle at room temperature until use.

Sample (3 g) was weighed and transferred into a cellulose cup and then kept into the Soxtec. The aluminum cup was weighed, filled with 50 mL n-hexane, and then kept into the Soxtec. The extraction was carried out at 135^oC, boiling time 40 min, condensation for 60 min, and a recovery time of 15 min. After completion of the extraction, the machine automatically shuts down. The aluminum cup was heated at 80^oC for 60 min to evaporate the solvent. The cup was allowed to cool down at room temperature and weighed. The oil extract weight was obtained from the difference between the initial weight and the aluminum cup's final weight. The percentage of oil extract was obtained from the extract's weight divided by the sample's total weight. This research also investigated temperature, sample weight, solvent volume, and boiling time. The temperature variations used were 100 - 145^0C, weight 1-6 g, solvent volume 40-90 mL, and boiling time 20-80 minutes.

2) Attenuated Total Reflection (ATR) procedure

For a liquid sample, 1 drop of oil was applied onto the prism measuring surface. Turned the handle counterclockwise to raise the pressure head and contact with the prism. Set the wavenumber to 400 – 4000 cm^{-1}. The prism was properly cleaned. The prism crystals' surface was cleaned with acetone and then waited for 10-15 min. The same steps were carried out for the flour samples.

3) Nuclear Magnetic Resonance (NMR) procedure

A total of 100 µL of oil sample was taken into the

NMR sample tube, then 600 µL of d-chloroform was added and then homogenized for 5 minutes. After that, 1 drop of TMS was added using a dropper, then the sample was homogenized again. The NMR sample tube was closed tightly and then inserted into the NMR machine. Before shimming, the temperature was set to 298K. The shimming process of NMR spectra was carried out to get symmetrical NMR peaks.

4) Gas Chromatography – Flames Ionization Detector (GC-FID) Procedure

The sample was prepared by saponification and esterification. Oil (0.05 g) was mixed with 1 mL n-Hexane. The mixture (1 mL) was taken and added with 1 mL of 1N NaOH in methanol. Saponification was carried out at 80^{0}C for 1 h and then proceeded with the esterification process at 75^{0}C for 2 h using 1 mL of 3N HCl in methanol. After completion of the esterification, 6 mL of saturated sodium was added to the mixture, centrifuged until it separated into two layers. After that, the organic layer was taken, and then 1 mL of n-hexane was added. It was filtered then analyzed with a GC-FID machine. The chromatography tube was set at an initial temperature of 130^{0}C, heating speed of 1^{0}C / min. The final temperature was set to 200^{0}C for 60 min. Front inlet mode was set to 28 psi, 250 ^{0}C, split ratio 50: 1, mobile phase was Helium, split-flow 33.3 mL/min.

3. Results and Discussion

1) Oil extraction

The intrinsic quality and distribution period of a food ingredient depends on the moisture content. Excess moisture can cause mold growth quickly to spoil the food. The market allows a maximum of 10% moisture in walnut [34, 35]. Most seeds and nuts are processed by roasting to release oil in the cells, thus facilitating oil extraction [16]. The drying process with different methods has been reported by Nejad [36]. This process does not significantly affect free fatty acids in pistachio nuts.

In our study, a drying temperature of 50^{o}C and a duration of 48-72 h have resulted in the lowest moisture percentages in five seeds and nuts (Fig. 1). According to Najad [36], products dried at low temperatures have good storage stability. Drying at a temperature of 30 -70^{o}C can reduce the activity of the enzymes in nuts. Drying for a shorter duration is better because it does not change the color of the product. Ajibola [37] has reported that with increasing moisture, the porosity can increase. The change in percent of moisture after drying in each sample was 6.1% to 2.42% (sunflower), 5.39% to 2.42% (peanut); 5.19% - 2.76% (sacha inchi), 3.86% to 1.77% (candlenut), and 1.92% to 1.56% (sesame) (Fig. 2). The percentage of moisture safe for storing peanuts is 6-8% [38, 39]. According to the report, high percentages of oil extracts can be obtained at low moisture levels. At high moisture, the obtained oil content is low [39].

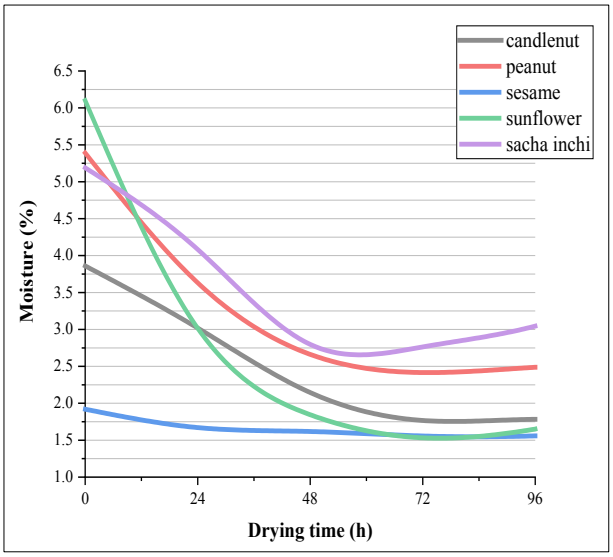


Figure 1. Percent of moisture in different samples

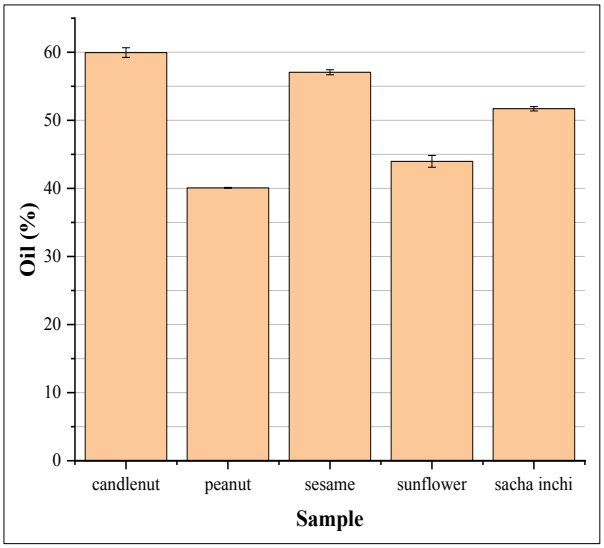


Figure 2. Percentages of oil in different samples

In the present study, oil extraction was carried out by the Soxtec 8000^{TM}. Compared with the Soxhlet, Soxtec 8000^{TM} has a short time for the extraction process, and the solvent recovery is automatic. While the maximum weight of the sample is 15 g. Some parameters that need to be optimized during the extraction by Soxtec are temperature, sample weight, type of solvent, solid-liquid ratio, and extraction time [40]. The optimum extraction conditions using Soxtec and n-hexane were: solvent volume 50 mL, boiling time 40 min, temperature 135^{o}C. the oil percentages in five seeds and nuts were: candlenut

(59.95%), peanut (40.08%), sesame (57.06%), sunflower (43.97%), sacha inchi (51.71 %) (Fig. 2).

Several researchers have used hexane as a solvent in the Soxhlet method [10, 41, 42]. The different solvents (n-hexane, ethyl acetate, petroleum ether, acetone) showed different chemical characteristics, affecting the potential nutritional value of oil. Extraction by n-hexane had higher monounsaturated fatty acid (C18:1) and lower polyunsaturated fatty acids (C18:2) [43]. Extraction by hexane solvent at cold temperature conditions produced oil extracts rich in linolenate [44].

2) ATR analysis

Seeds and nuts are common sources of protein. Besides, flour also contains carbohydrates, oil, minerals, and water. Functional groups found in candlenut, peanut, sesame, sunflower, and sacha inchi flours included amino groups, carboxyl groups, hydroxyl groups, carbonyl groups (aldehydes or ketones), esters, alkanes, and alkenes. IR spectra in Fig. 3 show the vibrational frequency of the molecules. The stronger the vibration, the higher is the absorbance value, which means a high concentration of these molecules in the sample.

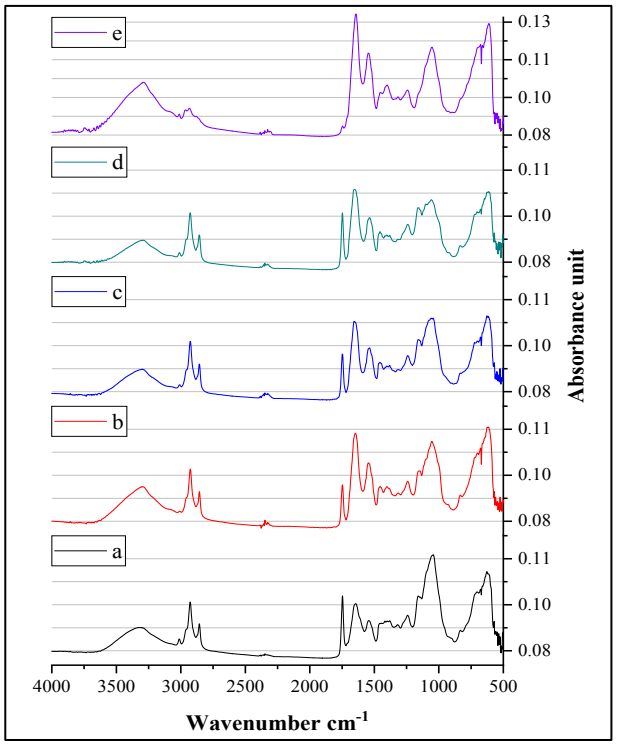


Figure 3. ATR spectra of candlenut flour (a), peanut flour (b), sesame flour (c), sunflower flour (d), and sacha inchi flour (e)

Table 1. IR spectra interpretation of candlenut flour (a), peanut flour (b), sesame flour (c), sunflower flour (d), and sacha inchi flour (e).

Bond	Wavenumber (cm^{-1})					
	Reference [21]	a	b	c	d	e
N—H	3300-3500	3320	3283	3295	3301	3283
C—N	1180-1360	1239	1239	1238	1237	1237
	3010-3095	3011				3013
	1500 -1600	1538	1544	1536	1533	1541
	675 - 995	627	624	627	613	612
C—H	2850-2970	2929	2927	2927	2927	2933
	1610-1680	1651	1645	1660	1651	1641
C—O	1050 -1300	1043	1053	1053	1058	1057
C=O	1690 -1760	1748	1748	1748	1748	
O—H	2500-3650	3320	3283	3295	3301	3283

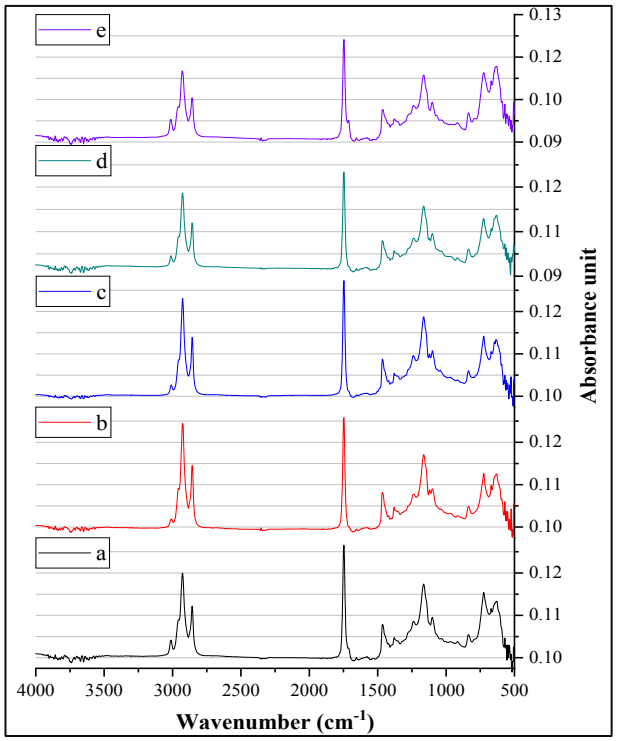


Figure 4A. ATR spectra of oil in candlenut (a), peanut (b), sesame (c), sunflower (d), and sacha inchi (e)

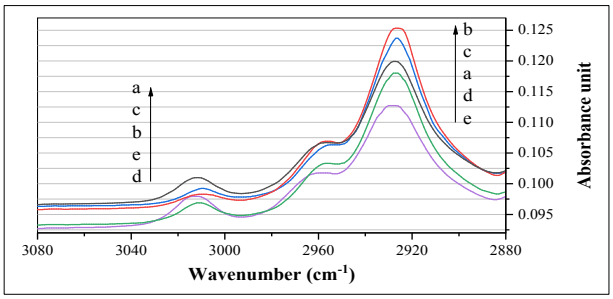


Figure 4B. ATR spectra of oil expand 2880 - 3050 cm^{-1} (B) in candlenut (a), peanut (b), sesame (c), sunflower (d), and sacha inchi (e)

The IR spectra of flour (Fig. 3) and oil (Fig. 4) have a difference in the wavenumber of 3400 cm^{-1}, indicating that the vibrational frequency of O−H molecules in oil is less than the flour. The extraction process using temperature above the boiling point of water may cause weak vibrations of O−H molecules in the oil. O−H vibrational frequency overlaps with N−H vibrational frequency in flour, allowing denaturation of the protein during oil extraction. Heating at a temperature above 60^0C caused denaturation of proteins. Many peaks overlapped at wavenumbers around 1200 cm^{-1} (Figure 3 and Table 1). It is due to single bond vibrations which have almost the same energy [28], such as C−N (1180 – 1360 cm^{-1}), C−H (1340 – 1470 cm^{-1}), C−O (1050 – 1300 cm^{-1}).

Oil is a mixture of glycerol and triglycerides composed of alkane, alkenes, and esters functional groups. IR spectra of oil are presented in Fig. 4A and the interpretation in Table 2. Sacha inchi oil has strong peaks in 3016 cm^{-1} (stretching vibration C=C), 2925 cm^{-1}, and 2855 cm^{-1} (stretching vibrations overlapping with C−H methylene group vibrations). The high absorbance value indicates that the oil is rich in polyunsaturated fatty acids [23]. The frequency of molecular vibrations at the wavenumber between 3009 - 3006 cm^{-1} occurred in non-oxidized oil (Fig. 4B). The peak at 3009 cm^{-1} is the vibration stretching of a cis double bond. The higher the oil concentration, the higher the peak height was [45]. Based on this, the strong vibrations of unsaturated fatty acids were found in candlenut, sesame, peanut, sacha inchi, and sunflower, respectively. The peak at 2952 cm^{-1} is vibrations of the aliphatic group attached to =CH, and the stronger vibration of that molecule was present in peanut, sesame candlenut, sunflower, and sacha inchi, respectively. The difference in vibrations' strength indicates a different type of unsaturated fatty acids in the sample.

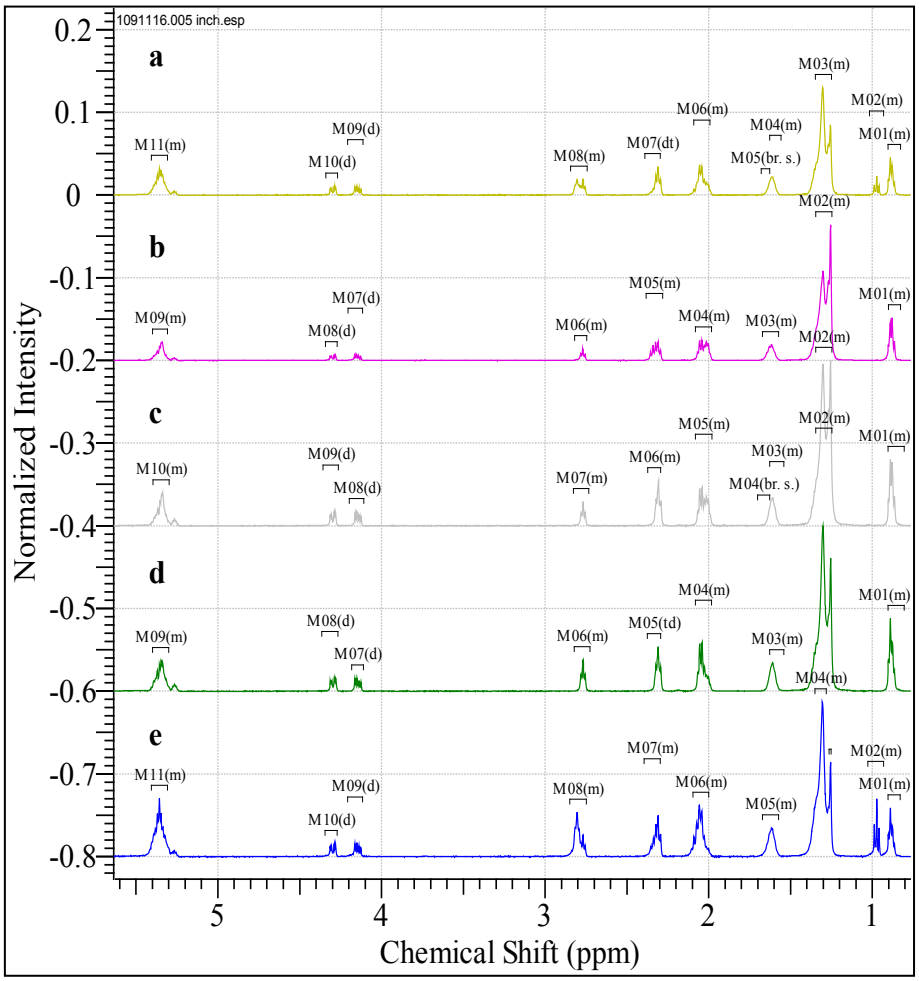
Table 2. IR spectra interpretation of oil in candlenut (a), peanut (b), sesame (c), sunflower (d), and sacha inchi (e)

Bond	Wavenumber (cm^{-1})					
	Reference [43]	a	b	c	d	e
	3010	3008	3000	3010	3012	3016
	1653	1652	1653	1649	1659	1659
	995-675	911 837 721 633	834 727 630	835 726 633	914 833 722 629	913 833 722 635
C—H	2927	2929	2929	2925	2925	2925
	2885	2859	2859	2857	2855	2855
	1461	1466	1463	1461	1464	1467
	1376	1378	1377	1377	1377	1376
C=O	1746	1744	1750	1747	1746	1749
C—O	1238	1239	1242	1237	1242	1237
	1163	1165	1165	1166	1161	1168
	1118-1120		1116	1123		
	1100	1104	1096	1102	1101	1099

The ester molecule's strong stretching vibration was also observed at wavenumber 1168 $cm^{-1,}$ indicating that the oil is rich in unsaturated fatty acids. The peak at 1749 cm^{-1} is a stretching vibration of the triglyceride molecule. Aliphatic functional groups that overlap with aryl were detected at wavenumber 1467 cm^{-1}. Based on the trend of peaks in all samples, it can be seen that all samples contain MUFAs and PUFAs. Overlapping of peaks has been reported earlier. The vibration of the methylene group (CH_2) overlaps with cis-alkene (cis-HC=CH), the stretching vibration ester (C-O), the bending vibration C-H, the stretching vibration ester (C=O), and hydroxyl group (OH) [24].

3) NMR analysis

NMR analysis was carried out on oils according to the methylene group both on α-CH_2 attached to and CH_2 as the long chain of fatty acids. NMR spectra in all samples showed different peaks but had a similar pattern. It indicates all samples have a similar functional group. (Fig 5). Unsaturated fatty acids methyl ester such as oleate (ω-9), linoleate (ω-6), and other acyl groups exist in a chemical shift of 0.83 - 0.93 ppm. ω-6 on sacha inchi appears at 0.889 ppm, ω-9 appears at 0.879 on linseed oil, while ω-3 was detected at 0.94 - 1 ppm [23]. ω-3 appears at 2.07 ppm (multiplet), ω-6 at 2.3 ppm (multiplet), ω-9 at 2.01 ppm (multiplet) [46]. Based on the data in Table 3 and Fig. 5, ω-3 was found in sacha inchi and candlenut, while ω-6 and ω-9 were found in all samples. In this experiment, candlenut and sacha inchi contained ω-3 because both had chemical shifts at 2.80 ppm (proton identities in C11 and C14 as a structure of linolenate). ω-3 was also found by Guillén [23] in sacha inchi oil. The NMR results were correlated with the detection of molecular vibrations in IR, which inform at peak 3010, 2927, 2889, 1168 cm^{-1} as PUFAs and MUFAs.



nd: M01: Multiplets 01; s (singlet); br.s (broadened singlet); d (doublet); dt (double triplet); m (multiplet)

Figure 5. ^{1}H NMR of oil in candlenut (a), peanut (b), sesame (c), sunflower (d), and sacha inchi (e).

Table 3. Identification of ω-3, ω-6, ω-9 and SFA in Candlenut (a), Peanut (b), Sesame (c), Sunflower (d), and Sacha Inchi (e)

Functional Group	Chemical shift (ppm)				
	a	b	c	d	e
ω-6 acyl	0.880	0.880	0.879	0.879	0.879
	2.03	2.03	2.03	2.03	2.04
ω-9 acyl	0.890	0.891	0.890	0.890	0.890
ω-3 acyl	0.975	nd	nd	nd	0.975
	2.06	nd	nd	nd	2.07
Methyl Saturated	1.256	1.256	1.256	1.256	1.256
Methyl Unsaturated	1.305	1.303	1.303	1.303	1.303
	2.02	2.02	2.02	2.02	nd

The proportion of protons in the fatty acid chain can be observed. For example, 28 protons contribute to the methylene group of C22:1 at a chemical shift of 1.2-1.4 ppm [47]. The height intensity of the peak at chemical shift 1.2 – 1.4 ppm belongs to the proton signal of the methylene group. Chemical shift 1.2 ppm belongs to methylene saturated while 1.3 ppm belongs to methylene unsaturated. The proportion of methylene saturated in candlenut (0.65), sunflower (0.81), and sacha inchi (0.60) was lower than methylene unsaturated. Sesame (1.02) has balanced proportions, and peanut has more proportion of SFA (1.52). The peak at 0.98 belongs to the ω-3 acyl group. The height of the ω-3 acyl group was equal to the ω-9 acyl group and ω-6 acyl group on sacha inchi. The proportion of ω-3 acyl group in the candlenut was less than ω-9 acyl group and ω-6 acyl group. The GC results in Table 4 support NMR data, sacha inchi rich in linolenate. The types of saturated fatty acids in vegetable oil were palmitate and stearate. Both of them did not have proton signals at chemical shifts of 5.36, 2.78, 2.03 ppm. This chemical shift was owned by protons in the functional groups =CH (Olefinic), =CH−CH−CH= (allylic), =CH−CH_2 (bis-allylic) [46–48]. Based on this, palmitate and stearate as SFAs were detected in all samples, including candlenut, peanut, sesame, sunflower, and sacha inchi. The ω-3 acyl group, ω-6 acyl group, ω-9 acyl group, and SFA have a functional group and chemical shift: −CH_2COOH (2.35-2.36 ppm), −CH_2CH_2COOH (1,61-1.64 ppm), −CH_2 (1.24-1.35 ppm) and −CH_3 (0.88-0.98 ppm). Different types of fatty acid containing the same functional group can cause peak overlap on the same chemical shift.

4) GC-FID analysis

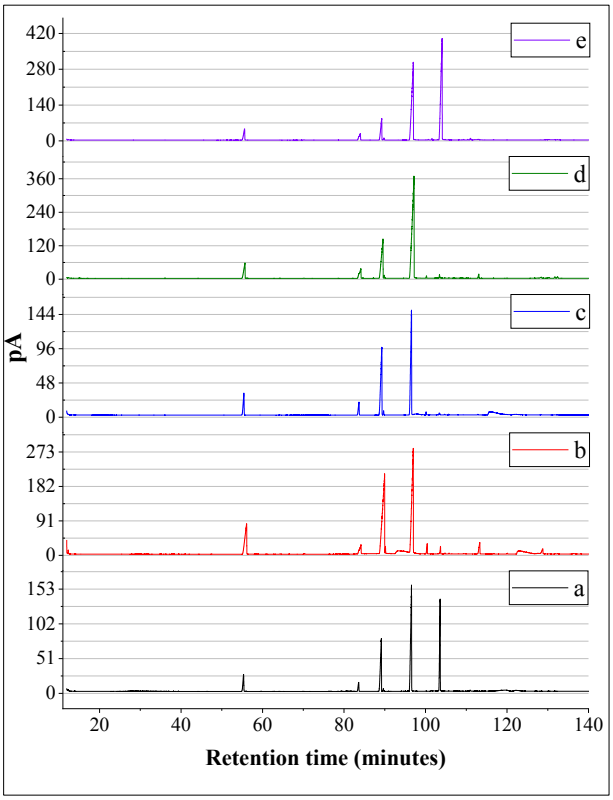


Figure 6. GC-FID chromatogram of oil in candlenut (a), peanut (b), sesame (c), sunflower (d), and sacha inchi (e).

Table 4. Identification of fatty acid in candlenut (a), peanut (b), sesame (c), sunflower (d), and sacha inchi (e).

Fatty acid methyl ester (FAME)		a (%)	b (%)	c (%)	d (%)	e (%)
Methyl Butyrate	C14:0	nd	0.16	nd	nd	nd
Methyl Palmitate	C16:0	3.79	7.01	5.40	5.26	2.15
Methyl Stearate	C18:0	1.81	0.71	2.96	4.02	1.52
Methyl Arachidate	C20:0	nd	0.80	0.32	0.26	nd
Methyl Behenate	C22:0	nd	1.43	nd	0.71	0.20
Methyl Oleate (ω-9)	C18:1	13.67	9.39	21.87	16.79	4.17
Methyl Eicosenoate	C20:1	14.78	0.37		0.48	nd
Methyl Linoleate (ω-6)	C18:2	25.69	20.15	26.38	16.12	20.74
Methyl Linolenate (ω-3)	C18:3	nd	nd	nd	nd	22.88
	SFA	5.59	10.11	8.68	10.25	3.87
Total	MUFA	28.45	9.75	21.87	17.27	4.17
	PUFA	25.69	20.15	26.38	16.12	43.62

nd: not detected; SFA: saturated fatty acid; MUFA monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

GC-FID analysis shows that each sample has various types of fatty acid methyl esters (Fig. 6, Table 4). There are five types of fatty acid methyl esters in candlenut: linoleate, eicosenoate, oleate, palmitate, and stearate. Peanut has eight types of fatty acid methyl esters such as linoleate, oleate, palmitate, behenate, arachidonate, stearate, eicosenoate, butyrate. Sesame has five types of fatty acid methyl esters: linoleate, oleate, palmitate, stearate, and arachidonate. Sunflower has seven types of fatty acid methyl esters: oleate, linoleate, palmitate, stearate, behenate, eicosenoate, arachidonate. Sacha inchi has six types of fatty acid methyl esters: linolenate, linoleate, oleate, palmitate, stearate, behenate. Only 0.157% butyrates were found in peanuts. Peanuts have been reported to contain butyrate as much as 127 mg/g [1]. Most SFAs were found in sunflower with palmitate and stearate. Most MUFAs were found in candlenut with the types of eicosenoate and oleic. Most PUFAs were found in sacha inchi with linolenate and linoleate. Only sacha inchi has linolenate as much as 22.88%. Some researchers have found linolenate in sacha inchi as much as 36% [49], 44% [30], and 50.8% [50]. Based on the NMR results, candlenut also contains linolenate, but it is not detected in the GC results. Linoleate and linolenate are PUFAs. These have the same functional group that caused the proton signal detection overlap. Linolenate is a fatty acid methyl ester that causes an oxidation reaction very quickly compared with stearate. The high levels of PUFAs and MUFAs in oil can cause an oxidative reaction, especially at high temperatures [51]. The double bond is sensitive to oxidation reaction, as the electrons become stronger, attracting protons around them [52]. Peanut has a peak in retention time of 89.3; 89.5; 89.7; 89.8; 89.9; 89.9 min. The retention time is identical to that of oleate. The SFAs type in peanuts was more varied than in other samples. This supports the NMR data, showing that the proportion of SFAs proton signals is more than unsaturated at 1.2 - 1.3 ppm

4. Conclusions

Extraction conditions like Soxtec 8000^{TM} at 135^{0}C, n-hexane 50 mL, boiling time 40 min, total extraction time 115 min, sample weight 3g resulted in different oil percentages in different seeds and nuts, e.g., candlenut (59.95%), peanut (40.08%), sesame (57.06%), sunflower (43.97%), and sacha inchi (51.71%). The IR spectra of oil and flour showed different peaks of the OH bond at 3400 cm^{-1}. The peak was stronger in flour compared to oil. Many peaks overlapped in the wavenumber around 1200 cm^{-1}. NMR results show that the chemical composition of the oil is diverse. Linolenate has a chemical shift of 0.975 ppm and was only found in candlenut and Sacha inch oils. The ATR, NMR, and GC-FID results showed that all samples contained unsaturated fatty acid. PUFAs and MUFAs were mostly found in candlenut, followed by sesame, sacha inchi, sunflower, and peanut. The types of fatty acid methyl esters varied among five different seeds and nuts, e.g., candlenut has five types of fatty acid methyl esters, while peanut has eight types, sesame five types, sunflower seven types, and six types in sacha inchi. Candlenut, peanut, sesame, and sacha inchi were rich in linoleate (ω-6) as much as 25.68 %, 20.15%, 26.38%, and 20.73%, respectively. Oleate (ω-9) was abundant in sesame (21.87%) and sunflower (16.78%). Linolenate (ω-6) (22.88%) was found only in sacha inchi. The maximum percent of the stearate was found in sunflower (4.02%) followed by sesame (2.96%), candlenut (1.81%), sacha inchi (1.52%), and peanut (0.71%). The area percentage ratio calculation showed that the largest area of the SFAs area was sesame. The MUFAs were mainly

found in candlenut, while the sacha inchi mostly have PUFAs.

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Competing Interests

Authors have no competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Authors' Contributions

SY and WJC conceived the research idea. SY, DSS, LHY, and CYC collected the data, developed the theory, verified and analyzed the data. SY prepared the manuscript draft. WJC supervised the research work. DCA made a substantial contribution to the revision of the manuscript. All authors discussed the results and contributed to the final manuscript.

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