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Identification of *Pistacia vera* and *Prunus amygdalus Batsch* seed oils using GC-MS as useful methodology for chemical classification

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Abstract. Two types of nut seeds, pistachio and almond, are used to study their chemical and physical properties as well as their saturated and unsaturated fatty acid content using Gas Chromatography- Mass Spectrophotometry technique. According to the results, variant physicochemical proprieties of the oils in peroxide value are 5.7 and 7.3, respectively while acidity values are 0.1753 and 0.3756, respectively. These results are comparable to the quality of unsaturated fatty acids. In both of the studied samples, oleic acid is found to be the primary fatty acid followed by linoleic acid. Oleic acid in both samples is 60.72% and 58.19%, respectively while linoleic acid content reached 29.05% and 30.72%, respectively. Palmitic acid C: 16 as saturated fatty acid in the pistachio and almond samples is found to be 6.52% and 7.22%, respectively. In conclusion, pistachio and almond oils are rich in oleic acid that is considered a beneficial source of unsaturated fatty acids for human health.

1. Introduction

The original habitat of pistachio trees was in the Western Asia region before it extended to include the Eastern Mediterranean countries 4000 years ago. Pistachio belongs to the Pestachia family which includes various species of produced pistachio trees. Interestingly, pistachio cultivation came to the US too late [1] as its production began in California in the mid-1970s, yet it became the world's second largest producer of pistachio.

Arabs have known Almond since ancient times. For instance, in Egypt it is called "Shweiki or Ain al-Jamal", in Libya it is called "loze khazanin " and in Morocco "gerge" and in Tunisia "zouz". In Egypt, husk Almond stalks are known as "Sewaq Almaarba." In Saudi Arabia pistachio is called "derm" "derma" or "derman" which is used for one of the cosmetics as in lips dye in a beautiful purplish red color. On the other

hand, the almond tree *Prunus amygdalus* belongs to family Rosaceae that is cultivated in Mediterranean countries [19]. Pistachio nuts are widely consumed globally and that is balanced by production of one million metric tons annually. 80% of the world's annual production of pistachio is provided by California [20].

According to the nutrient database of the United States Department of Agriculture (USDA), almond is a source of protein, monounsaturated fatty acids, polyunsaturated fatty acids, dietary fibre, polyphenols, α-tocopherol, essential minerals, and other phytochemicals. Their proportion is (21.15, 31.55, 12.33, 12.5, 0.195) %, respectively [21-25]. Furthermore, it has extensively been reported that almond oil contents have importance in medical, industrial, and nutritional products. For example, almond nutrients play significant roles in preventing cardiovascular diseases, lipid regulation, antioxidant activity, and weight control. Additionally, almond contents can be used in cosmetic formulations (anti-wrinkle and anti-ageing products) [26-28]. Numerous studies have proven that almonds can also be used in functional foods as a prebiotic and as an ingredient in snacks and other food products [20], [29]. In fact, "nuts" are considered in the forefront of food that have useful fats such as unsaturated and monounsaturated fatty acids to protect the heart against many biological diseases [3]. The results in [4] showed that nuts play the largest role in reducing the risk of heart diseases as the rate of infection was reduced by more than half. The research also showed that the high content of fatty acid (useful fats) in almonds reduced the rate of negative cholesterol (LDL) and lowered the overall cholesterol levels. Generally, nuts have many health benefits as they are rich in dietary fibre which can reach more than 3% in cashew and hazelnut and that has a positive health impact because dietary fibre is linked to the reduction of many infections such as colon infection as well as reducing cholesterol and triglyceride levels [3]. Therefore, nutritionists recommend eating acceptable amounts of nuts (50 grams daily) which helps in weight loss because eating nuts stimulates satiety. However, excessive amounts of nuts can lead to weight gain because they are rich in calories and fat [5].

Compared to other types of nuts and seeds, pistachio and almonds seeds are high in antioxidant compounds specifically carotenoids such as lutein, zeaxanthin, beta-carotene, and gamma-tocopherol. Notably, the scientific commission of ministry of Agriculture in U.S. say: "of antioxidants", an ounce of pistachios contains a higher amount than that found in a cup of green tea! when the researchers of this ministry reviewed the total of antioxidant capacity (TAC) of one for the most important types of phenols in one hundred types of food commonly eaten. Besides, they found that pistachios were among the highest containment food groups in comparison with other nuts. Pistachio contains the "lutein" antioxidant whose amount is 13 times its amount in hazelnut which comes second after pistachio [5]. Reportedly, that taking this type of antioxidant reduces the risk of deterioration of the integrity of the dark spot in the retina at old ages [5]. The goal of this study is to determine unsaturated fatty acids in seeds oil of pistachios and almonds by GC-MS technique and to study its physicochemical characteristics that prove its important roles in human health.

2. Materials and Methods

2.1 Samples Collection

The study was conducted at the Faculty of Agriculture, Basrah University's GC-MS Central Laboratory. Two types of fresh nuts were collected from Basrah's local market: (Pistacia vera) and (*Prunus amygdalus*).

2.2 Samples Preparation

The part covered were separated from the pulp for the nuts then the pulp was collected for each type and washed with distilled water to remove the remaining meat part of the pistachios and almonds. Then they were dried at 70 ◦C under vacuum. The next step was to grind the pulp of pistachios and almonds with a mill type ZN-200 with a capacity of 500 g. Then the powder of each type of nuts was collected.

2.3 Extraction of Oil and Lipid Components

The pistachio and almond oils were extracted by procedure [6] that is continuous of extraction using 99.8 % hexane solvent, where 40 g of pistachios and almonds were placed in the soxhlet units and the extraction process was carried out for 24 hours. The oil was decomposed from the hexane by rotary evaporator and the amount of oil obtained by weight difference was estimated [6].

2.4 Physical and Chemical Parameter of Oil

Refractive index of oil seeds at 25 °C was determined by an Abbe refractometer device [8]. A thermal-hydrometer and viscometer (Canon-Fenske calibrated, 15 cSt max. range) were used to measure the gravity and viscosity, respectively in accordance with the ASTM standards D1298 and D445 [30].

The oil's odor and color, using visual inspection at room temperature (7).

According to the European Official Method of Analysis (Commission Regulation EEC N-2568/91), specific extinctions (E1 percent 1 cm) at 232 nm (K232) and 270 nm (K270) were determined using [6]. The content of chlorophyll and carotenoids was measured as described by [8]. The acidity value was calculated according to the following equation:

$$\text{Acidity value } (A.V.) = 1ml\ NaoH \times NNaoH \times 5.009\ wt\ of\ sample$$

Peroxide value was evaluated according to AOAC [30]. 2g oil was weighed into a tube and 1g of powdered potassium iodide with 20ml of solvent mixture was added. The tubes were placed in boiling water for 30s. The content was transferred into a flask containing 20 ml of 5% iodide solution. Then, the tubes were washed with 25 ml of distilled water and titrated with 0.002N sodium thiosulphate solution using starch as indicator; a blank was prepared alongside the oil samples. Peroxide value was calculated according to the following formula:

$$P.v = 2(v1 - v2/_{w}$$

W= Weight of sample, V1= the samples under study, V2 = blank titer value.

2.5 Preparation of Fatty Acid Methyl Esters (FAME)

Total fatty acid content and fatty acid composition were determined simultaneously in seed oil samples. Triplicate fatty acid analysis consisted of two sequential steps, preparation of fatty acid methyl ester (FAME) and chromatographic analysis [9]. The method was performed to esterify lipid extract. FAME was prepared for esterification from the lipids extracted from the samples by heating first with methanolic NaOH, then with BF3 methanol. 5 ml n-heptane was added to recover methyl esters in organic phase. Saturated NaCl solution was added to the mixture. Then, the aqueous and organic layers were separated using a separating funnel. The upper layer contains fatty acid methyl esters Fatty Acid Methyl Aster in hexane (FAME) and the lower layer contains the soaps from the reaction. The upper phase was taken in 10 ml glass vials after the lower phase was discarded, it has been stored under sub-zero temperature evaluation until GC-MS measurements was performed.

2.6 Fatty Acid Methyl Ester (FAME) Analysis with Gas Chromatography Mass Spectrometry (GC MS)
The gas mass spectroscopy analysis of methylated fatty acids was performed on the capillary column of a Shimadzu QP2010 quadrupole Gas Chromatography Mass Spectrometry (GC-MS) equipped with a capillary column type DB5 MS (5% phenyl, 95% methyl polysiloxane) as a stationary phase, its dimensions are (30 m length, 0.25 mm diameter, 0.25µm thickness of stationary phase) one microliter (µl) sample was injected into the capillary column. Spontaneously, the injection in column is AOC-20i+s. Absolute helium with concentration 99.90% was used as the carrier gas. Temperature of both the injector and detector temperatures were set at 280°C. Injection was performed in split mode (1:30). The column temperature was programmed initially at 50°C for 1 minutes and then to increase at a rate of 5°C per min with a final temperature of 280°C for one minute. Fatty acid methyl esters were separated at constant pressure (100.1 kPa) and peaks were identified by comparing the mass rang with the mass spectral database. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008 [10].

Table 1. Fatty Acid Methyl Ester (FAME) Gas Chromatography Mass Spectrometry (GC MS) Experimental Conditi on.

Gas Chromatography	Mass Spectrometer
Column Oven Temp. :50.0 °C	IonSourceTemp :200.00 °C
Injection Temp. :280.00 °C	Interface Temp. :280.00 °C
Injection Mode :Split	Solvent Cut Time :3.00 min
Flow Control Mode :Linear Velocity	Start Time :3.00min
Pressure :100.1 kPa	End Time :31.00min
Total Flow :55.5 mL/min	ACQ Mode :Scan
Column Flow :1.69 mL/min	Event Time :0.50sec
Linear Velocity :47.2 cm/sec	Scan Speed :1666
Purge Flow :3.0 mL/min	Start m/z :50.00
Split Ratio :30.	End m/z :800.00

3.Results and Discussion

3.1 Oil yield
Results in Figure 1 showed that there are differences in oil yield content in the pistachio and almond seeds which were 40% and 55% respectively. These variations in seeds oil yields may be due to their varied nature, farming climate, ripening crop rang, genetic variation and environmental condition [11].

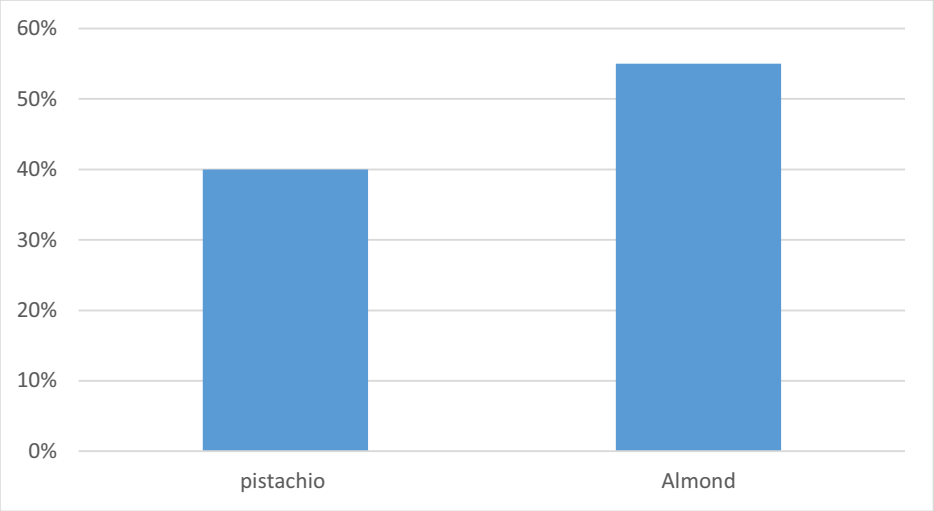


Figure 1. the percentages (%) of the pistachio and almond seeds oil content.

3.2 Physical and Chemical Characterization

It is clear from the results in Table 2 that a variation in the proportion of peroxides value were 7.3 and 5.7 in the samples of pistachios and almonds, respectively while the acidity value was 0.3756 and 0.1753, respectively. Table 2 displays the physicochemical characterization of pistachio and almond seeds oil. Conventionally, the physical properties of lipids reveal their chemical structures and functional groups. and significant influenced of lipids in foods and the techniques needed for their manipulation and processing. Moreover, it can be used to evaluate the purity or quality of lipid material in accordance with recognized types or preferred features [12]. The pistachio seeds oil was greenish yellow and the almond seeds oil were colorless. Color is one of the sensory properties with a controlling impact on its acceptance food by the consumer depends on a lot of characteristics such as the environmental condition, maturity, degree of processing and other features of food can be acquired. [13] states that the oil is fluid at room and fridge temperature. The refractive index of oils relies on their molecular weight, the length of the fatty acid chain, the degree of unsaturation and the degree of conjugation [14]. The pistachios and almonds seed oil showed refractive index of 1.5024and 1.5103, respectively, those findings were in excellent agreement with 1.46 in Turkish pistachio seed oil [15].

Table 2. Physical and chemical characterization of nuts oil.

Parameter	pistachios seed oil	Prunus seed oil
Refractive index	1.5024	1.5103
Peroxide value meq O2/kg	7.3	5.7
Acid value	0.3756	0.1753
Viscosity	32.0751	30.4121
Color	green yellowish	Colorless

3.3 Acid Value Determination

The acid value of pistachios and almonds seeds oil have been determined to be 0.3756 and 0.1753 mg KOH /g oil, respectively. Its maximum acceptable level of 4 mg KOH / g oil that is measured to free fatty acid content (saturated and unsaturated fatty acids), this is due to enzymatic activity, and radiation exposure which is indicative of spoilage. The results of this work showed good agreement with the results of [15] and [32] which indicated that saturated fatty acids in the contents of pistachios and almond oil increased with exposure to irradiation of gamma dose while unsaturated fatty acid decreased in the oil of these nuts decreased with gamma irradiation.

3.4 Peroxide Value Evaluation

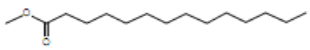
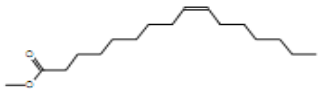
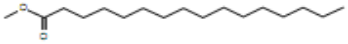
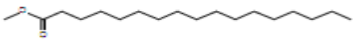
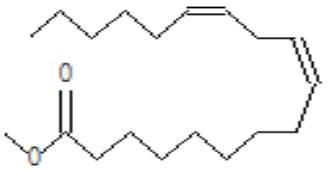
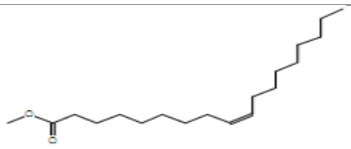
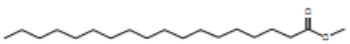
Also Table 2 provides the results of the peroxide value (PV) in pistachios and almond oils reaching (7.3, 5.7) meq O_2/kg oil, respectively. The high PV values indicate that increase the concentrations of oxidative rancidity this is agreement with [18] reported that similar to PV is 5.4 meq O_2/kg pomegranate oil. However, [32] found that increase in PV for each pistachio and almond oils to exposure gamma irradiation reaching (1.0-1.8) meq O_2/kg pistachio, (0.8-1.4) meq O_2/kg almond. The amount of peroxide value can be reduced using the addition of antioxidants, thus reducing the rate of rancidity [17].

3.5 Determination of the Fatty Acids Components by Using Gc-Ms Spectrometry

Gas-chromatography coupled with mass spectrometry analysis of fatty acids in the oil from seeds of pistachios and almonds have been recorded in Fig. 2 and Fig. 3 and Table 3. We found that the seven identified fatty acids included saturated, monounsaturated and unsaturated fatty acids which accounted Oleic acid methyl ester,C18:1 (58.19 , 60.72) % for pistachio and almond oils, respectively, followed by Linoleic acid, methyl ester C18:2 (30.72 , 29.05) % respectively , Palmitic acid methyl ester C16 (7.22, 6.52) % respectively, The saturated fatty acids identified include Palmitic acid methyl ester, Myristic acid methyl ester, palmitoleic acid methyl ester , stearic and margaric acid methyl ester. The results are similar to [15] who studied eight different varieties of pistachios that found an oleic acid is higher than unsaturated fatty reaching (58.19) %. In addition, Our results of almond oil oleic acid content was (58.19)% this agreed with [33] found which the major unsaturated fatty acid compounds of almonds reaching Oleic acid (77.8%; 75.3%), linoleic acid (13.5%; 15.8%) its application as antioxidants and anticancer, [16] who studied oil and lipid compounds from almond avium seeds. In addition, Fig 4 and 5 indicates that the chromatogram of pistachio and almond oil are determined by GC-MS which detected the retention time of oils compounds the highest percentage of saturated fatty acid **Stearic acid** was 21.750 followed by **Margaric acid was 20.525. Furthermore, the** retention time of unsaturated fatty acid were oleic acid reaching 21.517 followed by linoleic acid 21.383, this is results agree with [34], [35] that found the results similar to ours to analysis bioactive compounds using GC-MS.

In conclusion, our results indicated that the oil composition of ***Pistacia vera*** **and** ***Prunus amygdalus*** seeds were collected from local market in Basrah. It has been studied some of physical and chemical properties, the percentage of unsaturated fatty acids mainly, oleic acid of almond was higher than pistachio. The unsaturated fatty acid was investigated using GC-MS technique. Nuts are especially pistachio and almond oils rich in unsaturated fatty acid that its beneficial effects on human health as antioxidants, and anticancer.

Table3.fatty acid content of pistachio and almond seed oil

Peak no	Fatty acid name	Retention time (RT)	(%)		Structure
			pistachios seed oil	almond seed oil	
5	Myristic acid	6.481	0.09	0.04	
6	Palmitoleic acid	18.950	0.60	0.4	
7	Palmitic acid	19.25	7.22	6.52	
9	Margaric acid	20.525	0	0.04	
10	Linoleic acid	21.383	30.27	29.05	
11	Oleic acid	21.517	58.19	60.72	
13	**Stearic acid**	**21.750**	**0.86**	**1.45**	

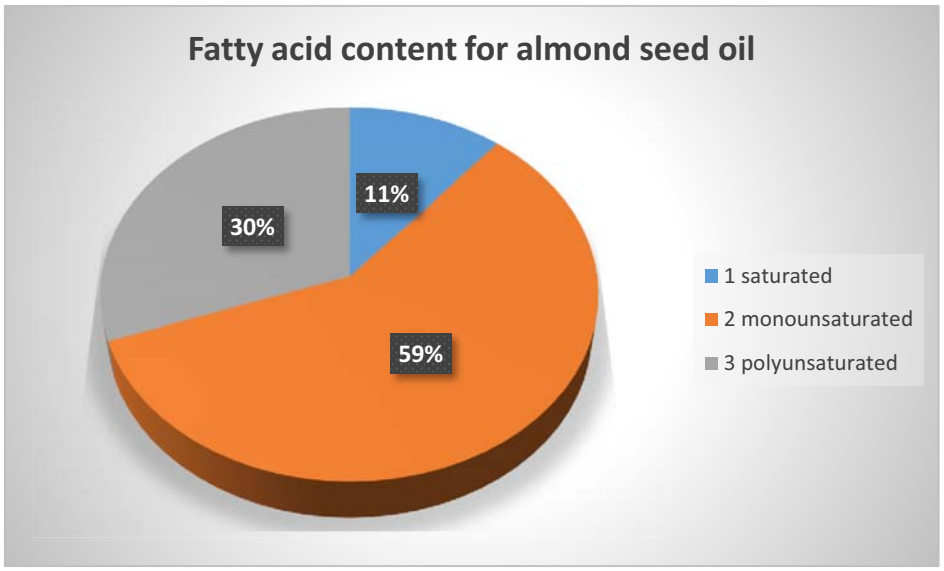


Figure 2. Fatty acids %content for almond oil.

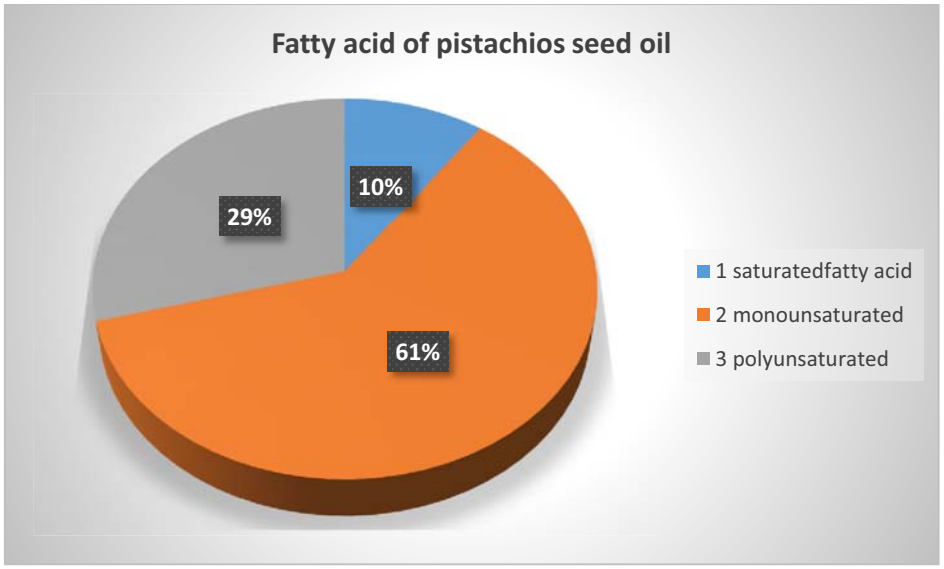


Figure 3. Fatty acids %content for pistachios seed oil.

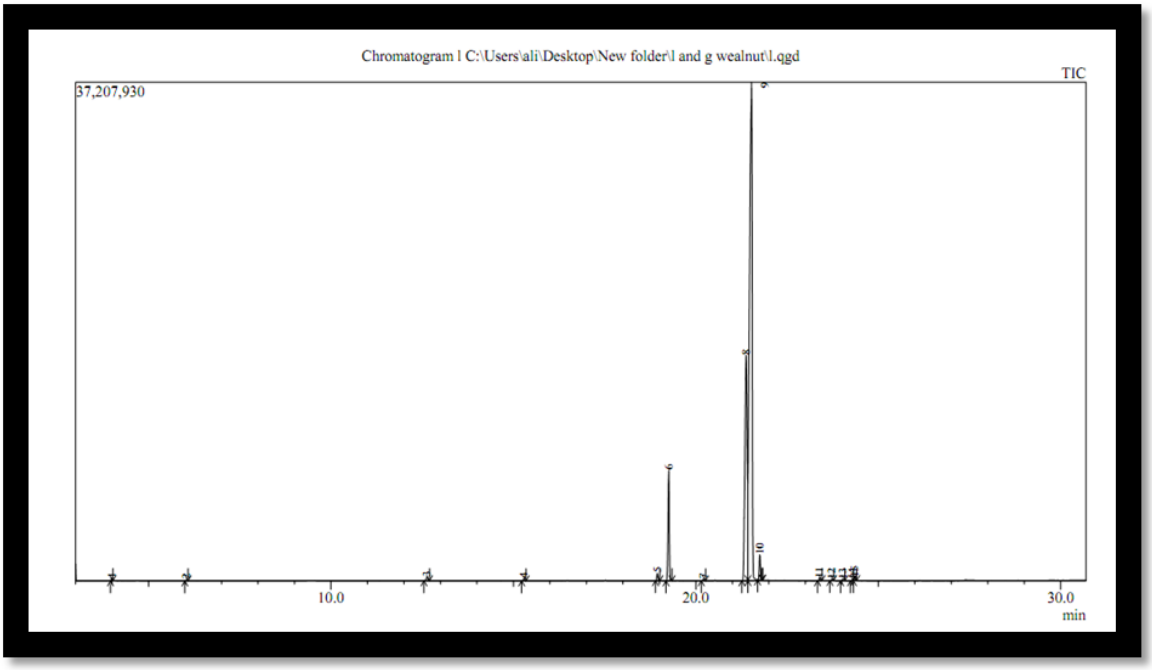


Figure 4. GC–MS Fatty acid composition of the pistachios seed oil with column DB MS-59

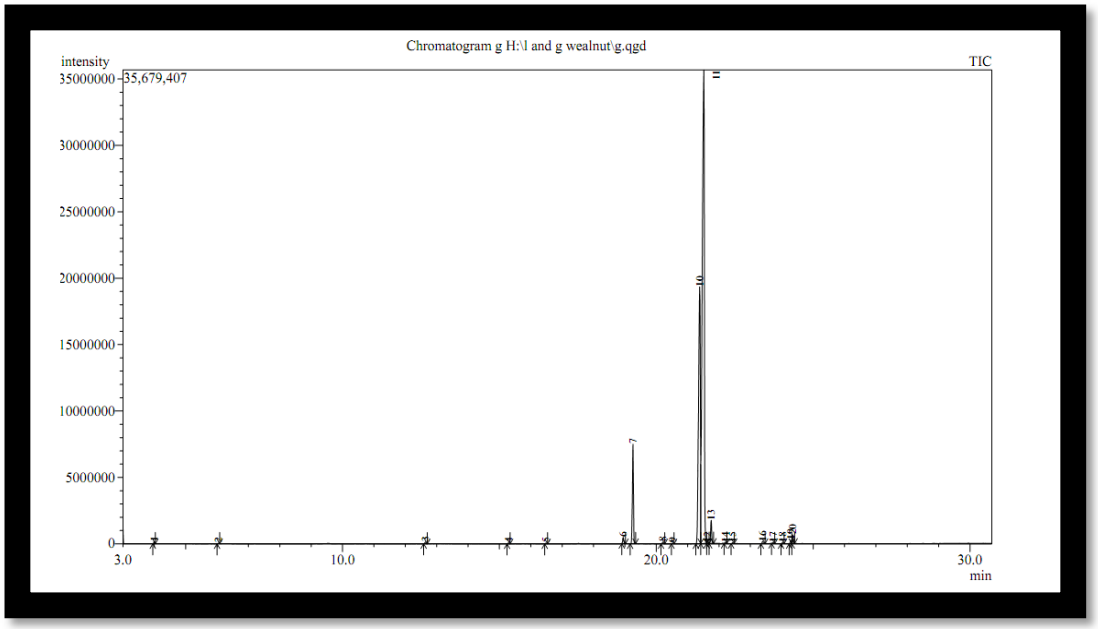


Figure 5. GC–MS Fatty acid composition of the almonds seed oil with column DB MS-59

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