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Research Article

# PHYSICO-CHEMICAL SCREENING OF ALGERIAN LINSEED OIL AND CHARACTERIZATION OF THEIR FREE ACIDS METHYL ESTERS (FAMEs)

**BENMEHDI HOUCINE,* AMROUCHE ABDELILLAH, MEZIANE ABDELKADER, ZAABOUB IMENE, CHABANE SARI MERIEM, DAOUDI CHABANE SARI**

**Faculty of sciences and technology, Department of technology, University of Bechar 08000 Algeria, Laboratory of LASNABIO, Department of Chemistry, University of Tlemcen -Algeria- Faculty of sciences and technology, Department of sciences, University of Bechar 08000 Algeria Laboratory of LAPRONA, University of Tlemcen, Algeria. Email: h_ben90@yahoo.fr**

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**ABSTRACT**

The aim of this work was to study the physico-chemical properties of Algerian linseed oil and to characterize their FAMEs from *Linum usitatissimum* L., a medicinal plant used in Algerian folk medecine. After extraction of oil from *L. usitatissimum* seeds, saponification and methylic esterification reactions of this oil were carried out. Colum chromatography separation of the crude esters extract allowed us to isolate a major fraction of esters which was analysed using IR, $^{1}$H NMR, $^{13}$C NMR and GC-MS. The FAMES consist essentially in esters of the two unsaturated linoleic and linolenic acids as well as in the two saturated palmitic and stearic acids.

**Keywords:** *Linum usitatissimum*, Methyl esters, Fatty acids,Viscosity, Gas chromatography, Mass spectroscopy

## INTRODUCTION

*Linum usitatissimum* L. is one of the important genuses of the family "Linoceae.". It is native in the region extending from the eastern Mediterranean to India and was probably first domesticated in the Fertile Crescent. Flax was extensively cultivated in ancient Ethiopia and ancient Egypt [1].

The flax seed oil is edible. Because of its quick drying property its oil is used for the preparation of paints, varnishes, printing ink, oil cloth, soap, patent leather, and waterproof fabrics [2]. Besides, it has been reported that oil from seeds removes biliousness [2]. Linseed taken in the diet may benefit individuals with certain types of breast [3,4] and prostate cancers [5]. It may also stunt the growth of prostate tumors [5]. Scientists at American National Cancer Institute singled out flaxseed as one of six nutraceuticals for food applications [6].

Flax may also lessen the severity of diabetes by stabilizing blood-sugar levels [7]. Therapy with oral flaxseed oil capsules (1 or 2 g/day) reduces ocular surface inflammation and improves the symptoms of keratoconjunctivitis sicca in Sjögren's syndrome patients [8].

A review of the literature on flaxseed yielded 13 categories for which flaxseed had been studied in humans, including for exemple constipation/laxative, attention-deficit hyperactivity disorder, hyperlipidemia, atherosclerosis/coronary artery disease and human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) [9]. Moreover, the study of Arvind (2006), explored the antioxidative properties of linseed oil in the prophylactic action against oxidative stress induced by a radiomimetic drug, cyclo phosphamide [2].

Our ethnopharmacological survey undertaken in southwest Algeria revealed that *Linum usitatissimum* L. has a long history of use in folk medicine as a treatment against various ailments such as those described above.

Chemically, flaxseed is an exceptionally rich source of mammalian lignan. Circular dichroism analyses confirmed the presence of two distinct optically active compounds present in the flaxseed extract i.e. two secoisolariciresinol diglucoside (SDG) diastereomers: the (2*R*, 2'*R*)-2,bis-[(4-hydroxy-3-methoxyphenyl)-methyl]-1,4-butanediyl-bis-β-glucopyranoside, the

predominant flaxseed lignan, and respectively, the (2*R*,2*S*)-2,3-bis[(4-hydroxy-3-methoxy- phenyl)methyl]-1,4-butanediyl-bis-β-glucopyranoside [10]. It is important to underline that SDG is present at levels 75-800 times greater than in any other plant known to date [2]. Flax seeds contain high levels of dietary fiber and omega-3 fatty acids [32].

The use of plants in medicine promoted the chemical analysis of medicinal plants for the active chemical constituents to be extracted, identified, and then synthesized [11-14]. In this context, the present study aimed to study and analyze the oil composition isolated from the seeds of *L. usitatissimum* by using the saponification and esterification reactions followed by column separation and spectroscopic analysis.

## RESULTS AND DISCUSSION

The petroleum ether-extracted oil content of *L. usitatissimum* seeds assayed from south west of Algeria was found to be (9.8 g). The isolation of fatty acids *via* saponification reaction conducted to saponifiable fraction, in the final step, methylic esterification of fatty acids provided (3.67 g) of crude esters. Column chromatography separation of the crude esters using the elution systems: petrolium ether (100%) and petrolium ether:chloroform(9:1; 8:2 and 7:3) as eluent systems allowed us to isolate the major fraction of esters (3.038 g; 82.8% yield).

In the other hand, we have undertaken to analyze the physicochemical parameters of the oil because of their relevance with respect to the nutritional properties. The quality of the oil from the peel of *L. usitatissimum* seeds was assessed using parameters such as the acid, acidity, peroxide, iodine and saponification values (Table 1).

The acid value is an important index of physicochemical property of oil which is used to asses the quality, age, edibility and suitability of oil for use in industries such as paint [15]. According to Demian, acid values are used to measure the extent to which glycerides in the oil have been decomposed by lipases and by physical factors such as light and heat [16]. Thus, for acid index, the data showed that *L. usitatissimum* has as value (1.59 mg KOH/g) according to codex norms (4 mg KOH/g) [17].

The peroxide value is used as a measure of the extent to which rancidity reactions have occurred during storage. The value of peroxide indice of *L. usitatissimum* was found to have occurred to be (4.74 meq $O_2$/kg) in agreement with codex norms smaller than (10 meq. of $O_2$/kg of oil).[17] This value indicates a relatively good quality of this oil. The low peroxide value of the linseed oil also corroborated the fact that the linseed oil has less resistance to lipolytic hydrolysis and oxidative deterioration [18].

The saponification value is a measure of the average molecular mass of fatty acids in oil samples. The high saponification value in the linseed oil (191 mg KOH/g) is within the range of some edible oils such as palm oil (196 - 205), groundnut oil (188 - 196), and corn oil (187 - 196) [19]. Therefore, the extremely high value of saponification of the linseed oil may extend its use in the saponification industry (production of liquid soap and shampoos) [20]. Furthermore, the higher iodine value of the linseed oil (II = 192) suggests that the oil chains are moderately rich in unsaturated fatty acids (karleskind 1992) [21].

Refractive index is very stable parameter and should be used for checking the identity of oil. The oil showed a low level of unsaponifiable matter at (1.7%).

**Table 1: Physico-chemical characterization of linseed oil**

| | |
|---|---|
| Oils | *L. usitatissimum* |
| Extraction yield (%) | 40 |
| color | Yellow straw |
| Physical caracterization | |
| Relative Density | 0.9319 |
| refractive index (20°C) | 1.4595 |
| freezing point (°C) | -4° |
| Chemical caracterization | |
| Saponification value (mg KOH/g) | 191 |
| iodine value | 192 |
| Insaponifiable matter (%) | 1.7 |
| Characteristics of alteration | |
| acid value (%) | 3.08 |
| Acidity (%) | 2.03 |
| peroxide value (meq $O_2$/Kg) | 4.74 |

As regards the viscosity of the linseed oil was found to have low value. It decreased with an increase in temperature at 12.5°C, 25°C, 35°C and 45°C as generally observed for other oils and liquids. In fact, this behaviour is observed in other cases since oil viscosity depends on molecular structure and decreases with the unsaturation of fatty acids [22].This may be partly due to the π bonds that prevents rotation around CC double bonds. Also, the extended chain makes the flow easier thus reducing viscosity [23]. Furthermore, the viscosimetric investigation allowed us to measure the optimal concentration of linseed oil corresponding to the best rheological behaviour. This concentration named C star (C*), it was found to have a value ($C_2$* = 5.63.10$^{-6}$ g/mL). The Figure 1 demonstrates the profile of variation of viscosity versus concentration.

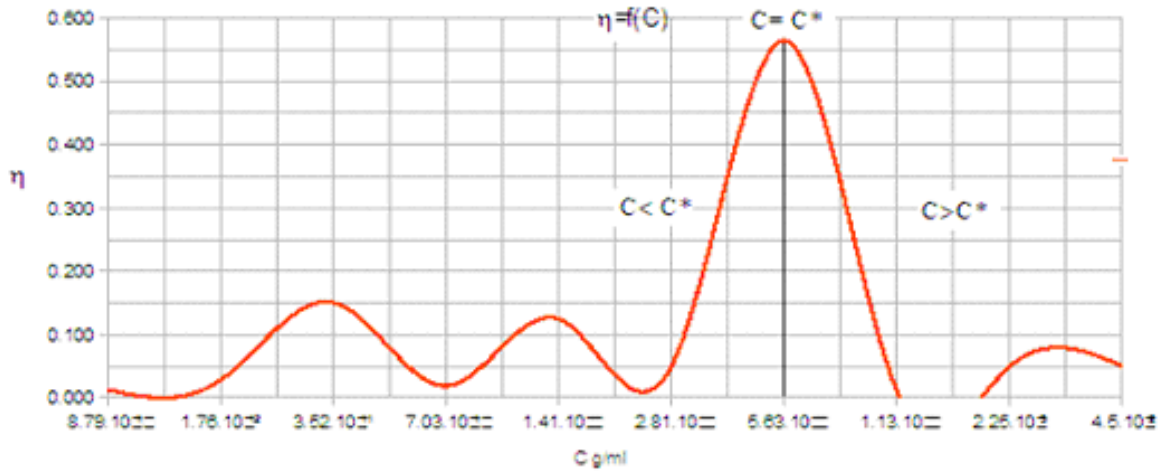


**Fig. 1: Specific viscosity profile of *L.usitatissimum* oil ($\eta_{sp}$ =f(C))**

The effect of concentration on viscosity of FAMEs fraction was examined at 6 concentrations. The experimental results showed that the specific viscosity measured at the same temperature increased as the concentration of FAMEs increased from (1.77.10$^{-7}$ to 3.46.10$^{-6}$ g/mL), then it changes as sinusoidal manner between (6.07.10$^{-5}$ and 2.56.10$^{-2}$ g/mL). (Table 2)

**Table 2: Variation of specific and reduced viscosities and absorbance versus concentration**

| C (g/ml) | 1.77.10$^{-7}$ | 1.95.10$^{-6}$ | 3.46.10$^{-6}$ | 6.07.10$^{-5}$ | 6.74.10$^{-5}$ | 1.13.10$^{-3}$ | 2.56.10$^{-2}$ |
|---|---|---|---|---|---|---|---|
| $\eta_{sp}$ | 0.039 | 0.211 | 0.243 | 0.222 | 0.114 | 0.239 | 0.127 |
| $\eta_{red} = \eta/C$ | 2.20.10$^5$ | 1.24.10$^5$ | 6.09.10$^4$ | 3.65.10$^3$ | 1.69.10$^3$ | 2.11.10$^2$ | 4.96 |

Further, the thin layer chromatography separation of esters gave the results presented in Figure 2. The eluent used in this separation was petrol ether : chloroform = 8/2 and 7/3). It appears clearly that several esters are present. It seemed thus important to carry out a purification of the crude esters *via* column chromatography. As seen in Figure 2b, a major fraction

of esters was separated successfully. In order to characterize this fraction, different spectroscopic methods were used and the results are shown above.


a

B

**Fig. 2: TLC profile of esters (a and b: before and after chromatography column separation)**

Firstly, we have used the Gas chromatography to characterise the chemical composition of linseed oil. The chromatogram presented in Figure 3 shows several compounds at various retention period.

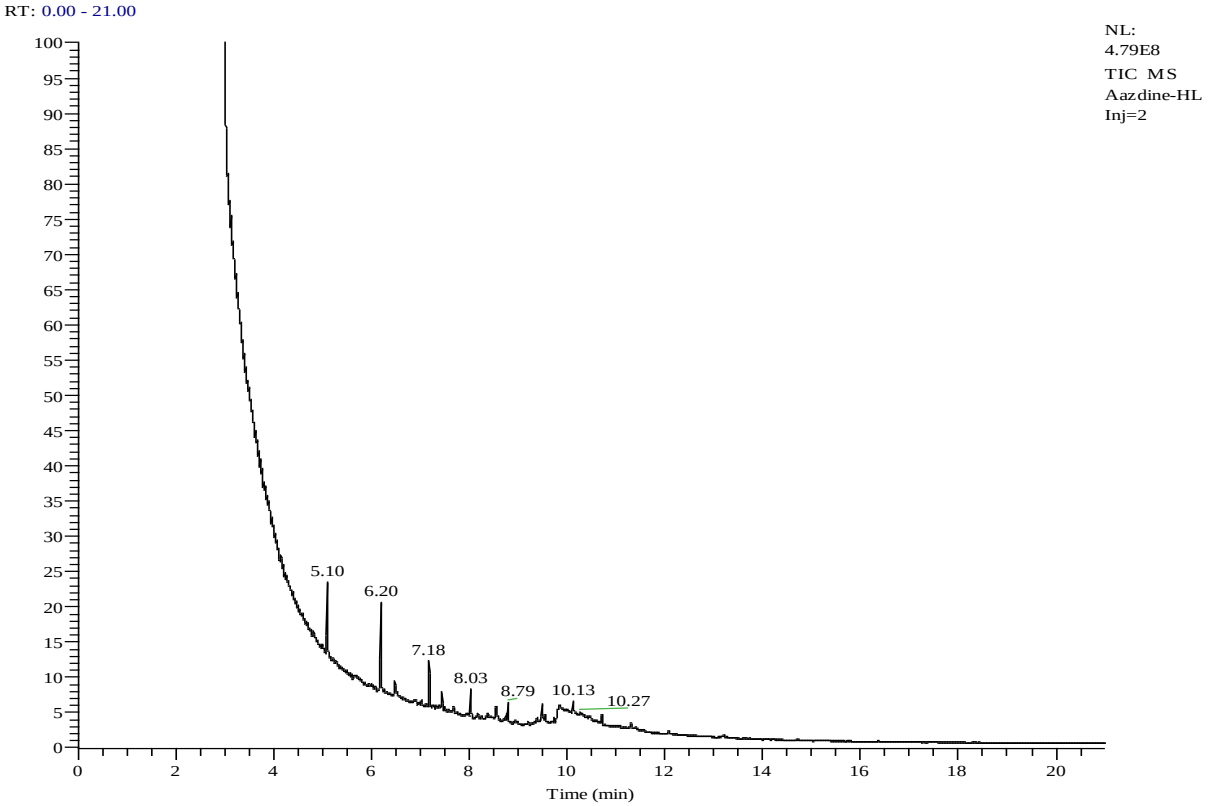


**Fig. 3: GC spectrum of linseed oil**

In order to analyze fatty acids by GC/MS, their methyl ester analogs (FAME) had to be prepared. The GC profile of FAMEs fraction is shown in Figure 4. GC/MS analysis of the free fatty acid methyl ester fraction showed the presence of four peaks with retention times (8.63, 9.48, 9.55 and 9.62 min), respectively.

Total run time for the separation of the esters was 10 min. A total of four FAMEs were identified from the relative retention times compared to those of known FAMEs using a comprehensive databank of NBS and Wiley library for identification, and mass spectroscopy. The peaks sited at (8.63, 9.48 and 9.55 min) corresponded to the palmitic, linolenic, linoleic acids methyl esters respectively.

The peak at (9.62 min) was attributed to the saturated stearic acid methyl ester.

The linolenic acid methyl ester has an abundant molecular ion (*m/z* = 292) and hydrocarbon ions of general formula $[C_nH_{2n-5}]^+$ tend to dominate the spectrum with the ion at (*m/z* = 78.8) as the base peak. A peak at (*m/z* = 150) is characteristic for methyl esters of polyunsaturated fatty acids with $[C_{11}H_{13}]^+$ moiety, while one at (*m/z* = 108) defines terminal group $[C_8H_{13}]^+$ [24-26].

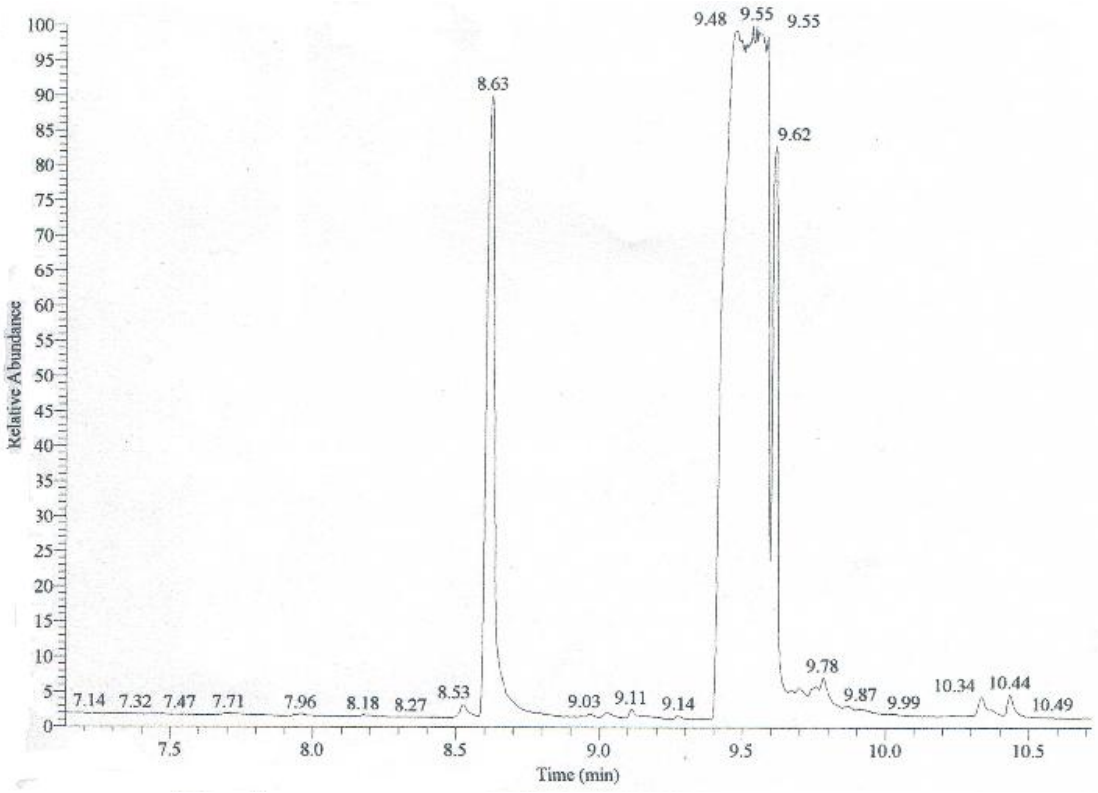


**Fig. 4: GC spectrum of FAMEs fraction isolated from linseed oil**

In addition, there are small ions formed by a similar cleavage at the carboxyl end of the molecule giving a fragment containing the first two double bonds

and the second methylene group (minus a proton) that could be termed the '*alpha*' ion.

The corresponding ion in the spectrum of methyl 9, 12, 15-octadecatrienoate is at (*m/z* = 236). This ion was first noted by Holman and Rahm (1971) [24], and was studied more systematically *via* chemical ionization methods in a paper by others that appears to have been largely overlooked [25].

As illustrated in Figure 5, the linoleic acid methyl ester has an abundant molecular ion (*m/z* = 294), and a prominent ion for loss of the McLafferty ion (*m/z* = 220), although the McLafferty ion (*m/z* = 74) is small [27]. The peak at (*m/z* = 263.1) corresponded to $[M-31]^+$ fragmentation. Hydrocarbon ions of general formula $[C_nH_{2n-3}]^+$ dominate in the lower mass range (*m/z* = 67, 81, 95, 109, 123, etc)

The reading of the mass spectrum of the peak at (8.63 min), revealed the presence of molecular ion at (m/z = 270). Besides, mass spectra of unbranched FAME (palmitic acid methyl ester) is characterised by fragment ions at (*m/z* = 74) (McLafferty rearrangement), (*m/z* = 87) (β-cleavage), and $[M - 31]^+$ (loss of OMe). Further fragment ions $[M - CnH_2n+1]^+$ (227, 185, 143, 129) arise from cleavage of the saturated unbranched alkyl chain.

Elucidation of mass spectrum of the peak at (9.62 min) (Figure 7) confirmed that it corresponds to stearic acid methyl ester. The results showed the molecular ion peak at (m/z = 298), fragment ions at (*m/z* = 74) (McLafferty rearrangement), loss of OMe (*m/z* = 267),

(*m/z* = 87) (β-cleavage) and fragment ions $[M - C_nH_{2n+1}]^+$ at (255, 241, 213, 199, 185, 157, 143, 129 and 101).

On the other hand, the mixture of FAMEs was also analysed by IR, $^1H$ NMR and $^{13}C$ NMR analysis. The IR spectrum of the FAMEs displayed strong carbonyl absorption at (1743.37 $cm^{-1}$). Absorption at (3011.34 $cm^{-1}$) is characteristic to olefinic (=C-H) stretching. Similarly, the absorption at (1652.84 $cm^{-1}$) correspond to C=C stretching.

The proton NMR spectrum (400 MHz) showed a large singlet at (δ 3.59 ppm) assigned to the ester methyl groups. Moreover, the presence of vinylic protons was confirmed by the signals between (5.27 and 5.30 ppm).

The $^{13}C$ NMR spectra of FAMEs showed carbonyl signals around (174.3 ppm), while signals between (130.26 and 127.11 ppm) indicated the presence of vinylic carbons (C=C). Furthermore, resonances corresponding to methylene carbons appeared between (34.09 and 22.67 ppm).

## MATERIALS AND METHODS

Esterification reaction was monitored by thin-layer chromatography on TLC plastic sheets (silica gel 60F254, layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (particle size 0.063-0.200 mm) from Merck without any special treatment. The elucidation of linseed oil composition was confirmed by GC-MS, IR, $^1H$ and $^{13}C$ NMR spectra. GC-MS spectra were enregistred on GCMS-QP2010 ultra device. IR spectra were recorded with an ATI Mattson Genesis Series FTIR infrared spectrometer (4000-600 $cm^{-1}$). $^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ with a Bruker AC 400 spectrometer. Chemical shifts (*δ*) were measured in ppm relative to internal TMS.

### Viscosity measurement

Viscosity was measured with a capillary viscosimeter ubbelohde-schott Gerat AVS400 [28]. The temperature is kept constant by a thermostat (25 ± 0.1)°C. Dilution of oil solutions was performed manually. The viscosity measurement is based on the determination of the flow time of a volume of the solution through a capillary of length "1" and diameter "a".

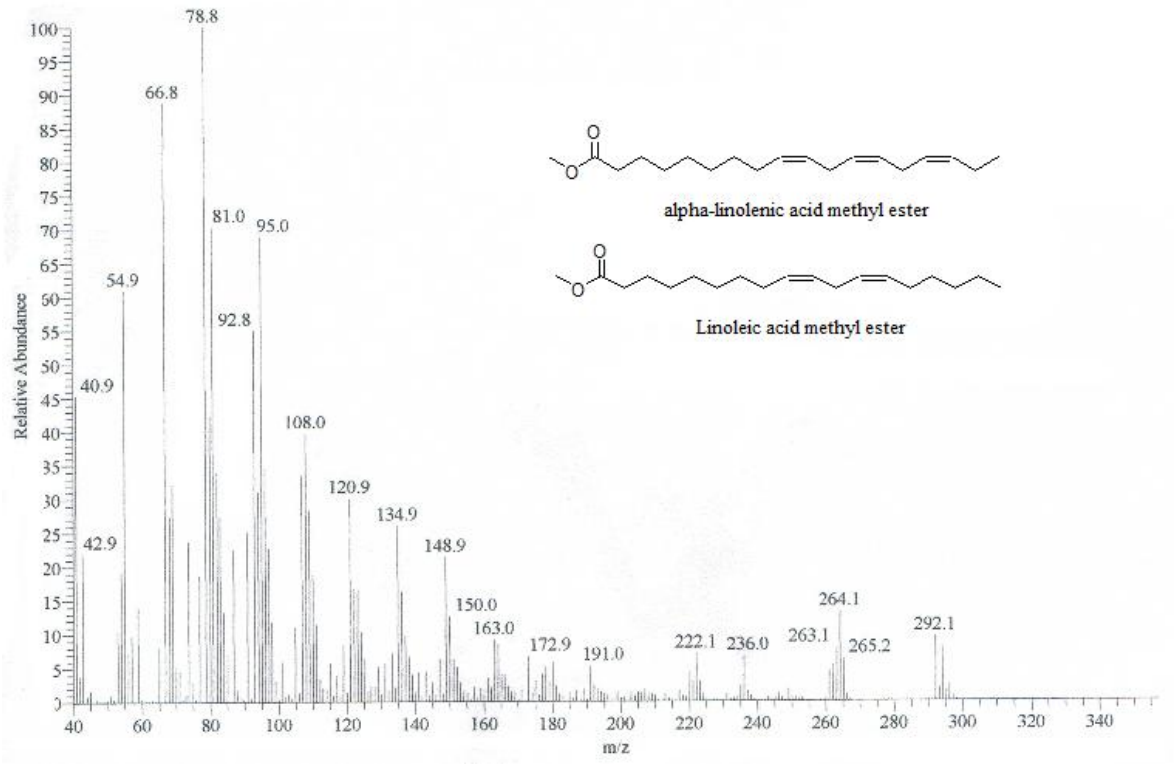


**Fig. 5: MS spectrum of linolenic and linoleic acids methyl esters**

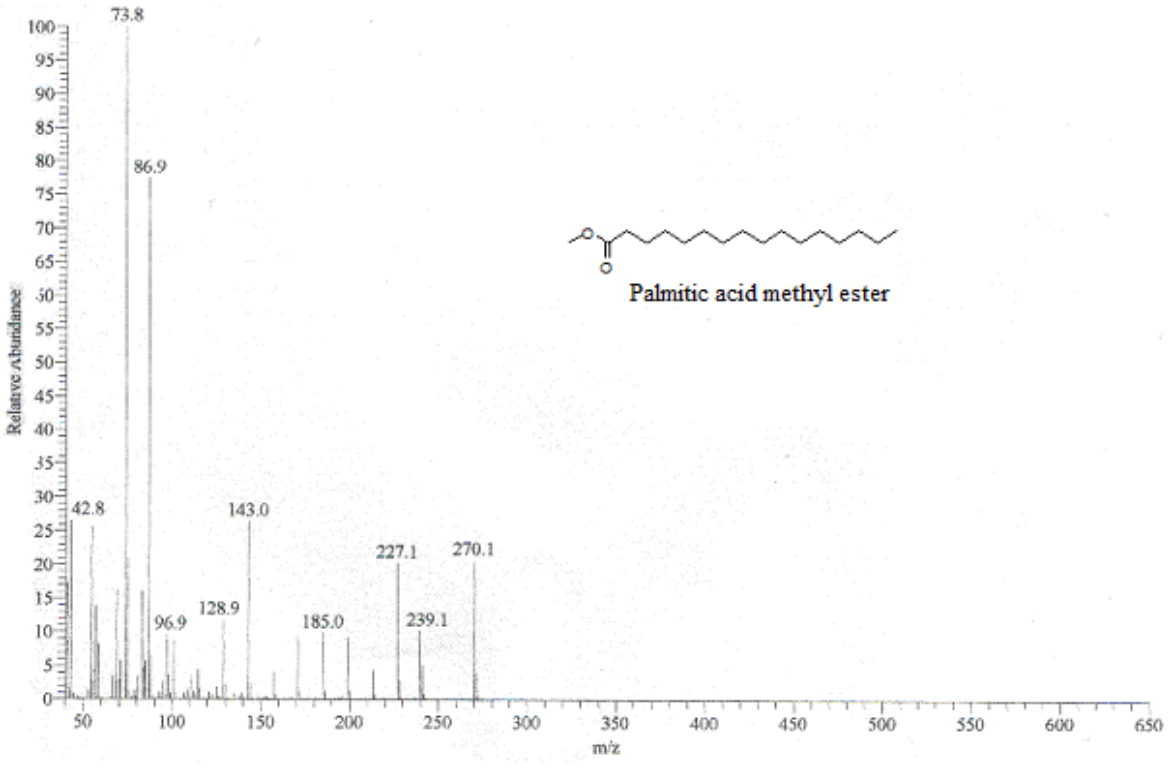


**Fig. 6: MS spectrum of palmitic acid methyl ester**

**Extraction of linseed oil**

Samples (100 g) of dried seed (powdered form) were taken into the soxhlet apparatus. A piece of cotton is placed at the top and bottom of the apparatus to evenly distribute the solvent as it drops on the sample during extraction.

Extraction was carried out with Petroleum ether for 6 hours without interruption by heating at (40 to 60°C). After the extraction, the solvent was evaporated on the *Buchi –Rotavapor* until no smell of solvent remains and the resulting oil was collected in a separate Becker for further analysis [29].

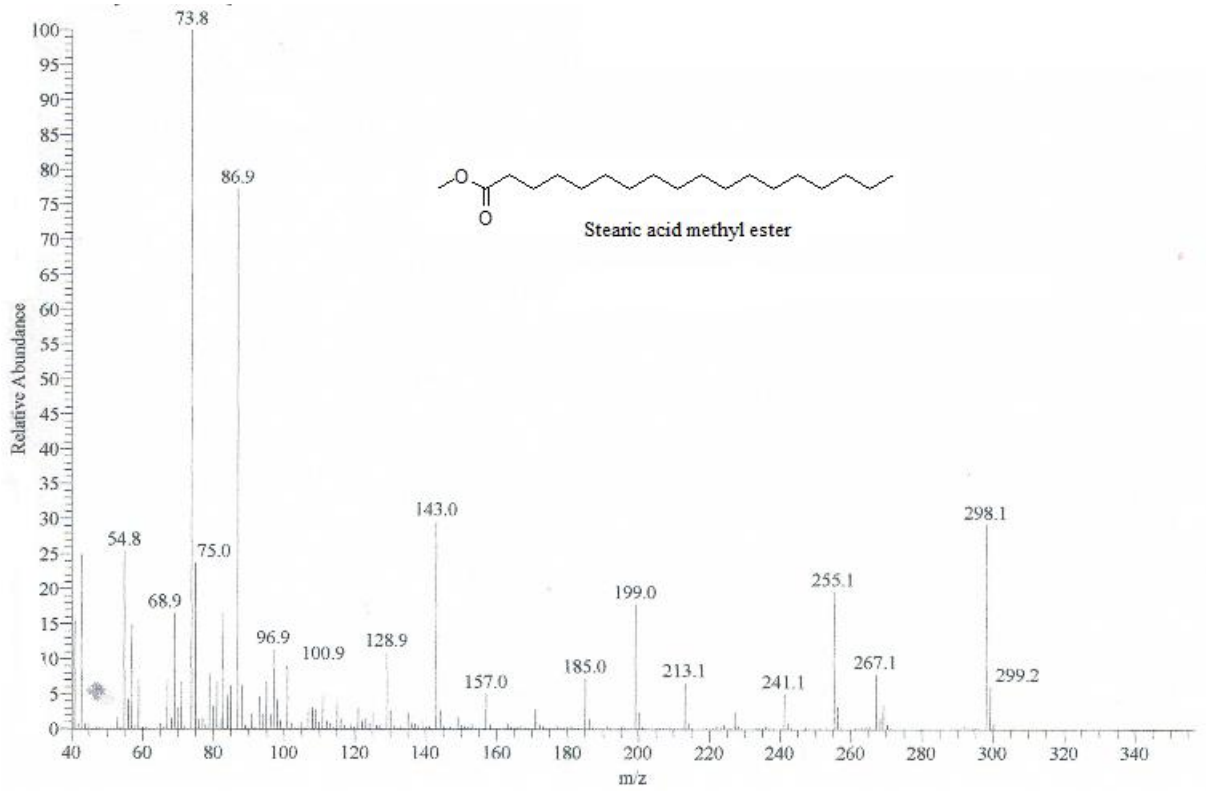


**Fig. 7: MS spectrum of stearic acid methyl ester**

**Separation of saponifiable fraction**

Into a (250 mL) round-bottomed flask, fitted with a reflux condenser, place (3.4 g) of oiliness residue were mixed with ethanol (15 mL), water (15 mL) and Sodium hydroxide (3g) and the mixture was refluxed for about (45 min). Ethanol was eliminated under reduced pressure using a rotary evaporator. Extraction was achieved adding to the solution (15 mL) of diethyl ether. The organic layer was added with concentrated HCl with (20 mL) of diethyl ether. After evaporation, fatty acids residue was obtained (3.24 g) [30].

**Methylation of fatty acids**

Into a (250 mL) three-necked flask equipped with a drooping funnel, a sealed stirrer unit a double surface condenser, place (3 g) of fatty acids and (25 mL) of methanol. Add slowly through the dropping funnel and with vigorous stirring a solution of concentrate sulfuric acid (1 mL). Reflux the mixture for about (2 h). Allow the reaction mixture to reach room temperature and to stand for (2 h), cool the mixture and pour onto (300 g) of crushed ice. The aqueous layer was then extracted with chloroform. Dried the Organic layer with anhydrous sodium sulfate. The solvent was distilled off to give (1.12 g) of methyl esters residue [31].

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