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Research Article

Diagnosis and Characterization by Using NMR, FT-IR, HPLC and G C Spectroscopies for Flaxseeds (Brown Variety) Oil Content from Libya

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Abstract

Objectives: Extraction of oil from *Linum u.* seeds, investigating, characterizing of this oil by using chemical and extensive spectroscopic methodologies.

Methods: The oil constituents were isolated by using the Soxhlet Apparatus Extractor in n-hexane as a solvent, and diagnosis, characterization by the Gas Chromatography Technique (GC), High-Performance Liquid Chromatography (HPLC), FTIR, H^1, C^{13} NMR, Oil Thermal Analysis (DSC) and Determination of Solid Fat Content (SFC %).

Results: The percentage yield of oil content of *Linum u.* was 39%. The Physiochemical Properties, the acid value was 4.4 mg/g, Free Fatty Acid content (FFA %) free oleic acid was 2.2%, the average molecular weight value was 848 and the density of oil ranged from 0.89 g/cm^3. According to triacylglycerols profiles (TAG)s in the oil were polyunsaturated of OLL with 17.45%, LnLnLn with 20.65 - 21.44%, OLnLn with 8.18 - 8.84%, PLLn with 6.72%, PLnLn with 7.97%. Whereas the FTIR Spectrum results of the presence of the functional groups of triacylglycerols were peaks at 3010- 3020, 2927-924, 1743-1743, 1462-1463, 1376-1377, 1238-1250, 1100-1110, and 722-721 cm^{-1}. Thermal Transition Properties for quality of the *Linum u.* oil after melting in the Differential Scanning Calorimetric were showed a simple thermo-gram. While the obtained results from the p NMR analysis of the Solid Fat Contents were decreased when the temperature increased until it reaches room temperature 25^{0}C, which means that the oil contains higher unsaturated fatty acid 89.5% more than the saturated fatty acid 10.5%. Whereas by H^1 and C^{13} NMR chemical shifts were observed that there were no significant differences between the *Linum u.* oil from Libya and *Linum u.* oil from Canada, and where both oils contain triacylglycerol TAG in which the NMR chemical shifts were consistent. Accordingly, the H^1 NMR spectrum shows the chemical shift at 0.868-0.986 ppm and 0.5 ppm referred to the methylene group, respectively, and the terminal methyl group which is between 1.25-1.343 ppm and 1.0 ppm, and for the methylene 2.005 ppm and 2.0 ppm, the acyl groups were at 5.253-5.39 ppm and 5.2 ppm. As well as the methylene groups of glyceryl were at 4.281-4.308 ppm and 4.5 ppm. In addition, the C^{13} NMR spectrum shows the main signals assignment at 172.76-173.17ppm refers to the carbon atom of the carbonyl group, and the signals at 127.12-131.92 ppm refer to the unsaturated carbon atoms 62.09-68.92 ppm due to the glyceryl carbon atoms, and were the methylene carbon atoms were further distinguished into those adjacent to one double bond, saturated at 29.0-29.7 ppm. As well, the results obtained by Gas Chromatography (G C) were 89.4% for unsaturated fatty acid and 10.1% for saturated fatty acid. And the unsaturated fatty acids present in the oil are dominated by 55% linoleic acid (C18.3), 15.9% linoleic acid and 17.8% oleic acid. Meanwhile, saturation fatty acids, present in the *Linum u.* oil consisted of 5.3% Palmitic acid (C16:0) and 4.4% Stearic acid (C18:0). The HPLC results showed that LnLnLn (21%) was dominated by triacylglycerols.

Conclusion: The findings of the study revealed that *Linum u*. oil has the potential uses as a source of pharmaceutical for treating diseases. Therefore flaxseed plants can be further assayed for pharmacological preparations.

Keywords: *Linum usitatissimum* (*Linum u.*); HPLC; G C; FTIR; NMR; Oil Thermal Analysis; Solid Fat Content

Introduction

Medical plants are usually used in conventional medicine as treatments for several diseases and contagious illnesses. Flaxseed, which is also called linseed, is important seed oil in the world. Flaxseed, *Linum usitatissimum (Linum u.)* is a multi-purpose crop. Two types of flaxseed are grown, the seed flax for the oil in its seed and the fibre flax for the fibre in its stem. It is mostly grown in Canada, Argentina, America, China and India [1].

The *Linum u.* plant range in height from 30 to 100 cm and have narrow leaves and flowers that are in different shades of blue. Its seeds contain about 36 to 40% of oil and have been long used in human and animal diets and industry as a source of oil and as the basic component or additive of various paints or polymers [2]. While the main components of the *Linum u.* are protein (21%), dietary fibre (28%), and oil (40%). As well, the nutritional components of the *Linum u.* are oil, protein, lignans, soluble fibre, minerals, vitamins, etc. *Linum u.* also has a unique fatty acid profile. It is high in polyunsaturated fatty acid (73% of total fatty acids), moderate in monounsaturated fatty acid (18%) and low in saturated fatty acid (9%). Linoleic acid, an omega-6 fatty acid, constitutes about 16% of the total fatty acids whereas alpha-linolenic acid (ALA) constitutes about 57% [3], the highest of any seed oil [4]. From the composition of *Linum u.* oil, that oil-rich source from omega -3, thus this gave to oil many beneficial for some diseases.

On another hand, the Omega-3 fatty acid is essential for growth and development as well as having been associated with the prevention and treatment of heart disease, arthritis, inflammatory, autoimmune diseases and cancer**.** In humans, omega-3 fatty acids have also been utilised to prevent cancer-associated cachexia and to improve the quality of life [5]. The results of animal studies have demonstrated that the consumption of omega-3 fatty acids can slow the growth of cancer xenografts, increase the efficacy and reduce the side effects of chemotherapy or cancer by molecular mechanisms such as suppressing the expression of cyclooxygenase-2 in tumours and decreasing the expression of AP-1 and ras, two oncogenes implicated in the tumour promotion [6]. It has been reported that the dietary n-3 fatty acids may significantly retard the growth of tumours and that (omega-6) fatty acids may cause the development of tumours**.**

In addition to being ant arrhythmic, omega-3 fatty acids are antithrombotic and anti-inflammatory. *Linum u.* has recently gained attention as a "functional food" because of its unique nutrient profile and potential to affect the risk and cause of cardiovascular disease and some cancers [4], particularly hormone-dependent cancers such as the prostate and breast [7]. Functional foods are those that correspond to traditional foods, but affect benefits beyond their nutrition and energy value in advancing health and preventing certain chronic diseases, especially cardiovascular disease, cancer, arrhythmia, autoimmune disorders, arthritis, and diabetes [8]. Thus, the use of *Linum u.* in food has increased during the past decade due to the presence of functional compounds alpha-linolenic acid (ALA), lignans, and fibre [9]. The *Linum u.* oil is stemmed from the seeds of the flax plant. Unrefined flaxseed oil from good seed has an attractive golden colour, a pleasant, nut-like flavour and mild odour. The oil from flaxseed is qualitatively different from the more familiar vegetable oils with high PUFA proportions, such as rape oil, soya oil, olive oil, sunflower oil, etc.

Flaxseed provides as much as 75-800 times the amount of lignans as that of other oil seeds, cereals, legumes, fruits and vegetables [4]. In Libya, the Flaxseed is considered a plant that has been around for 5.000 years. And two species grow from the *Linum u.* in two different colours (yellow colour and brown colour (*Linum u.*) and is an erect annual plant growing to 1.2m tall with slender stems. The leaves are glucose green, slender and approximately 20-40 mm long and 3 mm in diameter with five petals, where it is used locally by people in Libya for medical purposes such as cough cirrhosis of the liver and weight loss [10].

What is more, flaxseed is rich in bioactive constituents such as phenolic compounds like lignans, ferulic, p-coumaric acid and is also affluent by mucilage which is a benefit for intestinal function [11]. Besides the Flaxseed oil owns an assortment of health benefits, similar as reducing the risk of coronary heart disease, preventing mammary, colon cancers, lowering plasma low-density lipoprotein (LDL) levels, the prevention and treatment of type I and type II diabetes [12].

Thereupon, the aim of this study is the extraction of oil from seeds of *Linum u* plant (Flaxseeds), which investigation and characterises this oil by using chemical and extensive spectroscopic methodologies.

Materials and Methods

Collection of plant material and preparing it for extraction

The samples of Flaxseed (Brown Variety) *Linum usitatissimum* (*Linum u.*) plant were collected from the region of Muesrata, 300 Km East to Tripoli capital of Libya. The *Linum u.* seeds were washed by tap water, distilled water and dried in shadow for 5 days. Then the sample was stored in opaque airtight vials at below -20^{0}C until use.

Detection of the moisture content and ash value

The *Linum u.* seeds were ground by using an electrical mill. The moisture content and ash value were determined according to the AOCS Methods.

Preparation of the flaxseed extracts

Extraction of lipid content

The weight of the oil extracted from 600g of seed powder was determined to calculate the lipid content. The result is expressed as the percentage of lipids in the composition of the dry seed Powder.

Oil extractions

Oil extraction was carried out in the Soxhlet apparatus (Figure 1). The seeds were ground by an electrical mill to fine powder, 600g of this powder was weighed accurately and then it was placed in a thimble and placed in the extraction chamber of a 5000 ml capacity Soxhlet apparatus. The Soxhlet Apparatus, fitted with a condenser, was placed on a 500 ml distillation flask containing 2500 ml of the solvent sample which were then extracted under reflux with n-hexane for 8 hours, followed by solvent removal under vacuum at 40o C. This is by the slightly modified method of [13]. Then, the round glass bottle was cooled to room temperature. After the extraction, the main solvent was eliminated by a vacuum rotary evaporator. The content was then collected in the borosilicate bottle and stored in the refrigerator pending the chemical analysis.

Figure 1: Soxhlet extraction unit.

Acid value %, Free fatty acids (F FAS)

The acid value of seed oil was determined according to AOAC Official Method Cd 3a-63. Percentage free fatty acids (FFAS) were calculated using oleic acid as a factor.

Viscosity

The viscosity of seed oil was carried out using Brook field RV-I. Spindle of SO3 was used at 10 rpm at room temperature.

Fatty acid composition analysis by using G. C.

About 0.1 ml oil was converted to methyl ester using 1 ml Na O Me (1M) in 1 ml of n-hexane before being injected into the gas chromatography. The GC analysis was carried out in a Shimadzu 17A. Gas Chromatography, equipped with a flame ionization detector (Shimadzu, Japan) at 280°C. The GC was also equipped with a capillary column (30 m × 0.25 mm × 0.25 µ m films). The detector temperature was programmed at 280°C with a flow rate of 0.3 ml/min. The injector temperature was set at 250°C and Nitrogen was used as the Carrier gas. Identification of the peaks was performed by comparing the retention times with those of the genuine standards analyzed under the same conditions.

Triacylglycerol analysis using HP LC method

High-performance liquid chromatography was performed using the Dionex ultimate 3000 (Vernon Hills, Illinois, USA), with an evaporative light scattering detector (Figure 3). The separation was carried out on non-polar reversed-phase C18, column (250 mm 4.6 mm I. D, particle size 5um at 35°C. The mobile phase was acetone: acetonitrile (60:40 v/v). Samples were dissolved in the mobile phase and the analysis was carried out under isocratic conditions at a flow rate of 1 mL/min. Chromatograms were recorded by an evaporative light scattering detector.

Molecular structure determination using the (H^1&C^{13})

The H-NMR is useful for a variety of quantitative analytical purposes in the chemistry of fats and oils. This technique mainly focuses on the quantitative information that can be obtained from the oils by the 1H-NMR spectra, which regards both the minor and major components. One - or two-dimensional high-resolution NMR spectroscopy has been recognized as a viable technique to analyze oils for their fatty acid composition, authenticity, adulteration and nature of unsaturation [14]. The unsaturated fatty acids (oleic, linoleic, linolenic) in oil can be quantified using the H - NMR. H^1 and C^{13} NMR were carried out due to [15]. The experiment was performed on the Bruker 400 NMR spectrophotometer by using

deuterium methanol and DMSO as solvents. The oil was dissolved in these solvents for both the H^1 and C^{13} in about 2 mg/ml and introduced to the NMR tube.

Determination of functional groups by using FT-IR spectra

The infrared (IR) spectra contain significant information about the individual components of complex mixtures; Fourier transform infrared (FT-IR) spectroscopy can enhance greatly the quantitative analysis capabilities of IR spectroscopy. It has an important role in the analysis of edible oils by yielding simpler and more rapid techniques for determining common oil quality parameters and identification of molecular structure originates from the high information content of IR spectra and to assign specific absorption bands refers to functional groups [16]. To determine the functional groups in flaxseed oil must employ two methods of spectroscopy: the FTIR and NMR. The experiment was carried out in the Perkin Elmer spectrum GX spectrophotometer in the range of 400-4000 cm^{-1}. This system is used to measure the functional group of the seed oil. A very thin film of flaxseed oil was applied to the NaCl cells (25 mmi. d × 4 mm thickness) for analysis.

Oil thermal analysis DSC

Thermal properties, particularly the melting temperature and crystallization temperature, were determined by the differential scanning calorimeter analysis (DSC 822e Mettler Toledo calibration). The DS C was carried out following the methodology by Jumat., *et al*. (2006) [17]. 30 mg of the *Linum u.* oil was weighed into the 49 mL sealed aluminum pan and samples were heated in an oven for 30 mints (10°C/min), then put in the freezer for 90 min and then kept at room temperature for 48 hours before the DSC. The DSC thermogram were from -30C to 60C at a heating temperature rate of 2^0C/min. To obtain the crystallization curve, the sample is left at 60C for 10C minutes and then cooled to -30C at the rate of C/min. The Differential scanning calorimetric (DSC) is a solvent that is a less simple and relatively inexpensive method used to examine the physical state and properties of food components. The modified DSC can adequately describe changes related to the level of oxidation due to the structural conformations of the triacylglycerols. Since this technique is sensitive to composition changes resulting from oxidation; it may also be used as a rapid and effective method to characterize the Quality of *Linum u.* oil at different degrees of oxidation [18]. Applied the modulated DSC to extra the virgin flax oil and oil subjected to various accelerated oxidation treatments [19]. They discovered a very good correlation between the thermograph parameters selected for the main crystallization peak and the various off-flavours derived mainly from the linolenic acid degradation. Thermal properties (measured both in cooling and heating regimes) of monovarietal *Linum u.* samples were found to correlate well with the chemical composition [20].

Determination of the solid fat contents (SFC %)

The Solid fat contents (S F C) are determined by using a Bruker Minispec PC 120 Pulse Nuclear Magnetic Resonance (p-NMR) analysis (Karlsruhe, Germany) according to the procedure described by Jumat., *et al*. 2006 [17]. The sample in the p - NMR tube is first melted at 70°C for 30 min, followed by chilling at ^{0}C for 90 min. It is then held at each measuring temperature for 30 min before measurement. The SFC is measured in the temperature range of 0°C-70°C.

Results and Discussion

Oil contents

The percentage yield of the oil content is 39% of Libyan flax seed *Linum u.* (brown variety) which is similar to Canadian flaxseed (brown variety) percentage yield [21,22].

Physiochemical characteristics

Both the acid value and FFA% measures the free fatty acid content of fats and oils acid value where is the amount of sodium hydroxide required for neutralization. FFA is calculated as free oleic acid on a percentage basis for seeds oil [23].

Characteristics	*Linum u.* Oil (Libyan)	*Linum u.* Oil (Canadian)
Acid value (mg/g)	4.4	3.37
Free fatty acid as oleic acid	2.2 %	0.1-2.0
Viscosity	104	95
Average molecular weight	848	875
Color	/	/
a^*	2.9	/
b^*	0.65	/
L^*	41	/
Physical state at room temperature	Liquid	Liquid

Table 1: Physicochemical characteristics of *Linum u.* Oil (Libyan) in comparing with the property of *Linum u.* Oil (Canadian) characteristics.

As shown in table 1 the acid value of *Linum u.* oil (Libyan) is 4.4 mg/g, while 3.37 mg/g for *Linum u.* oil (Canadian) [22]. These dif-

ferences in acid value are due to the quality of the extracted oil-dependent on the soil and climate conditions where the samples were collected also the improperly storing of the seed samples affect the acid value [24]. While the average molecular weight value of *Linum u.* oil (Libyan) was 848 which depend on the saponification value of oil, the high the saponification value of oil means the oil is lower in molecular weight. Furthermore, the results show that the density of oil is relative to that of an equal volume of water (specific gravity) ranging from 0.89 g/cm^3 in the *Linum u.* oil (Libyan) which is close to the *Linum u.* oil (Canadian) which was found to be 0.93 g/cm^3. In addition, the *Linum u.* oil (Libyan) demonstrate a higher (L*) value than vegetable oil such as data seed oil and lower (a*) and (b*) values. This means the *Linum u.* oil (Libyan) is less than other oils (Figure 2).

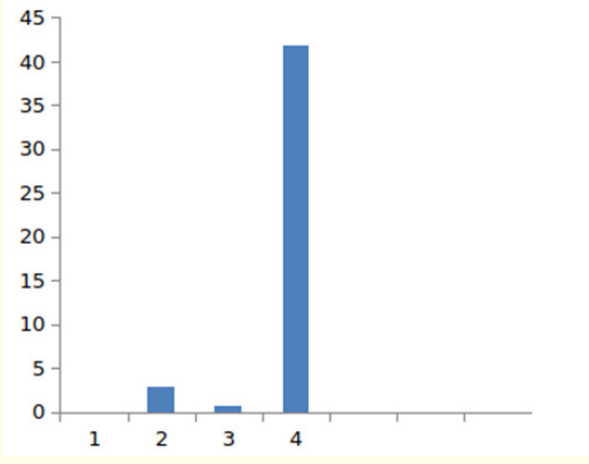

Figure 2: Cielab Coordinates (L*, a*, b*) Of *Linum u. oil* (Libyan).

Fatty acid composition

In this study also, a comparison was created of the results obtained for the composition of fatty acids for *Linum u.* oil (Libyan) with both *Linum u.* oil (Canadian) and *Linum u.* oil (Turkey) and the results are as shown in the table 2.

As shown in table 3 the fatty acid composition (%) of the *Linum u.* oil (Libyan), *Linum u.* oil (Canadian) and *Linum u.* oil (Turkey) where the *Linum u.* oil (Libyan), that is all eight fatty acids were present, three of which were unsaturated. Furthermore, the most abundant fatty acid of the *Linum u.* oil was the linolenic acid C18.3 in rang about 55% followed by oleic C18, linoleic C18.1, palmitic C16.0 5.3%, stearic C18.0 4.4% and another fatty acid present in the *Linum u.* oil (Libyan) with a small amount of beheric C22:0, Lignoceric C24:0, and arachidic acid C20.00 which together composed about 99.8% of the total fatty acids. However, a higher content found of linolenic acid 58% in the *Linum u.* oil (Turkey) and 58% in the *Linum u.* oil (Canadian) [25]. Canadians and Turkey, of the *Linum u.* oil may be regarded as linolenic oil because the linolenic acid was most abundant, followed by the oleic acid [25,26]. Where the *Linum u.* oil oil (Libyan) showed a similar percentage of unsaturated fatty acid of *Linum u.* oil (Canadian) which was 89.5%, 91.3%, respectively, thus, both oils showed higher unsaturated fatty acid than the *Linum u.* oil oil (Turkey) which was 87.3%. And also, as shown earlier in figure 2 the GS chromatography for the *Linum u.* oil (Libyan).

Fatty acid composition	*Linum u.*oil (Libyan) (%)	[a]*Linum u.*oil (Turkey) (%)	[b]*Linum u.* oil (Canadian) (%)
Palmitic $C_{16:0}$	5.3	6.8	5.3
Stearic $C_{18:0}$	4.4	4.5	3.3
Beheric $C_{22:0}$	0.2	/	/
Lignoceric $C_{24:0}$	0.1	/	/
Arachidic $C_{20:0}$	0.1	/	/
∑ Saturated	10.5	11.4	8.6
Oleic $C_{18.1}$	17.8	15	17.9
Linoleic $C_{18.2}$	15.9	13.9	14.7
Linolenic $C_{18.3}$	55.7	58.3	58.7
∑ Unsaturated (%)	89.5	87.3	91.3
([a][25] and [b][26].)			

Table 2: Fatty acid composition of *Linum u.* oil (Libyan), *Linum u.* (Turkey) and *Linum u.* (Canadian).

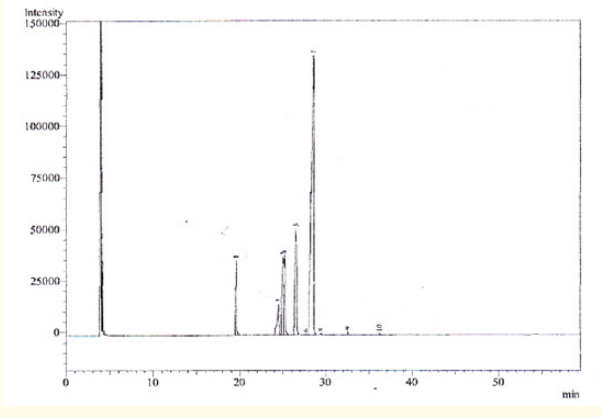

Figure 3: The fatty Acid Composition of *Linum u.* oil (Libyan) via the (G C).

Triacylglycerol's profile

The TAGs is separated according to the acyl chain lengths and the number of double bonds DBs. The retention time of the TAGs of the seed oil is governed by the equivalent carbon number ECN, which is defined as ECN = CN-2DB. This fact explains the relation between the carbon numbers CNs and DBs in all acyl chains. Triacylglycerol's TAGs is the main components of vegetable oils and the composition of flax acylglycerols is presented in table 3. As expected from the fatty acid composition, the main triacylglycerol's contain linolenic acid in their molecules.

ECN_S	Triacyglycerols	Libya %	[a]Canada %
42	PLnLn	7.9	7.9
44	PLLn	6.7	6.7
36	LnLnLn	20.6 - 21.4	20.9
44	OLnLn	8.1 - 8.8	8.4
46	POLn	4.3	4.0
48	POL	1.9	1.5 - 1.6
38	OLL	17.4	/
40	LLLn	0.6	/
50	PPS	0.5	/
46	POL	0.17	/
38	OOL	/	3.4

Table 3: TGA Composition of the *Linum u.* oil (Libyan).

Ln: Linolenic Acid, L: Linoleic Acid, O: Oleic Acid, P: Palmitic Acid, S: Stearic Acid, ECN_S: Equivalent Carbon Numbers. Source: [a] [9].

Due to the limitation in TAGs standard available commercially, the identified TAGs of the *Linum u.* oil (Libyan) was concluded by comparing the retention time of the standard TAGs peak in figure 4. And accordingly, table 3 shows the TAGs chromatograms detected in the *Linum u.* oil, correspondingly, the major TAG peaks in the studied oil were the polyunsaturated of OLL with 17.45%, LnLnLn with 20.65-21.44%, OLnLn with 8.18 - 8.84%, PLLn with 6.72%, PLnLn with 7.97%, for the *Linum u.* oil (Libyan). Meanwhile, the TAGs for the *Linum u.* oil (Canadian) also have higher contents in the polyunsaturated of PLnLn with 7.9%, PLLn with 6.7%, LnLnLn with 20.9%, OLnLn with 4.8%, POLn with 4.0%.

The functional groups (FT-IR spectroscopy)

The importance of IR spectroscopy is the identification of molecular structures that originate from the information content obtained and the possibility to assign certain absorption bands related to its functional groups. In fats and oils, most of the peaks and shoulders of the spectrum are attributable to the specific functional groups [27]. Triacylglycerols are the principal components in fats and oils.

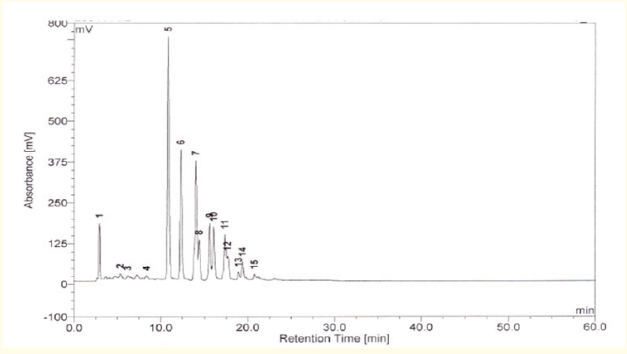

Figure 4: TAG Chromatograms of *Linum u.* oil (Libyan).

Wave number Libya	Wave number [a]Canada	Functional Group
2927-2854	2924	Asymmetrical and Asymmetrical stretching vibration of methylene group (-CH_2)
1743	1743	Ester carbonyl functional group of the triacylglycerols (C = O)
3010	3020	Cis double bond stretching or C-H stretching vibration (aliphatic)
722	721	Overlapping of the methylene(-CH_2) Rocking vibration and the out of plane vibration of cis - disubstituted olefins (C-H) group vibration (aliphatic)
1100	1110	Stretching vibration of the C-O-C ester groups.
1376	1377	Bending vibrations of the CH_2
1462	1463	Bending vibrations of CH_2 groups.
1238	1250	C-H or C-O
1163	1164	C-O

Table 4: The functional groups and modes of vibrations in the spectrum of the *Linum u.* oil (Libyan) and the *Linum u.* oil (Canadian).

Table 4 highlights the functional groups and modes of vibrations in the spectrum of the *Linum u. oil* (Libyan) are compared to the *Linum u.* oil (Canadian) of the FT-IR spectrum. The study showed that it is similar in the exact absorbance of values and the same functional groups. For the *Linum u*. oil (Libyan) and the *Linum u.* oil (Canadian), the observed peaks are at 3010-3020, 2927-2924, 1743-1743, 1462-1463, 1376-1377, 1238-1250, 1100-1110, and 722-721 cm^{-1}. Peaks at 2927 and 2924 cm^{-1} are due to the bands arising from the CH2 stretching vibrations, asymmetric and symmetric, respectively. Sharp peak around 1743 cm-1 results from the C = O stretching vibration of carbonyl groups. Correspondingly, peaks of 1462 and 1463 cm^{-1} bending vibrations for CH2 and CH3 groups. Also, regions between 1238-1250 cm^{-1} is due to stretching vibration of the C-O ester group and CH2 rocking vibration [28,29]. While figure 5 exhibits the FT-IR spectra of the *Linum u.* oil (Libyan) and showed the typical characteristics of the absorption peaks for common triacylglycerol's.

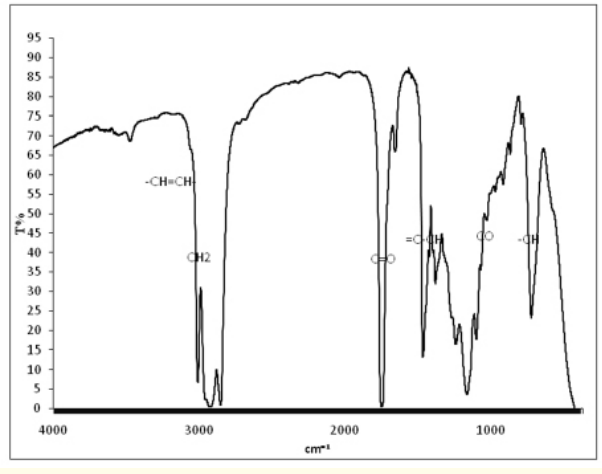

Figure 5: FT-IR Spectrum of *Linum u*. oil (Libyan).

Thermal transition properties

Differential Scanning Calorimetry (DSC) is a fast and direct way to assess the quality of oil [30]. Using this method, various physical properties of *Linum u.* oil (Libyan) can be studied. The *Linum u.* oil (Libyan) exhibits a simple thermogram after melting in the DSC. The endothermic in each melting point curve have been labeled in the order of increase in the temperature and the exothermic in each crystallization curve have also been marked in the order of the decrease in the temperature [31]. Figure 6 shows the DSC melting transition temperature (Tm) of the *Linum u*. oil (Libyan). The DSC shows one end thermal peak at - 21C0, but for the *Linum u.* oil (Canadian), it revealed peaks from -20C0 to -24^{0}C [23]. The DSC melting transition (Tm) temperature between them due to slight difference in TAGS composition, which showed that the DSC melting curve depends on the TAGs composition of oil sample, where the high saturated TAGS content shows to high transition temperature for melting curves [32]. Also, figure 6 shows the curve of DSC melting transition (Tm) the oil sample was heated, some of the less thermally-stable polymorphous melt earlier. The remaining TAG rearranges and recrystallizes into more stable polymorphous that melt later at higher temperatures. However, in the field of oils and fats, an exothermic associated with the crystallization may or may not be exhibited in DSC melting curves. These have been seen clearly in the thermal flax peak below. Figure 7 shows the DSC crystallization transition temperature (Tm) of the *Linum u*. oil (Libyan) at - 44C0. In this area, the TAG started to change from liquid to solid form indicating that the most saturated TAG has been solidified. In the cooling process of the *Linum u*. oil (Libyan) in a DSC, the oil exhibit less complicated exothermic than their respective melting curves. The subdivision of the crystallization curve of oil samples, into different exothermic regions. As the crystalline phase is forming, it will have a higher concentration of the TAG component which is kinetically favored. As the concentration of this TAG component is depleted, the less kinetically favored TAG component will enter the crystallization process until all TAG components are crystallized [32].

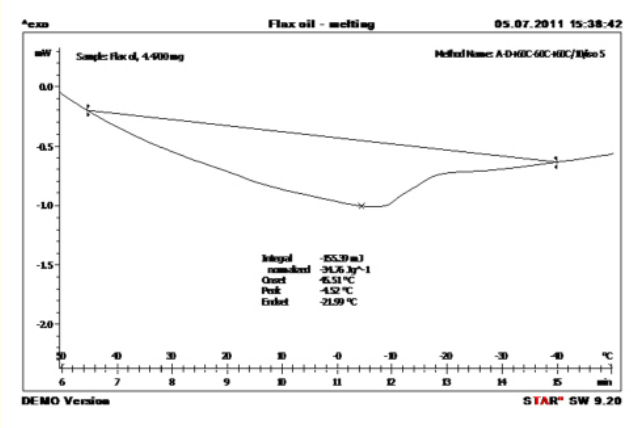

Figure 6: Melting Thermograms of *Linum u.* oil (Libyan).

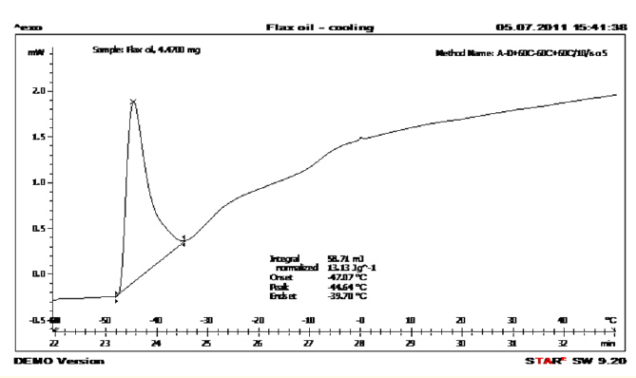

Figure 7: Crystallization Thermo grams of *Linum u.* oil (Libyan).

Solid fat content

The Solid fat content for fat was measured by the pulsed nuclear magnetic resonance (pNMR), as defined by the international standards. This technique, though non-destructive, is time-consuming due to the sample preparation. Figure 8, this signifies the relationship between the solid fat content of the *Linum u*. oil (Libyan) and the temperature which ranged between 0-25^{0}C. The solid fat content decreases when the temperature increases until it reaches room temperature 25^{0}C. This can be explained by the *Linum u*. oil (Libyan) which contains higher unsaturated fatty acid content of 89.5% than the saturated fatty acid at 10.5%.

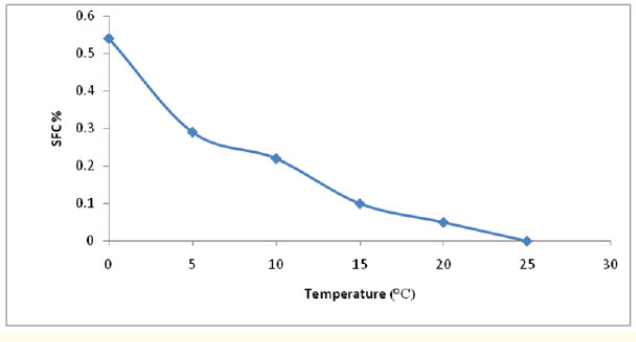

Figure 8: Solid Fat Content of Libyan flax seed oil.

The results of nuclear magnetic resonance (NMR) (^{1}H&^{13}C) spectroscopy analysis for *Linum u.* oil (Libyan)

Nuclear Magnetic Resonance spectroscopy has been increasingly being applied in the identification of lipid structure, particularly in the detection and often the location of the double bond systems in fatty acid chains. In this study, the NMR was used to determine the functional atoms of the flaxseed oil.

Libyan/Chemical shift (δ)	Canada/Chemical shift (δ)	Component Assignment
0.86 - 0.98	0.5	CH_3 Terminal
1.25 - 1.34	1.0	Saturated alphatic chain
1.59 - 1.61	1.8	CH_2Attached to one carbon atom
2.1	/	CH_2-O-C = O
2.0	2.0	CH_2-CH = CH
2.2- 2.3	/	-CH_2-Attatched- O-C-O
2.7 - 2.8	3.0	-C = C-CH_2-C = C-
4.1	4.1	CH-O-CO- in glyceryl
4.2 - 4.3	4.5	-CH_2-O-COR (Glycerol Group)
5.2 - 5.3	5.2	-CH = CH (acyl group)
/	5.7	Olefinic protons

Table 5: The summary of the Chemical Shifts of the *Linum u.* oil (Libyan) for H^1 NMR [33].

Linum u. oil (Libyan) Chemical shift (δ)	Assignment
20-27	Aliphatic carbons (single)
29.0-29.7	Aliphatic to carbonyl
62.1-68.9	C_1OF C_3 OF Glycerol
76.8-77.2	C_2OF Glycerol
127-131	CH_2-CH = CH-CH_2 (Olefinic carbon)
172-173	Carbonyl carbon

Table 6: C^{13} NMR Chemical Shift (ppm) of *Linum u.* oil (Libyan).

Tables 5 and 6 summarized the chemical shifts of the *Linum u.* oil (Libyan) for H^1 NMR and C^{13} NMR. It is clearly observed that there were no significant differences between the *Linum u.* oil (Libyan) in different countries such as the *Linum u.* oil (Canada). This similarity may be due to their fatty acid composition. Both the *Linum u.* oil (Libyan) and *Linum u.* oil (Canadian) contain triacylglycerol (TAG) in which the NMR chemical shifts were consistent. Indeed, the NMR is a very energetic tool for structure

elucidation and characterization of the proposed chemical compound. In order to identify exactly the fatty acid structure in the *Linum u.* oil (Libyan) and *Linum u.* oil (Canadian), the known standards of the expected fatty acids should be elucidated by the NMR and compared with the desired oil. Figure 9 shows the H^1 NMR spectrum of the *Linum u.* oil (Libyan). The distinguishable groups are protons of the terminal methyl of the fatty acid chin. The chemical shift at 0.868-0.986 ppm and 0.5ppm referred to the methylene group ($-CH_3$) of the Libyan and Canadian *Linum u.* oils, respectively. Next is the terminal methyl ($-CH_2$) which is between 1.25-1.343 ppm and 1.0 ppm. The methylene groups adjacent to one double bond, CH_2-CH = CH-2.005 ppm and 2.0 ppm and two double bond (-C = C-) acyle groups were at 5.253-5.39 ppm and 5.2 ppm. Others are the methylene groups of glyceryl (CH_2-O-COR) at 4.281-4.308 ppm and 4.5 ppm. Figure 10 is the C^{13} NMR spectrum of the *Linum u.* oil (Libyan). The C^{13} NMR spectrum shows the main signals assignment of the *Linum u.* oil (Libyan) as shown in table 6. The signals at 172.76-173.17 ppm refer to the carbon atom of the carbonyl group whereas the signals at 127.12-131.92 ppm refer to the unsaturated carbon atoms 62.09-68.92 ppm is due to the glyceryl carbon atoms. The methylene carbon atoms were further distinguished into those adjacent to one double bond, saturated at 29.0-29.7 ppm. The adjacent double bond and carbonyl group were believed to influence the methyl carbon atoms and produced a slight difference in their chemical environment.

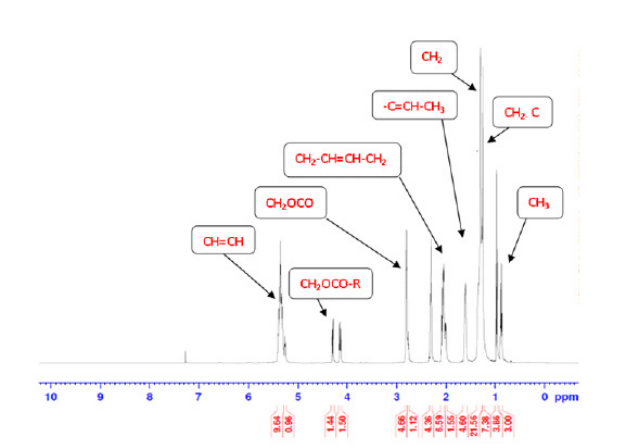

Figure 9: ^{1}H NMR Spectrum of Libyan Flax Seed Oil.

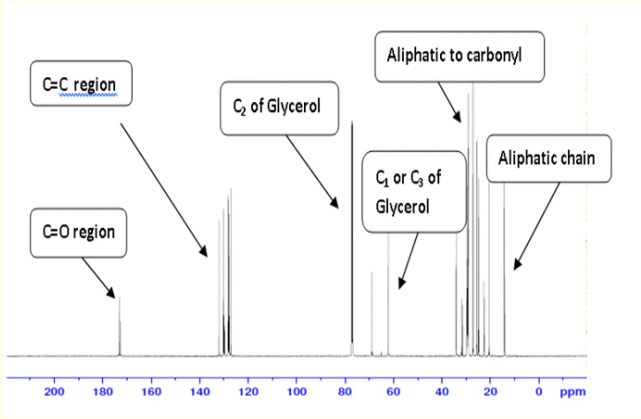

Figure 10: C^{13} NMR Spectrum of Libyan Flax Seed Oil.

Conclusion

In conclusion, in this a study was initiated to identify the medicinal importance of *Linum u*. oil (Libyan), which is used as traditional medicine by Libyans in ancient and current times, that can be used in medicine in the treatment of diseases such as diabetes, cough, skin, hair, lung, and another. The major fatty acids in *Linum u.* oil (Libyan) were linolenic acid, oleic acid, palmitic acid and stearic acid. Also, the most prominent TAGS *Linum u*. oil (Libyan) were LnLnLn, OLL. Additionally, the oil extracts exhibited good Physicochemical properties and could be useful as bio-diesel feed stock and industrial application. The results of the Physicochemical tests revealed the presence of active chemical compounds additionally the UV, FT-IR, 1 H NMR, 13C NMR, tests pointed to the existence of important chemical active groups, which also support the significance of *Linum u*. oil (Libyan) as a medicinal plant.

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