GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research / Araştırma
GIDA (2024) 49 (3) 397-407
doi: 10.15237/gida.GD23132

PISTACIA TEREBINTHUS L. OIL EXTRACTION BY LIQUID and SUPERCRITICAL CARBON DIOXIDE MODIFIED WITH A CO-SOLVENT AND EVALUATION OF PHENOLIC COMPOUNDS, FATTY ACIDS PROFILE AND TOCOPHEROLS

Atakan Sür[1], Esen Eyiler Kaya[2*], Halil Vural[1]
[1]Hacettepe University, Department of Food Engineering, Ankara, Turkey
[2]Akdeniz University, Korkuteli Vocational School, Antalya, Turkey

Received / Geliş: 21.11.2023; *Accepted* / Kabul: 04.04.2024; *Published online* / Online baskı: 22.04.2024

Sür, A., Eyiler Kaya, E., Vural, H. (2024). Pistacia terebinthus l. oil extraction by liquid and supercritical carbon dioxide modified with a co-solvent and evaluation of phenolic compounds, fatty acids profile and tocopherols. GIDA (2024) 49 (3) 397-407 doi: 10.15237/ gida.GD23124

Sür, A., Eyiler Kaya, E., Vural, H. (2024). *Pistacia terebinthus* l. yağının kosolvent ile modıfıye edilmiş sıvı ve superkritik karbondioksit ile ekstraksiyonu ve fenolik bileşikler, yağ asidi profili ve tokoferollerin analizi. GIDA (2024) 49 (3) 397-407 doi: 10.15237/ gida.GD23124

ABSTRACT
The *Pistacia terebinthus L* was extracted using liquid and supercritical carbon dioxide together with a co-solvent (ethanol). The effect of different temperatures (30 and 50°C), pressure (250, 300, and 350 bar), extraction time (60 and 120 min), and different percentages of co-solvent (0, 5, and 10%) was investigated. The amount of phenolic compounds, tocopherols, and fatty acid composition was determined. HPLC, UHPLC and GC were used for analysis of phenolic compounds, tocopherols, and fatty acid composition respectively. Quercetin was the main phenolic compound. The oil was rich in unsaturated fatty acids which were between 69.68 – 75.47%. Oleic acid was the predominant unsaturated fatty acid, and the main saturated fatty acid was palmitic acid. Total tocopherol content of the oil was between 13.07-245.3 ppm and the main tocopherol was β-tocopherol. The study showed that the amount of phenolic compounds, fatty acid composition, and tocopherol content were changed according to the parameters.
Keywords: *Pistacia terebinthus* L, supercritical carbon dioxide, phenolic compounds, tocopherols, fatty acid composition

PISTACIA TEREBINTHUS L. YAĞININ KOSOLVENT ILE MODİFİYE EDİLMİŞ SIVI ve SUPERKRİTİK KARBONDİOKSİT İLE EKSTRAKSİYONU ve FENOLİK BİLEŞİKLER, YAĞ ASİDİ PROFİLİ VE TOKOFEROLLERIN ANALİZİ

ÖZ
Çalışmada *Pistacia terebinthus L*, sıvı ve süperkritik karbondioksit ile yardımcı çözücü (etanol) kullanılarak ekstrakte edilmiştir. Bu amaçla farklı sıcaklık (30 ve 50°C), basınç (250, 300 ve 350 bar), ekstraksiyon süresi (60 ve 120 dakika) ve yardımcı çözücü yüzdelerinin (0, 5 ve 10%) etkisi

* *Corresponding author* / Sorumlu yazar
✉: eseneyiler@gmail.com ☎: (+90) 242 643 5000 🖷 (+90) 242 643 5005

Atakan Sür; ORCID no: 0000-0001-6083-294X
Esen Eyiler Kaya; ORCID no: 0000-0002-4052-4656
Halil Vural ORCID no: 0000-0001-6758-2912

araştırılmıştır. Ekstraksiyon sonrasında elde edilen yağların fenolik bileşik ve tokoferol içerikleri ve yağ asidi kompozisyonu belirlenmiştir. Fenolik bileşiklerin analizi HPLC, tokoferollerin analizi UHPLC ve yağ asidi kompozisyonunun analizi için GC kullanılmıştır. Fenolik bileşikler arasında en yüksek oran Kuersetine ait bulunmuştur. Elde edilen yağın doymamış yağ asitleri bakımından zengin olduğu bulunmuştur (69.68 – 75.47%.). Doymamış yağ asitlerince zengin olan yağda oleik asit baskın, doymuş yağ asitleri içinde ise palmitik asidin ana bileşen olduğu bulunmuştur. Yağın toplam tokoferol içeriğinin 13.07-245.3 ppm arasında ve ana tokoferolün β-tokoferol olarak bulunmuştur. Çalışma, fenolik bileşiklerin miktarının, yağ asidi kompozisyonunun ve tokoferol içeriğinin parametrelere göre değiştiğini göstermiştir.
Anahtar kelimeler: *Pistacia terebinthus* L, süperkritik karbondioksit, fenolik bileşikler, tokoferoller, yağ asidi kompozisyonu

INTRODUCTION

Terebinth (*Pistacia Tehrebinthus* L.) is a member of the *Anacardiaceae* family and is native to Asia and the Mediterranean widely grown in the southeast parts of Türkiye (Gecgel and Arici 2009; Kizil and Turk 2010). It is known as Menengiç or Melengic in Türkiye. The plant's fruits are small and globular and have a dark greenish color when ripe (Gecgel and Arici 2009). Menengiç paste is consumed as a hot beverage like coffee and the powder of the fruit is utilized in seasoning mixtures, especially in the Southeast Anatolian region of Türkiye (Kavak et al. 2010). Various parts and extracts of this fruit have long been used for different purposes including the treatment of gastric issues, cough, rheumatism (externally) and gastralgia (internally) (Kavak et al. 2010). Further, species of Pistacia can be used for treating eczema, renal stones, and throat infections, also they have anti-inflammatory, antibacterial, antiviral, and antipyretic effects (Giner-Larza et al. 2002). Studies have indicated that terebinth fruits are rich in oil (35-47%) and composed of mainly unsaturated acids which are oleic and linoleic acid (Gecgel and Arici 2009; Kizil and Turk 2010). Furthermore, the fruits are rich in carotenoids, phenolic compounds, tocopherols, tannins, and dietary fiber (10%) (Matthaus and Özcan 2006). Researchers have investigated the bioactive characteristics, fatty acid composition, mineral content, volatile compounds, physicochemical properties, and anti-microbial properties of terebinth fruits or leaves in various studies (Kordali et al. 2003; Matthaus and Özcan 2006; Kavak et al. 2010; Durmaz and Gökmen, 2011; Orhan et al. 2012). However, in these studies, the extractions were performed by classical extraction methods using different solvents such as ethanol, chloroform, and ethyl acetate. The main drawbacks of solvent extraction methods include high consumption of solvent, long extraction time, and matrix interactions between the extract and the solvent which makes it difficult to remove the solvent from the extract. Additionally, these methods may require high temperatures leading to the degradation of heat-sensitive compounds such as (phenolic compounds. Supercritical fluid extraction (SFE) is a promising method for the extraction of oils from different matrices as it eliminates the degradation of heat-sensitive compounds and minimizes solvent residues in extracts (Senyay-Oncel et al. 2011). Carbon dioxide is widely utilized in supercritical fluid extractions due to its non-toxic, cheap, and has non-flammable properties. When extracting phenolic compounds, a co-solvent like ethanol or methanol should be used in supercritical carbon dioxide extraction since carbon dioxide is non-polar and phenolic compounds cannot be extracted without a co-solvent.

In this study the extraction of phenolic compounds and tocopherols along with the oil from *Pistacia terebinthus* L by using $scCO_2$ with ethanol as a co-solvent was performed. Also, the fatty acid composition of the oils obtained was investigated. The effect of different parameters, such as temperature (30 and 50°C), pressure (250, 300, and 350 Bar), co-solvent percentage (0, 5, and 10%), and extraction time (60 and 120 min.) was investigated.

MATERIALS AND METHODS

Materials

Terebinth fruits (*Pistacia terebinthius*) were obtained from a local bazaar in Gaziantep during the

growing season. The fruits were cleaned to remove foreign materials such as dirt, stones, and chaff. Prior to each extraction, 30 g of fruits were crushed to homogenize the samples and increase the surface area for supercritical extraction. The standards used for HPLC analysis were obtained from Sigma-Aldrich (Steinheim, Germany). Ethanol, used as a co-solvent, and methanol, utilized for the extraction of phenolic compounds from the terebinth fruit oil was obtained from Riedel de-Haen (Germany). HPLC grade acetic acid (100%) was from Sigma-Aldrich (Germany) and Acetonitrile was from Merck (Darmstadt, Germany).

Supercritical fluid extraction of terebinth fruits

The extraction of oil and the phenolic compounds from the terebinth fruits was performed by using an analytical supercritical fluid extractor (SFE-100-2-FMC10, Thar Instruments, PA, USA). The instrument was equipped with an Automated back pressure Regulator, 100ml extraction vessel, 500 ml collection vessel, six-zone temperature controller, high-pressure P-50 Series pump, cooling systems filled with glycol, and a series III pump for co-solvent (which cannot operate over 400 bar). The co-solvent pump was purged before each extraction to ensure that co-solvent entered the system (Thar Instruments, Series III pump, Manuel)

The terebinth fruits obtained from the local market were cleared of foreign substances. 30 g of terebinth fruits were weighed for extraction, and they were smashed in a porcelain mortar for 10 min, to increase the surface area before extraction. Then they were sieved and the ones that were between 425 and 230 μm were used for extraction. The smashed fruits were then placed in the extraction vessel. The flow rate of CO_2 was set to 5 g/min and was the same for all the extraction parameters. Since CO_2 is a non-polar solvent, it is not possible to extract the phenolic compounds without a polar co-solvent. In this study, CO_2 was modified by using ethanol as a co-solvent. Three different co-solvent (ethanol) percentages were tested which were 0, 5, and 10% (weight %). Other parameters studied in the research to observe their effects on phenolic compounds, fatty acid profile, and tocopherol contents, were temperature (30 and 50ºC), pressure (250, 300, and 350 Bars), and extraction time (60 and 120 min.). The parameters were chosen based on the studies of Eyiler-Yılmaz et al. (2011). The oil extracted was collected in a 250ml volumetric flask along with the co-solvent and the ethanol in the collected samples was removed with a rotary evaporator (Büchi, B465, Switzerland) at 45°C, 110rpm. After removal of the ethanol, the samples were stored at 4°C until further analysis. The extractions with SC-CO_2 were performed in duplicate.

Phenolic compound analysis of terebinth extracts.

The samples obtained after the extraction process were analyzed by RP-HPLC according to the method of Pirisi et al. (2010). According to this method, 1 ml of oil sample was transferred to a centrifuge tube and 1 ml of methanol was added. The mixture was vortexed for 2 min before being centrifuged at 3000 rpm for 10 min (Nüve, NF 1215, Istanbul, Turkey). The supernatant obtained was then transferred to a test tube for further analysis.

The samples were analyzed using HPLC (Agilent 1100 RP-HPLC) equipped with a nucleosil C18 HPLC column (250*4.6 mm, Supelco Inc., Bellefonte, PA, USA) and a DAD detector set at 280 nm for catechin and gallic acid, 370nm for myricetin and quercetin. The flow rate of the mobile phase was maintained at 1ml/min and the injection volume was 20 μl. A gradient flow method was used with 2 mobile phases which are 2% acetic acid (in water Mobile Phase A) and 100 % acetonitrile as phase B, the total analysis time was 30 min. The gradient program was as follows: the concentration of acetonitrile was increased to 50% in 20 min, isocratic for 5 min, then the concentration of acetonitrile was decreased to 0% in 1 min and was isocratic for the last 4 min. The retention times for gallic acid, catechin, myricetin and quercetin were 4.4, 9.7, 15.7 and 18.2 minutes respectively. Each experiment was performed in 2 parallels.

Tocopherol analysis

The analysis of α-, β-, γ- and δ-tocopherol was performed with ultra-high-performance liquid chromatography (UHPLC, Dionex Ultimate 3000), by using LiChrosorb SI 60-5 column (4.6 mm internal diameter * 250 mm length and 5μm particle size). 2 g of oil samples were weighed into 25 ml volumetric flask and dissolved with some hexane then completed to 25 ml with hexane. The mixture was vortexed for 15 min then the prepared mixture was passed through 0.45 μm syringe-type HPLC filters (PVDF, Millipore Millex-HV) and transferred to an HPLC vial. The tocopherols were differentiated from each other with isopropanol: hexane (0.5:99.5%, v/v) mobile phase under isocratic conditions at a flow rate of 1ml/min, at 292 nm and the temperature of the column was 30°C. The analysis time was 30 min and the injection volume was 100μl. the tocopherol isomers were identified according to the retention times of the prepared standards and the amount was determined as ppm level by using the area under the peaks obtained from UHPLC (AOAC 2017). The retention times for α, β, γ and δ -tocopherol were 8.01, 14.12, 15.87, and 28.77 minutes respectively. The experiments were performed in 2 parallels.

Determination of fatty acid composition

The fatty acid composition of the samples was determined by using TRACE Ultra GC (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatography equipped with flame ionization detector and capillary column (0.25 mm i.d. × 100 m, 0.25μm film thickness) (European Commission Regulation 2017). The injection detector temperatures were set to 240 and 250°C respectively. The initial temperature was 100°C and the temperature ramp rate was 4°C/min. Helium was used as carrier gas with a flow rate of 1mL/min. The split ratio was set as 40:1. For the preparation of samples, 0.1 g of extracted oil sample was weighed, and 2ml heptane and 0.2 ml 2 M of methanol (11.2 g KOH dissolved in 100 ml HPLC grade methanol) were added. The mixture was vortexed for 15 min. then was centrifuged at 5000 rpm for 5 min. the supernatant obtained after centrifugation was used for the GC analysis. The experiments were performed in 2 parallels.

Statistical analysis

The results of the research were evaluated with factorial design variance analysis using SAS 9.4 statistical program. The means that were significantly different were compared by applying the Tukey multiple comparison tests.

RESULTS AND DISCUSSION

Phenolic compounds determined in the Terebinth oil samples.

To comprehensively assess the combined impact of pressure, co-solvent percentage, temperature, and extraction time on phenolic compounds, Table 1 presents the average values obtained across all parameters corresponding to the same pressure, co-solvent percentage, temperature, and extraction time for each specific compound.

The results indicate that changes in pressure and temperature did not significantly affect the amount of quercetin (Table 1, $P > 0.05$). However, an increase in co-solvent percentage and extraction time significantly increased the amount of quercetin ($P < 0.05$). These findings highlight the significance of co-solvent percentage and extraction time as the primary factors influencing quercetin levels in terebinth oil. These results align with the findings of Martino and Guyer (2004), who emphasized that co-solvent was the most influential parameter for quercetin extraction.

The results indicate that changes in temperature, pressure, and extraction time did not significantly affect the amount of catechin (Table 1). However, increasing the co-solvent percentage from 0% to 5% resulted in a significant increase in catechin levels ($P < 0.05$). Murga et al. (2000) discovered in their study that when the co-solvent level was below 5%, only gallic acid could be extracted from grape seeds. Although our study involved a different matrix, the results in Table 1 suggest that catechin extraction without co-solvent is possible at 50°C and 350 bars of pressure which is the supercritical condition. At high pressure and temperature, it becomes feasible to disrupt

analyte-matrix interactions and extract catechin from terebinth, which is consistent with the findings of Berna et al. (2001). (Table 1). According to the studies by Spencer Chatwell et al. (2021) and Bassing and Siegfried Braeuer (2021) when CO_2 is mixed with ethanol the critical conditions changes because the critical condition for ethanol is higher. Therefore, in our study the mixture obtained at 10% ethanol might not reach critical conditions which could be the reason for the decrease in the level of phenolic compounds.

Table 1. Effect of Pressure, Co-Solvent Percentage, Temperature and Extraction Time on Quercetin and Catechin

Main Factors	Quercetin (QUE) (mg/kg)	Catechin (CAT) (mg/kg)
Pressure (Bar)[1]		
250	72.66±20.65[a]	30.45±5.47[a]
300	79.87±27.64[a]	21.06±6.11[a]
350	72.75±23.07[a]	30.67±6.31[a]
p	0.8564	0.2668
Co-Solvent Percentage (%)[2]		
0	15.90±1.16[a]	11.12±5.59[a]
5	36.23±3.13[a]	41.42±2.18[b]
10	172.90±19.78[b]	29.61±6.15[ab]
p	0.0001	0.0005
Temperature (°C)[3]		
30	78.79±19,34[a]	25.88±4.92[a]
50	71.42±19.19[a]	29.14±4,97[a]
p	0.5460	0.5716
Extraction Time (min)[4]		
60	55.25±10.67[a]	31.57±4,69[a]
120	94.96±24.17[b]	23.46±5,03[a]
p	0.0026	0.1654

a-b: Means within the same factor and the same column with different letters are different ($p < 0.05$). ND: Not Detected.

[1] Each number represents the average value of each parameter for all samples with the same pressure.

[2] Each number represents the average value of each parameter for all samples with the same Co-solvent percentage.

[3] Each number represents the average value of each parameter for all samples with the same temperature.

[4] Each number represents the average value of each parameter for all samples with the same extraction time.

The results of gallic acid were not included in the factorial design statistical analysis because the obtained results were not suitable for the design. It was not possible to extract gallic acid in most of the extraction parameters.

The solvation power of CO_2 is a function of temperature and pressure, and it is well-known that an increase in pressure increases the solvation of supercritical CO_2. On the other hand, increasing the temperature decreases the solvation of CO_2 (Brunner 2005). Additionally, it should be noted that the impact of temperature is more complex, such as it was reported in the literature that at low pressures (10 – 15 MPA), temperature negatively affects the SFE of phenolic compounds. However, above these pressure thresholds, it was stated that temperature has s positive effect on the extraction of polyphenols. In our study, the pressure values exceeded these thresholds. Nevertheless, increasing the temperature while holding all other parameters constant did not lead to a significant increase in the concentrations of quercetin, catechin, and gallic acid. Similar findings have been documented in the literature, with this behavior being attributed to the thermal degradation of phenolic compounds (Ferrentino et al. 2018). Furthermore, Chafer et al. (2007) found that increasing the temperature decreased the amount of gallic acid in their study.

Myricetin was also analyzed by HPLC from the extracted oil however it was not possible to detect the compound. This may be attributed to the possibility that myricetin was not extractable under the parameters employed in this study.

As mentioned above temperature and pressure are the primary factors influencing the solubility of CO_2 as they define the density of supercritical fluids (Lee et al. 2006). In supercritical and near-critical solvents, the solubility of low-volatility substances typically decreases with temperature at low pressures due to the rapid decrease in fluid density as temperature increases near critical pressures. However, at higher pressures (over 200 Bar), the effect of temperature on density is diminished, and vapor pressure becomes the predominant factor affecting the density of the

supercritical fluid (Brunner, 2005). It can be observed from the results that the higher levels of catechin were extracted at 50°C where CO_2 is in a supercritical state. There was a slight, albeit non-significant decrease in quercetin levels with increasing the temperature. Since different compounds could be extracted at their maximum levels at different parameters, extractions should be conducted according to the selected compound. It can be emitted from the obtained results that the percentage of the co-solvent used was the most effective parameter for the extraction of phenolic compounds from terebinth fruits.

Fatty acid profile of the Terebinth oil

Thirteen different fatty acids were observed in the extracted terebinth oils. However, only the results of the five predominant fatty acids: palmitic, stearic, palmitoleic, oleic, and linoleic acids were presented in Table 2. Table 2 provides an overview of the combined effects of pressure, co-solvent percentage, temperature, and extraction time on the fatty acid profile. The values represent the average value of all the parameters within the same pressure, co-solvent percentage, temperature, and extraction time for the specific compound. The results indicate that changes in pressure did not significantly affect the levels of saturated and unsaturated fatty acids ($P > 0.05$). However, oleic and stearic acid levels were significantly increased with higher co-solvent percentages (Table 2). On the other hand, the levels of palmitic, palmitoleic, and linoleic acids were decreased when the co-solvent percentage was increased. Both temperature and extraction time were found to be significant factors in reducing the palmitic acid level ($P<0.05$). A decrease in the level of palmitic acid is favorable because it is a saturated fatty acid and as mentioned above saturated fatty acids increase LDL and HDL cholesterol.

Table 2. Effect of Pressure, Co-Solvent Percentage, Temperature, and Extraction Time on Fatty acid composition of Terebinth oil.

Main Factors	Palmitic acid (%)	Stearic acid (%)	Palmitoleic acid (%)	Oleic acid (%)	Linoleic acid (%)
Pressure (Bar)[1]					
250	22.18±0.20^{a}	1.68±0.03^{a}	3.61±0.34^{a}	44.25±0.27^{a}	22.23±0.12^{a}
300	22.29±0.17^{a}	1.70±0.02^{a}	3.87±0.07^{a}	44.69±0.15^{a}	22.22±0.10^{a}
350	22.37±0.20^{a}	1.72±0.02^{a}	3.20±0.43^{a}	47.53±0,32^{a}	21.94±0.13^{a}
p	0.5699	0.1581	0.1946	0.4274	0.1235
Co-Solvent Percentage (%)[2]					
0	22.75±0.06^{a}	1.63±0.02^{a}	4.07±0.03^{a}	47.15±0.24^{a}	22.27±0.12^{a}
5	22.42±0.18^{a}	1.69±0.02^{b}	3.90±0.06^{a}	47.30±0.31^{ab}	22.28±0.08^{a}
10	21.67±0.14^{b}	1.78±0.01^{c}	2.70±0.47^{b}	48.03±0.11^{b}	21.85±0.12^{b}
p	0.0001	0.0001	0.0013	0.0343	0.0137
Temperature (°C)[3]					
30	22,31±0.16^{a}	1,71±0.02^{a}	3,47±0.30^{a}	47,47±0.21^{a}	22,14±0.11^{a}
50	22,26±0.15^{b}	1,69±0.02^{a}	3,66±0.22^{a}	47,52±0.21^{a}	22,13±0.09^{a}
p	0.7347	0.1887	0.5223	0.8555	0.9546
Extraction Time (min)[4]					
60	22,42±0.15^{a}	1,69±.0.02^{a}	3,24±0.35^{a}	47,35±0.27^{a}	22,13±0.27^{a}
120	22,14±0.15^{b}	1,71±0.02^{a}	3,88±0.06^{b}	47,64±0.13^{a}	22,13±0.13^{a}
p	0.0735	0.2111	0.0367	0.3095	0.9825

Means within the same factor and the same column with different letters are different ($p < 0.05$).

[1] Each number represents the average value of each parameter for all samples with the same pressure.

[2] Each number represents the average value of each parameter for all samples with the same Co-solvent concentration.

[3] Each number represents the average value of each parameter for all samples with the same temperature.

[4] Each number represents the average value of each parameter for all samples with the same extraction time.

These results were lower than the findings of Gecgel and Arici (2009) and Durak and Uçak (2015) which could be attributed to differences in climate, soil type, and environmental factors where the fruits were obtained. Sodeifian et al. (2016) investigated the extraction of fruit oil from *Pistacia khinjuk* stocks using supercritical carbon dioxide and reported that the main component of UFA was oleic acid consistent with our study. The oleic acid content in *Pistacia khinjuk* stocks fruit was approximately 57% which was higher than the findings of our study. It was stated by Satil et al. (2003) that the amount of oil and the fatty acid composition of the terebinth samples were influences by the climatic conditions and type of soil of the area they were grown.

Pistacia terebinthus (Terebinth) and Pistachio nuts belong to the same family. It was shown by Satil et al. (2003) that the amount of oleic acid in the pistachio nuts was nearly 60% which is higher than that in *Pistacia terebinthus*. Olive oil has long been known and used oil, especially in the Mediterranean because of its positive effects on health such as the prevention of coronary heart disease and certain cancer types (Visioli et al. 2018). It is believed that this health benefit of olive oil comes from the unsaturated fatty acid which is mainly oleic acid. According to the results of Belbaki et al. (2017), the olive oil extracted using supercritical CO_2 included fatty acids from C_{16} to $C_{20:1}$, and oleic acid was the main unsaturated fatty acid with a ratio of 59.3% which is higher than our results. The amount of linoleic acid in the extracts was between 21.85 – 22.28%. These results were found to be higher than its relative pistachio nuts (Satil et al. 2003) and olive oil (Belbaki et al. 2017).

Palmitic acid was the main saturated fatty acid found in the fatty acid profile analysis (21.67 – 22.37%), while stearic acid content was lower, between 1.63 – 1.78%. Similar findings were reported by Gecgel and Arici (2009). Saturated fatty acids have been linked to increased serum low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels. Lauric and myristic acids exhibit the strongest effect on LDL and HDL cholesterol levels among saturated fatty acids, whereas palmitic acid, although increasing cholesterol levels, does so to a lesser extent than lauric and myristic acid. On the other hand, stearic acid does not affect LDL and HDL cholesterol (Mensink 2013). Based on this knowledge, it is important to reduce the amount of saturated fatty acids in the diet. It was found by Chowdbury et al. (2007) that the amount of palmitic acid in palm oil was 41.78% which is higher than that in terebinth oil. Palm oil is one of the most used oils in the food industry. Given the lower saturated fatty acid content in terebinth oil compared to palm oil, it could serve as a viable alternative in food applications. Nevertheless, it should be noted that the amount of saturated fatty acids in the study was higher than that of pistachio nuts and olive oil (Satil et al. 2003; Belbaki et al. 2017). Olive oil is one of the most suggested oils for consumption due to its high unsaturated fatty acid content. The results of the study showed that the unsaturated fatty acid levels of terebinth oil are lower when compared to olive oil. While olive oil usage in the food industry is somewhat limited due to its higher cost, terebinth oil presents an attractive alternative due to its high oleic acid and low saturated fatty acid content.

The ratio of saturated fatty acids to unsaturated fatty acids (SFA/UFA) is a criterion for the evaluation of the nutritional and functional properties of the oil (Sodeifian et al. 2016). The average value of SFA/UFA ratios of the samples was between 0.31 – 0.38 which is close to the results of Sodeifian et al. (2016). However, these values are relatively low when compared to other vegetable oils such as soybean, peanut, and olive oil (Fasina et al. 2008). Besides the effect of saturated fatty acids on cholesterol levels, it is believed that a high intake of saturated fatty acids may cause cardiovascular diseases and it has been found that a high intake of saturated fatty acids can lower insulin sensitivity which is an important factor in metabolic disorder and diabetes (Nagao and Yanagita 2010). Due to this reason, the extraction parameters should be chosen carefully to decrease the amount of saturated fatty acids. Also as mentioned previously the fatty acid composition of terebinth oil can be affected by the climatic conditions, soil type, and

environmental factors where the plant is grown (Satil et al. 2003).

Tocopherol content of the terebinth oil

The average values of each parameter for all the samples were given in Table 3. The total tocopherol content of the oil samples was between 13.07-245.3 mg/kg which was lower than the findings of Matthaus and Özcan (2006) and Durmaz and Vural (2011). Unlike the findings of previous studies (Matthaus and Özcan 2006; Durmaz and Vural 2011), the main tocopherol found in the study was β- tocopherol.

Table 3. Effect of Pressure, Co-Solvent Percentage, Temperature and Extraction Time on Tocopherol Content of Terebinth oil

Main Factors	α – tocopherol (mg/kg)	β- tocopherol (mg/kg)	γ- tocopherol (mg/kg)	δ- tocopherol (mg/kg)
Pressure (Bar)[1]				
250	18.23±12.79[a]	90.06±19.33[a]	8.79±0.76[a]	5.67±1.29[a]
300	7.24±6.15[a]	103.99±18.64[a]	8.47±0.78[a]	2.06±0.80[b]
350	8.62±4.02[a]	70.87±19.40[a]	6.36±0.60[b]	3.70±0.72[ab]
p	0.5514	0.3462	0.0411	0.0282
Co-Solvent Percentage(%)[2]				
0	21.90±13.41[a]	33.99±8.70[a]	6.59±0.72[a]	1.86±0.76[b]
5	2.61±1.13[a]	127.41±16.79[b]	8.53±0.62[a]	4.61±0.91[a]
10	9.58±5.08[a]	103.53±19.02[b]	8.51±0.86[a]	4.96±1.27[a]
p	0.2158	0.0007	0.0987	0.0406
Temperature (°C)[3]				
30	19.43±9.38[a]	91,34±16.57[a]	8,14±0.51[a]	4,47±0.99[a]
50	3,28±1.04[a]	85,28±14.82[a]	7,61±0.73[a]	3,14±0.65[a]
p	0.0789	0.7433	0.5135	0.2099
Extraction Time (min)[4]				
60	21,30±9.22[a]	92,27±18.67[a]	7,69±0.70[a]	3,41±0.85[a]
120	1,42±0.34[b]	84,34±12.03[a]	8,06±0.56[a]	4,21±0.86[a]
p	0.0329	0.6682	0.6523	04488

a-b: Means within the same factor and the same column with different letters are different ($p < 0.05$).

[1] Each number represents the average value of each parameter for all samples with the same pressure.

[2] Each number represents the average value of each parameter for all samples with the same Co-solvent concentration.

[3] Each number represents the average value of each parameter for all samples with the same temperature.

[4] Each number represents the average value of each parameter for all samples with the same extraction time.

The amount of α-tocopherol decreased with increasing temperature while keeping other parameters constant. α-tocopherol is susceptible to oxidation at high temperatures, potentially leading to its degradation. Similarly, the extraction time had a negative effect on α- tocopherol levels. As depicted in Table 3 increasing extraction time significantly decreased the amount of α-tocopherol (P <0.05).

According to the results an increase in the co-solvent percentage significantly increased the amount of β- tocopherol (P <0.05, Table 3). Conversely, temperature, pressure, and extraction time showed no significant effect. Moreover, time, co-solvent concentration, and temperature had no significant effect on the γ- tocopherol content. However, increasing the pressure to 350 bars led to a significant decrease in the amount of γ- tocopherol (P <0.05). The only parameter that significantly reduced the δ- tocopherol content was pressure (P <0.05). while, temperature, time, and co-solvent percentage showed no significant effect on δ- tocopherol content. The results demonstrated that changing the parameters had diverse effects on each tocopherol type. Generally

increasing the temperature from 30°C to 50°C led to a decrease in tocopherol levels, which may indicate the degradation of these substances at higher temperatures. Therefore, if it is desired to obtain higher amounts of tocopherols in the extract, temperatures lower than 50°C could be selected for SFE.

The tocopherol contents of terebinth oil in our study were lower when compared to the results of Mathaus and Ozcan (2006) which could be due to the differences in the environmental and climatic conditions and the differences in the soil where the terebinth plants were grown. The terebinth oil's tocopherol content was lower compared with other oils like palm oil (Tan et al. 2009) and olive oil (Uluata et al. 2021).

CONCLUSION

In this study, the extraction of oil from *Pistacia terebintus* L with liquid CO_2 and $ScCO_2$, followed by the analysis of phenolic compounds, tocopherols, and fatty acid composition was conducted. It was observed that Supercritical Fluid Extraction (SFE) offers several advantages, such as requiring less or no solvent, operating at low temperatures, and being oxygen-free, which prevents the degradation of easily degradable substances during extraction. Terebinth oil has a lower amount of unsaturated fatty acids and tocopherols when compared to olive oil. However, olive oil is an expensive type of oil and due to this, it is difficult to use in the food industry. In comparison, terebinth oil emerges as a promising alternative to oils like palm, soy, or rapeseed oil. Additionally, due to its high content of unsaturated fatty acids and tocopherols, terebinth oil can be used in the meat industry in low-fat meat products. It is important to note that changing the extraction parameters changes the amount of the phenolic compounds, tocopherols, and fatty acid composition. Therefore, researchers or producers should choose the extraction parameters carefully based on the substance they aim to extract at the highest concentration. This ensures optimal extraction efficiency and product quality.

ACKNOWLEDGMENTS

We would like to thank Gizem Öner and Dr. Esmael Ghanbari for their kind support for the laboratory analysis and Msc Fatma Yardibi for her help with the statistical analysis with SAS.

AUTHOR CONTRIBUTIONS

Prof. Halil Vural was involved in the conceptualization and planning of the study, Atakan Sür was involved in planning the experiments, execution of experiments, generating the data. Esen Eyiler Kaya contributed to statistical analysis and interpretation of the results followed by manuscript writing and editing for publication.

FUNDING

The research was financially supported by Hacettepe University, Research Center Office, Project no: FHD –2015-5503.

REFERENCES

AOCS Official Method 2017 Ce 8-89.

Bassing, D., Siegfried Braeuer, A. (2021). The influence of temperature and pressure on macro- and micro-mixing in compressed fluid flows; mixing of carbon dioxide and ethanol above their mixture critical pressure. *Journal of Supercritical Fluids*, 167. 105036. https://doi.org/10.1016/j.supflu.2020.105036

Belbaki, A., Louaer, W., Meniai, A.H. (2017). Supercritical CO_2 extraction of oil from crushed Algerian olives. *Journal of Supercritical Fluids,* 130: 165-171.

Berna, A., Chafer, A., Monton, J., Subirats, S., (2001). High-pressure Solubility Data System Ethanol (1) + Cathecin (2) + Carbon Dioxide (3). *Journal of Supercritical Fluids*, 20 (2): 157-162.

Brunner, G. (2005). Supercritical fluids, technology and application to food Processing. *Journal of Food Engineering,* 67 (1-2): 21-33.

Chafer, A., Fornari, T., Berna, A., Stateva, R.P. (2004). Solubility of quercetin in supercritical CO2 + ethanol as a modifier: measurements and thermodynamic modeling. *Journal of Supercritical Fluids*, 32: 89-96.

Chafer, A., Fornari, T., Stateva, R., Berna, A., Garcia-Reverter, J. (2007). Solubility of Natural Antioxidant Gallic acid in Supercritical Carbon dioxide+ethanol as cosolvent. *Journal of Chemical Engineering Data,* 52 (1): 116-121.

Chowdhury, K., Banu, L.A., Khan, S., Latif, A. (2007). Studies on the Fatty Acid Composisition of Edible oil. *Bangladesh Journal of Scientific and Industrial Research,* 42(3): 311-316.

Durak, Z.M., Ucak, G. (2015). Solvent optimization and characterization of fatty acid profile and antimicrobial and antioxidant activities of Turkish *Pistacia terebinthus* L. extracts. *Turkish Journal of Agriculture and Forestry,* 39: 10-19. https//doi.org/ 10.3906/tar-1403-63.

Durmaz, G., Gökmen, V. (2011). Changes in oxidative stability, antioxidant capacity, and phytochemical composition of *Pistacia terebinthus* oil with roasting. *Food Chemistry,* 128: 410–414.

European Commission Regulation (2013). Amending Regulation no. 2598/91, EU no. 1348/2013. Characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of the European Communities*, vol. L338, pp. 31–67.

Fasina, O., Craig-Schmidt, M., Colley, Z., Hallman, H. (2008). Predicting melting characteristics of vegetable oils from fatty acid composition. *LWT-Food Science and Technology,* 41: 1501–1505.

Ferrentino, G., Morozova, K., Mosibo, O.K., Ramezani, M., Scampicchio, M. (2018). Biorecovery of antioxidants from apple pomace by supercritical fluid extraction. *Journal of Cleaner Production*. 186, 253-261.

Gecgel, U., Arici, M. (2009). Studies on Physico-chemical Properties, Fatty Acid Composition of Terebinth (*Pistacia terebinthus* L.) Oil and Presence of Aflatoxins in Fruits. *Asian Journal of Chemistry,* 21 (2): 1559-1564.

Giner-Larza, E.M., Manez, S., Giner, R.M., Recio, M.C., Prieto, J.M., Cerda-Nicolas, M., and Rios, J.R. (2002). Anti-Inflammatory Triterpenes from *Pistacia terebinthus* Galls. *Planta Medica,* 68 (4): 311-315.

Kavak, D.D., Altiok, E., Bayraktar, O., Ulku, S. (2010). *Pistacia terebinthus* extract: as a potential antioxidant, antimicrobial, and possible β-glucuronidase inhibitor. *Journal of Molecular Catalysis B: Enzymatic,* 64: 167–171.

Kizil, S., Turk, M. (2010). Microelement contents and fatty acid compositions of *Rhus coriaria* L. and *Pistacia terebinthus* L. fruits spread commonly in the southeastern Anatolia region of Turkey. *Natural Product Research,* 24 (1): 92-98.

Kordali, S., Cakir, A., Zengin, H., Duru, M.E. (2003). Antifungal activities of the leaves of three Pistacia species grown in Turkey. *Fitoterapia* ,74: 164–167.

Lee, M.R., Lin, C.Y., Li, Z.G., Tsai, T.F. (2006). Simultaneous analysis of Antioxidants and Preservatives in Cosmetics by Supercritical Fluid Extraction combined with Liquid Chromatography-mass spectrometry. *Journal of Chromatography*, 1120 (1-2): 244-251.

Martino, K.G., Guyer, D. (2004). Supercritical Fluid Extraction of Quercetin from Onion Skins. *Journal of Food Processing Engineering,* 27 (1):17-28.

Matthäus, B., Özcan, M. (2006). Quantitation of fatty acids, sterols, and tocopherols in turpentine (*Pistacia terebinthus*) growing wild in Turkey. *Journal of Agricultural and Food Chemistry,* 54: 7667–7671.

Mensink, R.P. (2013). Fatty acids: Health Effects of Saturated Fatty acids. In Caballero, B., Allen, L., Prentice, A. (Eds) *Encyclopedia of Human Nutrition.* Pg: 215-219. Academic Press:USA.

Murga, R., Ruiz, R., Beltran, S., Cabezas, J. (2000). Extraction of Complex Phenols and Tannins from Grape Seeds by using Supercritical mixtures of carbon dioxide and alcohol. *Journal of Agricultural and Food Chemistry.* 48 (8): 3408-3412.

Nagao, K., Yanagita, T. (2010). Medium-chain fatty acids: Functional lipids for the prevention and treatment of the metabolic syndrome. *Pharmacological Research,* 61: 208-212.

Orhan, I.E., Senol, F.S., Gulpinar, A.R., Sekeroglu, N., Karta, M., Sener, B. (2012). Neuroprotective potential of some terebinth coffee brands and the unprocessed fruits of

Pistacia terebinthus L. and their fatty and essential oil analyses. *Food Chemistry,* 130: 882–888.

Pirisi, F., Cabras, P., Cao, C., Migliorini, M. (2010). Phenolic compounds in Virgin Olive Oil. 2. Reappraisal of the Extraction, HPLC Separation and quantification procedures. *Journal of Agricultural and Food Chemistry,* 48 (4): 1191-1196.

Satil, F., Azcan, N., Basar, K.H.C. (2003). Fatty acid composition of Pistachio nuts in Turkey. *Chemistry of Natural Compounds,* 39 (4): 322-324.

Senyay-Oncel, D., Ertas, H., Yesil-Celiktas, O. (2011). Effects of Supercritical Fluid Extraction Parameters on Unsaturated Fatty Acid Yields of *Pistacia terebinthus* Berries. *Journal of American Chemical Society,* 88: 1061-1069.

Sodeifian, G., Ghorbandoost, S., Sajadian, S.A., Ardestani, N.S. (2016). Extraction of oil from Pistacia khinjuk using supercritical carbon dioxide: Experimental and modeling. *Journal of Supercritical Fluids,* 110: 265-274

Spencer Chatwell, R., Guevara-Carrion, G., Gaponenko, Y., Shevtsova, V., Vrabed, J. (2021). Diffusion of the carbon dioxide–ethanol mixture in the extended critical region. Royal society of Chemistry, 23, 3106. https://doi.org/10.1039/D0CP04985A

Tan, C.H., Ghazali, H.M., Kuntom, A., Tan, C.P., Ariffin, A.A. (2009). Extraction and Physicochemical Properties of Low Free Fatty Acid Crude Palm Oil. *Food Chemistry,* 113: 645-650.

Uluata, S., Altuntaş, U., Özçelik, B. (2021). Characterization of Turkish Extra Virgin Olive Oils and Classification Based on Their Growth Regions Coupled with Multivariate Analysis. *Food Analytical Methods* 14: 1682-1694.

Visioli, F., Franco, M., Toledo, E., Luchsinger, J., Willett, W.C., Hu, F.B., Martinez-Gonzalez, M.A. (2018). Olive oil and prevention of chronic diseases: Summary of an International conference. *Nutrition, Metabolism & Cardiovascular Diseases* 28: pp. 649-656

Yilmaz, E.E., Özvural, E.B., Vural, H. (2011). Extraction and identification of proanthocyanidins from grape seed (Vitis Vinifera) using supercritical carbon dioxide. *The Journal of Supercritical fluids*, 55:3, 924-928.