

## Characterization of Extracted Oil from Seeds of Terebinth (*Pistacia Terebinthus L.*) Growing Wild in Turkey

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### Abstract

In this study for which terebinth seeds obtained from Elazığ were used and the obtained oil physical/chemical properties are investigated. Because these properties are quite effective on oil quality. At first the composition of terebinth was determined in terms of nutritional elements (crude oil, crude protein, crude cellulose, etc.). According to soxhlet method, it was determined that terebinth seeds included 47% (w/w) oil. Some physical and chemical properties of oil samples such as free acidity, peroxide value, saponification number, iodine number, quantity of unsaponifiable matter, sediment, viscosity, specific gravity and refraction point were defined by using various types of solvents. It was concluded that the terebinth oil extracted with petroleum ether was different in terms of peroxide value and it was not sufficient when compared to oil samples obtained by other solvents. Finally, oil acidity composition was determined by using GC-FID and it was confirmed that terebinth oil was composed of about 74% unsaturated fatty acids and 26% saturated fatty acids. Through mean values, it was determined that terebinth oil included 45.8% oleic, 23.93% linoleic ( $\omega$ -6), 0.47% linolenic ( $\omega$ -3), 3.78% palmitoleic, 24.27% palmitic and 1.7% stearic acid. Oleic acid, linoleic acid and palmitic acid were considered as main fatty acids of terebinth oil.

**Key Words:** Terebinth oil, composition of fatty acids, oil characterization, *Pistacia terebinthus L.*

## Türkiye’de Yabani Olarak Yetişen Menengiç (*Pistacia terebinthus L.*) Tohumlarından Ekstrakte Edilen Yağın Karakterizasyonu

### Özet

Elazığ’da temin edilen menengiç tohumlarının kullanıldığı bu çalışmada, elde edilen yağın fiziksel ve kimyasal özellikleri incelenmiştir. Çünkü bu özellikler yağın kalitesi üzerinde oldukça etkili ve belirleyicidir. İlk olarak menengiçin besin öğeleri açısından (ham yağ, ham protein, ham selüloz v.b.) bileşimi belirlenmiştir. Soxhlet metoduna göre, menengiç tohumlarının yağ içeriği % 47 olarak tespit edilmiştir. Farklı çözücü türleri kullanmak sureti ile elde edilen yağ örneklerinin serbest asitlik, peroksit değeri, sabunlaşma sayısı, iyot sayısı, sabunlaşmayan madde miktarı, tortu, viskozite, özgül ağırlık ve kırılma indisi gibi bir takım fiziksel ve kimyasal özellikleri tayin edilmiştir. Petrol eteri ile ekstrakte edilen menengiç yağının peroksit değeri açısından farklılık gösterdiği ve elverişli olmadığı sonucuna varılmıştır. Son olarak GC-FID kullanmak sureti ile yağ asidi bileşimi belirlenmiş ve menengiç yağının yaklaşık olarak % 74 oranında doymamış, % 26 oranında ise doymuş yağ asitlerinden ibaret olduğu tespit edilmiştir. Ortalama değerler üzerinden menengiç yağının % 45.8 oleik, % 23.93 linoleik ( $\omega$ -6), % 0.47 linolenik ( $\omega$ -3), % 3.78 palmitoleik, % 24.27 palmitik ve % 1.7 stearik asit içerdiği saptanmıştır. Oleik asit, linoleik asit ve palmitik asit menengiç yağının ana yağ asitleri olarak dikkate alınmıştır.

**Anahtar Kelimeler:** Menengiç yağı, yağ asidi bileşimi, yağ karakterizasyonu, *Pistacia terebinthus L.*

### 1. Introduction

Terebinth (*Pistacia terebinthus L.*) is a part of the vegetation of our country and a kind of self-growing plant without needing farming. In Figure 1, terebinth tree and its unripe fruit are seen. *Pistacia terebinthus* (terebinth or turpentine tree) has a strong resin smell and it is

small. As a member of Anacardiaceae (pistacia, pistachio) family, this kind of tree is not only native to Asia and Mediterranean but it also grows widely in Southern parts of Turkey [1]. It especially grows in the pinewoods of Toros mountains at 1600 m [2]. It is also known that it grows widely in rural areas of East Anatolia and Southeast Anatolia regions.

The reddish purple flowers of terebinth blossom in March and April. The fruit of terebinth is small and globular and when it ripens its changes colour from green to blue. Its seeds ripen between August and September. Being cold and drought resistant, this kind of tree has a

good drainage and it grows better on dry, hot, calcareous and rocky fields. It grows best on alkaline soil. It does not get taller much, its growth rate is low and its need for light is quite high [3-5].



a) b)  
**Figure 1.** a) Terebinth tree, b) unripe fruits

*Pistacia terebinthus* could be turned into pistachio by grafting. The number of pistachio trees in our country is 42 million and about 14 million of them were obtained by grafting wild species like terebinth [6].

There have been many studies on vitamine and essential oil composition and antimicrobial, antifungal and antioxidant qualities of terebinth used widely in folk medicine. Recently, it has been attracting attention of researchers as a food stock. In a study conducted by Özcan [7], it was stated that terebinth oil extracted with diethyl ether included 0.1% lauric, 0.1% myristic, 21.3% palmitic, 3.4% palmitoleic, 2% stearic, 52.3% oleic, 19.7% linoleic, 0.6% linolenic, 0.1% eicosanoic and 0.2% eicosenoic acid. In an another study done by Matthäus and Özcan<sup>5</sup> in the same field, similar data regarding to fatty acid composition were reported.

Studies about terebinth oil are quite new. Therefore, there has not been any comprehensive study on fatty acid composition and physicochemical properties of terebinth oil obtained under different extraction conditions.

Within the scope of this study, it is aimed to close the existing gap in the literature.

## 2. Material and Methods

**2.1. Plant material:** Terebinth seeds were taken from the region of Elazığ-Harput. The seeds brought into the laboratory were dried by natural air circulation and then were cleared of contamination such as stem, waste and dust. The samples were maintained at about 4°C in closed containers.

**2.2. Pre-treatments** After the seeds were ground by an automatic mortar until they become 0.55 mm-particles, they were put into conditioning process for 15 minutes in a drying oven at 90°C. During this process, homogenisation was provided by adding a sample in every 5 minutes. Finally, 15% water was added to the sample and it was cooked at 115°C. Cooking process was continued until the sample was dry [8, 9].

**2.3. Determination of the composition of terebinth seeds:** In line with this purpose, the analysis of crude oil, crude protein, crude cellulose, ashes and total carbohydrate was conducted. The determination of oil was realized by soxhlet method and crude protein determination was done according to kjeldahl method [9-11].

**2.3.1. Ash determination:** The pulp degreased and dried at 105°C was taken into a porcelain crucible that was brought to stable weighing ( $m_1$ ) at 105°C previously. The crucible filled with the dry sample was put into ash oven and burned at 550 °C. Burning process was continued until the crucible reached stable weighing ( $m_2$ ). The quantity of ash (A) was calculated as percentage by weight as follows [10].

$$A = \frac{m_2 - m_1}{m} \cdot 100 \quad (1)$$

m: the sample weight on a dry basis, g

**2.3.2. Crude cellulose determination:** The pulp degreased and dried at 105°C was taken into a flask with 1000 ml volume. Afterwards, 200 ml 0.255 N  $H_2SO_4$  was added into the flask and it was boiled under condenser by mixing. Right after this process, acid solution was spilled into a filtering cone and it was filtered under low-vacuum. After filtering the acid, the cone was rewashed with some hot water. The presscake was dried and was boiled with 200 ml 0.313 N NaOH. It was again filtered under low-vacuum in the filtering cone. After the basic solution was filtered, it was washed with hot water one more time. After being washed with hot water, the presscake was washed with 1 % HCl (w:v) once and then was washed with hot water, and finally it was washed with ethanol twice and with ether three times. The presscake obtained was taken into a porcelain crucible that was brought to stable weighing at 105°C previously.

The sample full of crucible was brought to stable weighing by being dried in a drying oven at 105°C ( $m_1$ ). After drying process, the crucible filled with the dry sample was put into ash oven and burned at 550°C. Burning process was continued until the crucible reached stable weighing ( $m_2$ ). The quantity of crude cellulose

(RC) was calculated as percentage by weight as follows [11,12].

$$RC = \frac{m_1 - m_2}{m} \cdot 100 \quad (2)$$

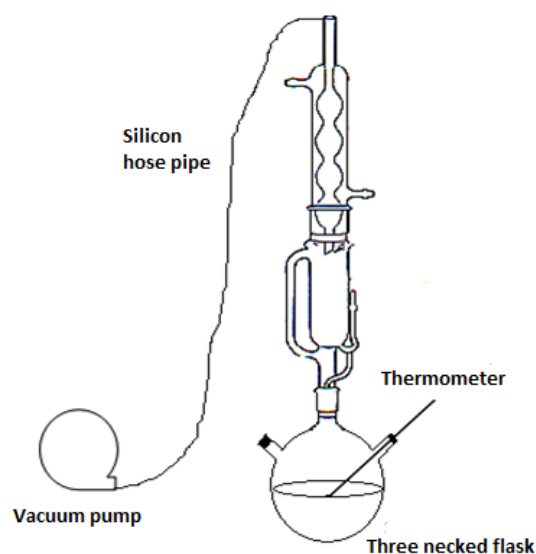
m: the sample weight on a dry basis, g

**2.3.3. The determination of total carbohydrate:** It was obtained by subtracting fat, protein, fiber and ash values from the dry based sample weight [13].

$$\text{Carbonhydrate \%} = 100 - (\text{crude oil} + \text{crude protein} + \text{cellulose} + \text{ash}) \quad (3)$$

**2.4. FTIR analysis of crude oil:** IR analysis of oil sample was performed by ATI Unicam Mattson 1000 FTIR device.

**2.5. Determined of extraction conditions for oil samples:** A series of extractions were performed prior to the study. Extraction studies was performed by using Soxhlet extraction the method. As the solvent was used n-hexane, n-heptane, petroleum ether and  $CCl_4$ . The extracted oil yield was examined depending upon extraction time, temperature and the dosage of terebinth seeds. As a result of, the optimum extraction conditions (Table 1) were determined according to extracted oil yield. The apparatus used in the extraction operation is shown in Figure 2.



**Figure 2.** Soxhlet extractor under the vacuum

**2.6. Determination of physical and chemical properties:** In this context, the terebinth oil samples of analysed were obtained under the

different extraction conditions. The extraction conditions under which the oil samples are obtained are stated in Table 1.

**Table 1.** The extraction conditions considered optimum

Solvent type	Extraction time (min.)	Extraction temperature (°C)	Sample weight (g)	Particle size of sample (mm)
<b>n-hexane</b>	30	48	10	0.55
<b>n-heptane</b>	40	98	10	0.55
<b>Petroleum ether</b>	20	40-60	10	0.55
<b>CCl<sub>4</sub></b>	40	56	10	0.55

The determination of free fatty acids (FFAs) was realized by solving the oil sample in neutralized diethyl ether-ethanol mixture and by titrating with 0.1 N KOH solution. The determination of saponification number (SN) was done by saponifying the oil sample by 0.5 N KOH solution and by determining the consumption through titrating the alkalosis in solution environment with 0.5 N HCl. After the oil sample was saponified with 2 N KOH solution, the mixture obtained was transferred into a separating funnel. It was washed with water and petroleum ether here. When phase separation was observed in separating funnel, wet soap phase below was taken into another container and the phase with petroleum ether composing the part that is unsaponifiable was processed distillation under low-vacuum in a balloon with a known tare. The quantity of unsaponifiable matter was calculated by weighing difference. Iodine value was determined according to Wijs method. To determine the peroxide number, the oil sample was solved by adding acetic acid-chloroform solution. 1 ml out of saturated KI solution was added to the mixture and it was shaken swiftly and it was kept in darkness for 5 minutes. At the end of this time, 50 ml water was added to the mixture and until the change in colour was observed, it was titrated with 0.002 N sodium thiosulphate solution in the presence of starch indicator [7, 9, 11, 14-16]. Sediment content was determined at 6000 rpm at the end of a 10-minute-process. The specific gravity was determined by using a pycnometer with thermometer. While refractive index was being measured, measurements were done after the heat of refractometer was stabilised with a water bath at 40°C. Viscosity was determined with a

device measuring by TuningFork vibration method.

**2.7. The preparation of fatty acid methyl esters (FAMES):** So as to prepare methyl esters of oil samples, about 50 mg oil extract was taken into 30 ml-test tube and 1 ml hexane/isopropanol mixture was added, and it was solubilised with the help of vortex. It was mixed by adding 5 ml methanol that includes 2% sulphuric acid. This mixture was kept in methylation for 15 hours in a drying oven at 50°C. Then, the tube was taken out of the oven and cooled until it reached room temperature and it was mixed by adding 5 ml 5%-sodium chloride thoroughly. Fatty acid methyl esters generated in the tube were extracted with 5 ml hexane, and after hexane phase was taken with a pipette, it was processed with 5 ml 2% KHCO<sub>3</sub>, and it was kept for 4 hours to separate the phases. Then, the mixture including methyl esters was heated at 45°C under nitrogen gas flow and its solvent was evaporated. The residue was resolved with 1 ml hexane and prepared for GC analysis [17]. By applying this procedure to each sample of terebinth oil extracted under the conditions considered as optimum with n-hexane, n-heptane, petroleum ether and CCl<sub>4</sub> solutions, oil samples were prepared for GC analysis.

In addition, pure fatty acid standards were derivatized for GC analysis. Therefore, after 36.7 mg palmitic acid, 39.7 mg stearic acid, 30 µl oleic acid, 30 µl palmitoleic acid, 30 µl linoleic acid and 30 µl linolenic acid were put into a 30 ml-test tube, derivatisation procedure defined above was applied as it is.

**GC operating conditions:****Table 2.** GC conditions for analysis of fatty acid methyl esters

<b>Instrument</b>	SHIMADZU 17 Ver.
<b>Column</b>	Stainless steel (25mx0.25µmx25µm)
<b>Dedector</b>	FID
<b>Carrier gaz</b>	N <sub>2</sub>
<b>Carrier gaz flow rate</b>	0.5 ml/min
<b>Injection temperature</b>	240 °C
<b>Dedector temperature</b>	280 °C
<b>Column temperature programmes</b>	Was initiated by 120 °C, 5 °C/min with a heating rate was set to 200 °C, from 200 °C to 220 °C with 4 °C/min heating rate was brought.
<b>Injection amount</b>	1 µl

**2.8. Statistical analyses:** Experimental results were evaluated with ANOVA by SPSS 20.0 package program.

**3. Result**

One of the reasons why terebinth seeds are focused on in this study is that they have a high fat content. The results of the experiments done to determine the content of the seeds are given in Table 3.

**Table 3.** Composition of terebinth seeds

<b>Component</b>	<b>Values (weight %)</b>
Crude oil	47
Crude protein	9.1
Cellulose	17.52
Ash	5.08
Total carbohydrate	21.3

While the results were somewhat higher than the results found in the study conducted by Özcan<sup>7</sup> in terms of crude oil content, they showed similarity with the results in the studies by Matthäus and Özcan [5] and Geçgel and Arıcı [18]. Geçgel and Arıcı stated that fat content of various terebinth samples changed between 35.26% - 47.52%. Matthäus and Özcan determined the chemical content of 14 various terebinth samples and stated that the terebinth samples taken from Mersin (Silifke) included average 45.1% fat.

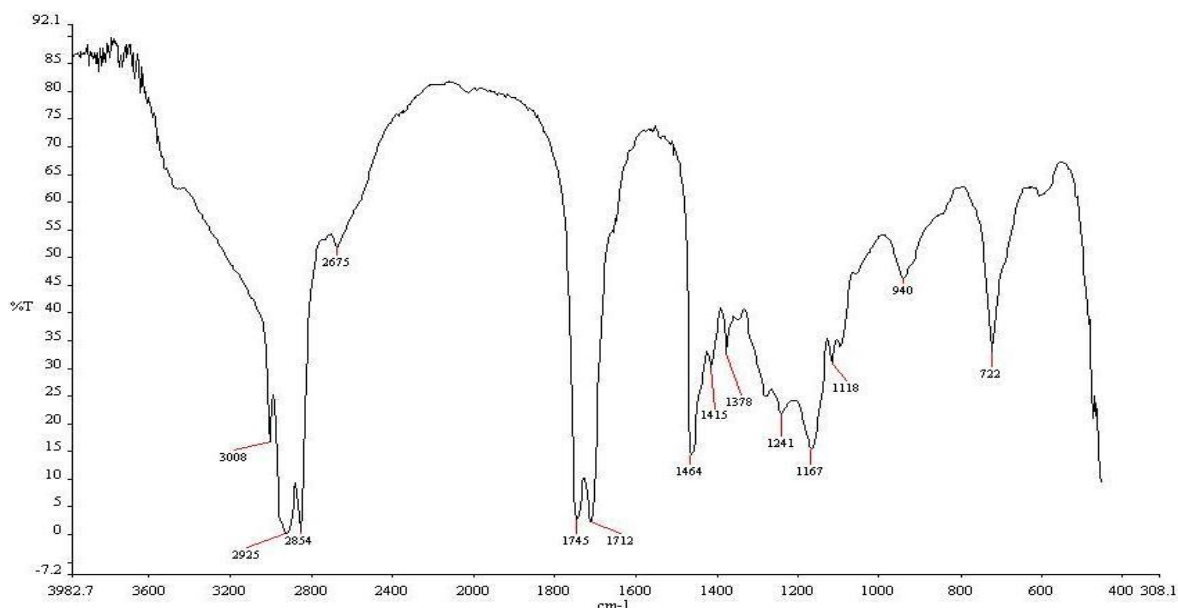
IR spectrum of raw terebinth oil extracted with hexane is seen in Figure 3. When IR

spectrum was analysed, the peak belonging to aromatic ring C-H stretching vibration at 3007 cm<sup>-1</sup>, the peaks belonging to aliphatic C-H stretching vibration at 2923 and 2854 cm<sup>-1</sup> (2923 cm<sup>-1</sup>: C-H assymetry and 2854 cm<sup>-1</sup>: C-H symmetry), C=O peak at 1747 cm<sup>-1</sup>, the peak belonging to -C=C- cis position at 1655 cm<sup>-1</sup>, C-H shearing peak at 1464 cm<sup>-1</sup>, C-O peak at 1163 cm<sup>-1</sup> and C-H rocking peak at 722 cm<sup>-1</sup> were observed. Except from the aromatic ring peak defined in the spectrum above, other peaks are the main ones presenting triglyceride functional groups [19].

In Table 1, the physical and chemical qualities determined for crude terebinth oil obtained under prescribed conditions for each solvent are given in Table 4.

As it is also understood from Table 4, except for the peroxide value, physicochemical properties of terebinth oil extracted with n-hexane, n-heptane, petroleum ether and carbon tetrachloride showed similarities and the determined values are in accordance with the ones reported in other studies.

In a study in which diethyl ether was used as a solvent, the physicochemical properties of the oil obtained from terebinth such as specific gravity ( $d_{20}^{20}$ ), fraction index ( $n_D^{40}$ ), free acidity degree (% oleic), peroxide value (meq/kg), saponification number, the quantity of unsaponifiable matter (mg/kg) and iodine value were determined as 0.9742, 1.477, 0.86±0.16, 0.47±0.09, 156.7±14.64, 15.7±3.3 and 89.06, respectively<sup>7</sup>. In another study, the peroxide value of terebinth oil was stated to be between 0.45 and 0.76 meqO<sub>2</sub>/kg [18].



**Figure 3.** IR spectrum of crude terebinth oil

**Table 4.** Chemical and physical characteristics for crude oil obtained with different solvents

	hexane	heptane	Petroleum ether	CCl <sub>4</sub>	Mean value	Standard deviation
FFAs (oleic %)	0.684	0.675	0.67	0.637	0.666	0.018
Saponification number	180.75	167.45	162.44	185.28	174	9.35
The quantity of unsaponifiable matter (g/kg)	5.3	4,25	6.54	6.36	5.61	0.92
Iodine value	87.03	83.34	82.33	81.33	83.51	1.98
Peroxide value (meq/kg)	0.44	0.427	<b>13.535</b>	0.686	0.518	0.12
Refractive index ( $n_D^{40}$ )	1.469	1.466	1.463	1.462	1.465	0.003
Viscosity at room temperature (cp)	57.4	61,2	59	60.1	59.4	1.4
Specific gravity ( $d_{20}^{20}$ )	0.917	0.913	0.918	0.909	0.914	0.003
Sediment content (weight %)	4	5	4	6	4.75	0.83
<b>p value = 1.000</b>						

As seen in Table 4, the peroxide value of the oil extracted with petroleum ether was determined to be higher than the oil samples obtained with other solvents.

In the scope of a project searching for the valuation means of terebinth, only petroleum ether was used as a solvent and it was determined that peroxide value was minimum 13.09 meq/kg [13].

The peroxide value presenting peroxide concentration is a measure for active oxygen content in oils and is a significant parameter as to determine the degree of degradation progress. Although peroxides are not directly responsible for the degradation of smell and taste, they have an accelerating role in degradation.

In addition, according to Turkish Food Codex Communiqué on Edible Vegetable Oils Called by Plant Names, it was stated that the peroxide



number for refined oil could be maximum 10 meqO<sub>2</sub>/kg and the peroxide number for cold pressed oils and extra virgin oils could be maximum 15 meqO<sub>2</sub>/kg [20].

In the light of these data, it was agreed that terebinth oil extracted with petroleum ether was not favourable compared to other oil samples in terms of the peroxide number of terebinth oil. However, crude oil was extracted with petroleum ether is not known why high peroxide value.

In the scope of this study, oil was also extracted with polar solvents like ethanol and acetone. However, it was noticed that crude oil obtained by these solvents hardened and became jellylike at 80°C. In spite of these extraordinary conditions, significant deviations were not determined in physicochemical properties of crude oil that became jellylike. Fraction index, free fatty acidity (% oleic), peroxide number and iodine number of the crude oil extracted with ethanol and acetone were determined to be ( $n_D^{40}$ )1.4645, 1.5-1.9, (meq/kg) 0.7 and 85.11, respectively. As understood, only free acidity degree was stated to have a higher value than the required degree. Terebinth is a quite colourful material and it is thought that polar solvents like ethanol and acetone, by solving some pigmentary substances, polar groups, oxidized fatty acids and resins within terebinth, carry them to the oil environment and these carried materials change the existing physical condition of oil mass by degrading at high temperature.

In the method for the solution of this problem, it was observed that terebinth sample was first treated pre-extraction with water for 1-2 hours (thus, most of the polar groups in the structure were extracted) and then was extracted with ethanol and acetone and the existing problem did not occur again.

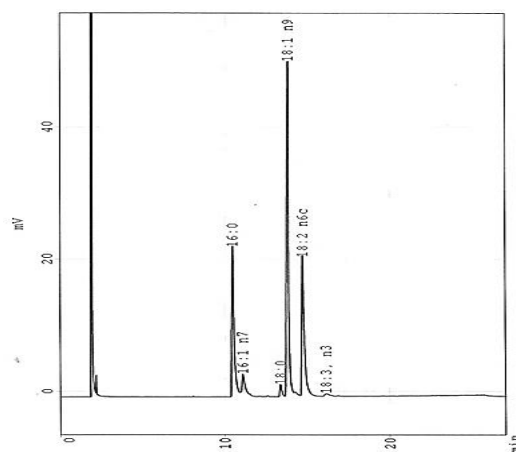
As a result, the solvent type has an influence on the physical and chemical properties of fats and oils. However, this interaction is not statistically significant ( $p=1.000$ ).

The data about fatty acids composition of terebinth oil obtained by various solvents are given in Table 5 and related chromatograms Figure 4-7.

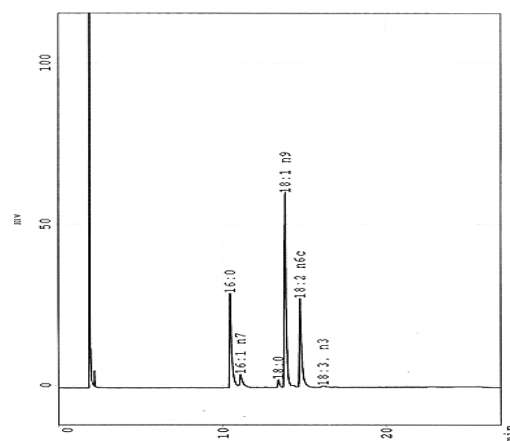
When Table 5 is generally evaluated, it is seen that oil samples are not influenced by solvent type in terms of fatty acid composition. This state has been proven statistically

( $p=1.000$ ). At mean values, it was determined that terebinth oil included 45.82% oleic, 23.93% linoleic, 0.47% linolenic, 3.78% palmitoleic, 24.28% palmitic and 1.7% stearic acid and the composition of terebinth oil was composed of about 74.04% unsaturated fatty acids and 25.96% saturated fatty acids. Oleic acid, linoleic acid and palmitic acid were regarded as the main fatty acids of terebinth oil.

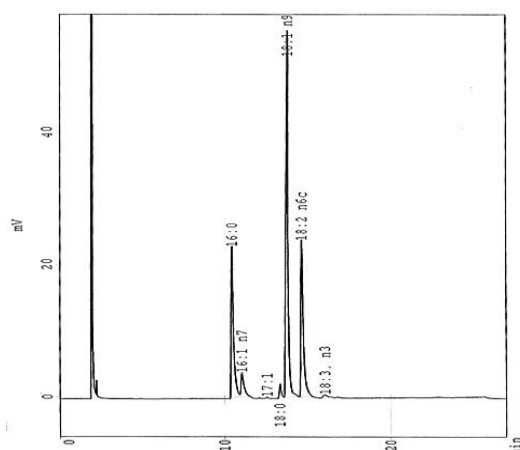
It is possible to classify vegetable oil according to their fatty acid compositions. In this context, oils could be grouped as lauric acid oil, palmitic acid oil, oleic/linoleic acid oil, high oleic acid oil and linolenic acid oil [21]. In the light of these data, it is likely to say that terebinth oil is relatively oleic/linolenic acid oil.



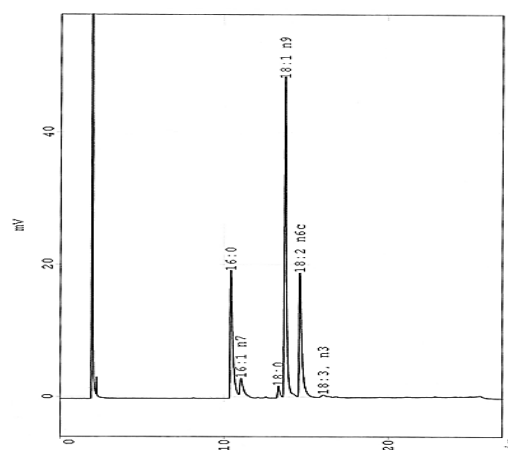
**Figure 4.** GC chromatogram of terebinth oil obtained with n-hexane



**Figure 5.** GC chromatogram of terebinth oil obtained with n-heptane



**Figure 6.** GC chromatogram of terebinth oil obtained with petroleum ether



**Figure 7.** GC chromatogram of terebinth oil obtained with CCl<sub>4</sub>

**Table 5.** Fatty acid composition of crude oil obtained with different solvents

	hexane	heptane	CCl <sub>4</sub>	Petroleum ether	Mean value	Standard deviation
Palmitic acid (%)	24.66	24.6	24.31	23.53	24.28	0.45
Palmitoleic acid (%)	3.8	3.63	3.79	3.9	3,78	0.09
Stearic acid (%)	1.62	1.72	1.77	1.7	1.7	0.05
Oleic acid (%)	45.4	45.88	45.98	46	45.82	0.24
Linoleic acid (%)	24.16	23.8	23.63	24.12	23.93	0.22
Linolenic acid (%)	0.36	0.375	0.53	0.617	0.47	0.11
Total saturated fatty acids	26.28	26.32	26	25.23	25.96	0.44
Total unsaturated fatty acids	73.72	73.68	74	74.77	74.04	0.44

**p value = 1.000**

#### 4. Discussion

It was found that terebinth seeds included 47% oil. It is seen that terebinth has more oil than oily seeds/seeds like soy bean, cottonseed, corn and olive which are industrially important and has equivalent fat composition with oily seeds/seeds like sunflower, safflower, colaseed and palm. In this context, it is possible to state that terebinth is an important raw material in terms of oil content.

As understood from the results of the experiments, the type of the solvent used in the process of extraction affected oil quality (physicochemical properties), but it did not have a significant influence on fatty acid composition. Because the solvent cannot solve fatty acids since fatty acids are chemically bound to glycerine with ester bound. In other words, it has

to solve whole molecule of triglyceride. Therefore, the type of the solvent does not have a significant effect on fatty acid composition. Although the material we define as oil is mainly composed of triglyceride, it also includes many minor lipid components and some impurities. According to the polarity of the solvent used, these minor components (phosphatides, sterols, terpenes, oxidation products etc.) are extracted into the oil environment. Thus, the quality of the oil changes with regard to the type and quantity of minor component within the environment. The type of the solvent used in this direction is an active parameter on the quality of oil. As seen from the results within the scope of this study, the oil obtained with petroleum ether was not found to be favourable and qualified in terms of peroxide value, and the oil extracted with ethanol and acetone wasn't found to be



favourable and qualified in terms of physical condition.

According to fatty acid composition, physicochemical properties and total unsaturation degree, it seem possible to use terebinth oil as edible oil. However, we think that testing this oil on alive organisms and determining the positive and negative effects could be useful before terebinth is consumed as a food product.

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