# Physicochemical Characterization and Bioactive Compounds of Cold Pressed Pine Nut Oil

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**Abstract**

This study was conducted to determine some physicochemical characteristics and bioactive compounds of Turkish pine nut cold pressed oil. The moisture content, total protein amount and crude oil yield of the pine nut were 3.18%, 31.46% and 35.58% respectively. The fatty acid profile, tocopherol and sterol contents of this oil were characterized. Linoleic acid (46.39%) was found to be the predominant fatty acid followed by palmitic (6.3%), stearic (3.5%), gadoleic (0.81%), linolenic (0.58%), eicosadienoic (0.49%), oleic (37.73%), behenic (0.13%), palmitoleic (0.1%), margaric (0.08%), myristic (0.06%) and heptadecenoic acid (0.05%). Regarding tocopherol composition, α-tocopherol (174.48 µg/g), γ-tocopherol (485.92 µg/g) and α-tocotrienol (2004.65 µg/g) were major ingredients in the pine nut oil. Regarding sterol composition, pine nut oil was determined to have remarkably high content of β-sitosterol (76.15%). The other sterols present in the oil were campesterol (15.60%), sitostanol (6.46%), D-5,24-stigmastadienol (1.43%) and ergosterol (0.36%). The most abundant triacylglycerol (TAG) was LLL (trilinolein) (11.5867%) followed by OLnL (Oleolinolenolinolein) (0.7302%), PLnL (Palmitolinolenolinolein) (0.1422%) and PoLL (Palmitoleodilinolein) (0.0826%).

## 1. Introduction

Pine nuts are seeds that are widely used in world cuisine, obtained from pine cones (*Pinaceae* family, *Pinus* genus), with 29 currently known edible species [1, 2]. Pine nuts, nutrient-rich and popular food, contain about 32% protein and 45% fat [3, 4]. The remainder consists of moisture, soluble sugar, ash and minerals such as potassium, phosphorus and magnesium [3]. Although it is commonly referred to as a "nut", it actually belongs to the class of "seeds" because it contains an edible part (embryo) surrounded by a hard shell. Pine nuts have been harvested for human consumption since prehistoric times [1]. Pine nuts are often used raw, as a roasted snack, or as an ingredient in various products that often require a roasting process, such as cakes, breads or desserts [5, 6]. If the shelled pine nuts are kept dry, they can be stored for a long time without any deterioration. Unshelled pine nuts are prone to rapid deterioration and rancidity [1]. Pine nut shells, which have a large annual production, are very suitable raw materials for the production of porous carbon due to their lignin and cellulose contents up to 40%, low ash content, high hardness and fixed carbon content [7, 8]. Pine nut shells are considered as food by-products in the agriculture and food industry. However, pine nut shells have excellent antioxidant properties and functional properties [9]. It is known that the oil yield for 100 g of pine nuts is between 45-65 g and this amount varies depending on the extraction method (cold pressing or solvent). The fatty acids found in pine nuts are polyunsaturated fatty acids (50%), monounsaturated fatty acids (40%) and saturated fatty acids (10%) [6]. Pine nut oil is mainly composed of unsaturated fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, eicosenoic acid and pinolenic acid. Pinolenic acid (PLA) is known as the active compound or reference material of

pine nut oil and is effective in wound healing, immune and inflammatory diseases, and cancer [10]. Besides, pine nut oil is also known to reduce body fat, to alleviate hyperlipemia and hypertension [4, 10]. Pine nut oil also contains fat-soluble antioxidants, including phytosterols and squalene, as well as tocopherols [6]. Linoleic acid (LA) is the most common fatty acid in the range of 40-60% of total fatty acids (FAs) and the predominant polyunsaturated fatty acid (PUFA) in pine nut oil (PNO). The high linoleic acid content in PNO is similar to many other seed oils. Oleic acid (cis-9 18:1) is the second most prevalent fatty acid and a significant monounsaturated fatty acid, which constitutes 12-30% of total FAs. PLA is the most common non-interrupted fatty acid (NMIFA) and typically constitutes 14-19% of total FAs in *P. koraiensis and P. sibirica* . It is reported that taxoleic and sciadonic acid constitute approximately 2% and 1-1.2% of the total FAs in *P. koraiensis*and *P. sibirica* , respectively. Delta-7 eicosatrienoic acid (ETA) (all cis-7,-11,-14 20:3) is only present in small amounts (1–3%) in PNOs. Matthaus et al. determined that PLA, taxoleic and sciadonic acids were found in some pine nut oils. Baker et al. found that PLA was much lower, whereas linoleic acid and oleic acid were higher in oils obtained from *P. eldarica, P. excelsa, P. pinea* and *P. torreyana*cultivars [6].

The top three consumers of pine nuts and PNO are Korea, the United States, and Russia. The oil obtained from *P. sibirica* hazelnuts consists of 99.4% non-polar lipids and 0.6% polar lipids by weight. Triacylglycerols (TAG) are important components of nonpolar lipids and Acheampong et al. detected 58 different TAG species in the oil of*P. koraiensis* . Due to their high TAG content, pine nuts and pine nut oil naturally contain high levels of FA (esterified to TAGs). The functional benefits of these fatty acids, as well as the underlying mechanisms of action, are yet unknown. PLA's biological impacts might be significant since it could provide a long-term terrestrial alternative to long-chain omega-3 PUFAs, which have been demonstrated to have a variety of health advantages, including inflammation reduction [11, 12].

In this study, some physicochemical properties (total crude oil and refractive index value) as well as fatty acids, triglycerides (TAG), tocols and sterol contents of oils obtained by cold pressing from pine nuts grown in Turkey were investigated.

## 2. Material and Methods

### 2.1. Materials

Pine nut (*Pinus pinea* ) samples were obtained from a pine nut producer in Bursa, Turkey. Pine nuts were supplied in shell and their shells were cracked with the help of a pine nut crusher. In this way, shell-kernel separation was provided.

Moisture content of pine nut was determined using Ohaus MB45 model automatic moisture meter. The moisture content of the pine nuts was determined as percent (%) by measuring the decrease in weight of approximately 3 g of ground pine nut samples at 105°C.

The protein content of pine nuts was measured by the express combustion method according to Dumas using the express analyzer Rapid N Cube (Elementar, Germany).

### 2.2. Chemicals and reagents

The analytical standards used to determine the tocols (tocopherols and tocotrienols), sterols and fatty acids composition were obtained from Merck KGaA (Darmstadt, Germany). All organic solvents of HPLC grade were purchased from Sigma-Aldrich through Delta Kimya (Adana, Turkey). All other chemicals (analytical quality) were purchased from Sigma-Aldrich and Merck.

### 2.3. Physicochemical characteristics of pine nut oils

#### 2.3.1. Oil extraction

A cold press oil machine (Karaerler brand NF500 model, Turkey) was used to extract the oil from pine nuts. The pine nuts were pressed at a frequency of 15 Hz at 45°C. The cold press oil machine was preheated and the temperature was set to 100 °C. The amount of cold pressed pine nut oil (%) was determined from the

ratio of the amount of pine nut that was initially cold pressed and the amount of oil obtained as a result of the process. After filtering the pine nut oil, it was stored in dark bottles at 4 degrees under refrigerator conditions.

2.3.2. Refractive index value

The refractive index (RI) value of pine nut oil was calculated using the Krüss AR2008 model Abbe refractometer (Germany) at 20 degrees and given as "nD 20 °C".

2.3.4. Tocols (tocopherols and tocotrienols) analysis

Analysis of tocols (tocopherols and tocotrienols) in oil of pine nut was carried out using "Shimadzu Prominence-I LC 2030C 3D Plus HPLC" according to TS ISO 9936 (2004) method. Depending on the tocols concentration, approximately 1 g of oil sample was weighed into a 20 mL test tube. A 10 mL of hexane was added and the tube was vortexed for 2 minutes to dissolve the sample. It was then filtered through a 0.45 μm nylon filter and added to a 1.5 mL vials [13, 14].

The chromatographic column was an Inertsil $NH_2$ 5μm 250×4.6 mm with 5 μm particle size. The mobile phase consisted of n-hexan/acetic acid/iso-propanol (IPA) (1000 mL/5 mL/6 mL) in isocratic conditions at a flow rate of 1 mL/min. The tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) were detected by UV where the wavelengths were set up at 296 nm. The injection volume was set at 10 μL. The column temperature was set in room temperature (˜20°C). Peak identification was carried out by comparing retention times of authentic standards of tocopherols and tocotrienols.

2.3.5. Fatty acids analysis

Determination of the fatty acid composition of pine nut oil was carried out by modifying TS EN ISO 12966-2 and Method 4 methods [15, 16]. The pine nut oil was extracted and fatty acid methyl esters (FAME) were prepared. After derivatization, they were injected to a GC instrument with a flame ionization detector (Agilent 7820A GC-FID) and a capillary column (HP-88 column; 100 m x 250 μm i.d. x 0.25 μm film). Injector temperature of 250 °C, detector temperature of 280 °C and helium (1.0 mL/min) as carrier gas were used as chromatographic conditions. The amounts of individual fatty acids were determined by comparison with the retention times of the standard solutions and expressed as a relative percentage.

2.3.6. Triacylglycerols analysis

Triacylglycerol analysis was carried out by modifying the COI method (2017) and the method used by Louati et al. (2014) [15, 17]. Agilent Infinity II 1260 HPLC device with a reverse phase column and refractive index detector (RID) was used. Triacylglycerols were separated from other oil components by column chromatography. The oil sample dissolved in petroleum ether was placed on a previously conditioned chromatography column containing a silica gel absorber. An ACE 5 C18 column (250 mm x 4.6 mm x 5 μm) was used for separation. Chromatography conditions; mobile phase acetonitrile/acetone (36.5:63.5), column temperature 35°C, flow rate 1.0 mL/min and injection volume of samples 20 μL. Triacylglycerols were identified by comparison with a reference chromatogram [18, 19].

2.3.7. Sterol analysis

Sterol analysis was performed according to ISO 12228 standard "Animal and vegetable fats and oils-Determination of individual and total sterol content-Gas chromatographic method" and TS EN ISO 12228-1: "Determination of individual and total sterol content-Gas chromatographic method-Part 1: Animal and vegetable fats and oils" [16]. The lipids with betulin added as internal standard were saponified and the unsaponifiable matter was extracted as above. On a simple silica gel layer, bands corresponding to sterols and triterpenic alcohol fractions of alcohol were isolated by thin layer chromatography (TLC). The sterols from the plate were transferred to an ethanol-diethyl ether mixture and the mixture analyzed using the Agilent 6850 GC with FID detector and an HP-5 (30 m x 320 μm x 0.25 μm) column. Chromatographic conditions: injection at 280 °C at 7.9 psi, HP-5 column and column temperature 260 °C, detector temperature 290 °C, injection volume 1.0 μL and split ratio 10:1, flow rate 35 mL/min, hydrogen as carrier gas was used.

## 3. Result and Discussion

### 3.1. Determination of Some Physicochemical Characteristics

Physicochemical properties of the pine nut oil were shown in Table 1. The moisture content, total protein amount and crude oil yield of the pine nut were 3.18%, 31.46% and 35.58% respectively.

### 3.2. Determination of Tocols Compositions

In this study, α-tocopherol, α-tocotrienol and γ-tocopherol were found to be 174.48 μg/g, 2004.65 and 485.92 μg/g, respectively. In a study conducted by Nasri et al., 2009, α-tocopherol and γ-tocopherol were found to be 15.34 and 1681.75 ppm, respectively [20].

In another study, tocopherol content was determined by hexane and chloroform/methanol extraction in pine nut samples. α-tocopherol contents by hexane and chloroform/methanol extraction were 114.6 and 166.3 mg/kg and γ-tocopherol contents were 229.5 and 247.4, respectively [21]. Lixia et al., 2018 determined some physicochemical characteristics of some pine nut oils with different extraction method. The results showed that α-tocopherol (16.14 and 12.96 mg/100 g) and γ-tocopherol (11.29 and 10.29 mg/100 g) were higher by cold press than by hot press technology [22]. There are some differences between these results and our study. These differences are thought to be because of their geographic origin.

### 3.3. Determination of Fatty Acids Content

Fatty acid content including total saturated fatty acids (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFA) was shown in Table 2. An example of the fatty acids chromatogram is presented in Figure 1.

When the fatty acid contents of the oils obtained by cold pressing method from pine nuts are examined, it was determined that it is rich in unsaturated fatty acids (86.16%) and the total saturated fatty acid content (10.7%) is much lower.

Linoleic acid (46.39%) was found to be the predominant fatty acid followed by palmitic (6.3%), stearic (3.5%), gadoleic (0.81%), linolenic (0.58%), eicosadienoic (0.49%), oleic (37.73%), behenic (0.13%), palmitoleic (0.1%), margaric (0.08%), myristic (0.06%) and heptadecenoic acid (0.05%).

In our study, 13 kinds of fatty acid were detected which is higher than that found in most studies in the literature. Colic et al., 2017 identified 16 kinds of fatty acids in 23 almond genotypes from Serbia [20]. Wang et al., 2019 detected 10 kinds of fatty acids in kernel oil from five wild almond species in China [21]. Nergiz et al., 2004 and Vanhanen et al., 2017 found that 12 kinds of fatty acids were present in different *Pinus* species [23, 24]. Meshgi et al., 2019 conducted a study to investigate the fatty acid composition in different pine species and they determined 5 kinds of fatty acids in different samples [25]. Lixia et al., 2018 detected 9 kinds of fatty acids in *Pinus koraiensis* L. nut oils [22].

The most abundant fatty acids were linoleic, oleic, palmitic and stearic acid in this research. This result is very consistent with other studies in the literature. The studies performed by Vanhanen et al., 2017 and Sen et al., 2016 showed that the major fatty acids present in some pine nut samples are linoleic, oleic, palmitic and stearic acid, respectively [24, 26]. In another study, palmitic and stearic acid were found to be the dominant saturated fatty acids [22]. Some studies showed that linoleic and oleic acid were found to be the dominant unsaturated fatty acids in some pine nut oils [21, 22]. Nasri et al., determined that linoleic acid is the major fatty acid followed by oleic, palmitic and stearic acids in the kernels of *Pinus pinea* L. [27].

Studies in the literature and our study have shown that the most common saturated fatty acids in pine nut or pine nut oil are palmitic and stearic acid, while the most common unsaturated fatty acids are linoleic and oleic acid.

### 3.4. Determination of Phytosterols Composition

The sterol contents of pine nut oils are given in Table 3 and their chromatogram is given in Figure 2.

The level of total sterol in this research was found to be 978 mg/kg. The most abundant sterol was β-Sitosterol (76.15%) followed by campesterol (15.60%), sitostanol (6.46%), Δ5-D24-stigmastadionol (1.43%) and ergosterol (0.36%). The predominance of β-Sitosterol and campesterol is typical for most vegetable oils [21]. When previous studies with pine nuts were examined, it was determined that β-sitosterol and campesterol were the most abundant sterols in almost all of them [28–32]. In a study conducted by Tukan et al., 2013, fatty acid, sterols and amino acid contents of the kernels of Aleppo pine (*Pinus halepensis* Mill.) cultivated in Jordan were analyzed and Δ5-D24 stigmastadionol content was found to be 1.43% which is exactly the same result as our study [31].

The results of our study are very close to many studies in the literature. As with many other seed or kernel oils, pine nut oil has been found to be a very good quality oil in terms of nutritional value and oxidation stability.

3.5. Determination of the Triacylglycerols (TAG) Content

The triacylglycerols (TAG) content of pine nut oils are given in Table 4 and their HPLC chromatogram is given in Figure 3.

In our study, 9 kinds of TAG were determined and the most abundant TAG was found to be LLL (trilinolein) with the TAG quantity of 11.5867% in total crude oil. It was followed by OLnL (Oleolinolenolinolein) (0.7302%), PLnL (Palmitolinolenolinolein) (0.1422%) and PoLL (Palmitoleodilinolein) (0.0826%), respectively. Similar to the results in our study, Nergiz and Dönmez, 2014 investigated the TAGs of some pine nuts with an ECN of 42 and LLL (10.8%) was found to be the predominant TAG followed by OLnL (2.23%) and PLnL (0.83%), respectively [23]. In another study, 3 kinds of TAG with an ECN of 42 were analyzed and the most abundant TAG was found to be LLL (12.96%) followed by OLnL (2.10%) and PLnL (0.65%), respectively [18]. Previous studies are very close to our study in terms of triacylglycerol content of pine nut oil.

## 4. Conclusion

It has been determined that the amount of tocopherol and tocotrienol is quite high in oils obtained by cold pressing compared to hot pressing. Hot pressing destroys the structure of tocopherol and tocotrienol in the composition of the oil and reduces their amounts. It is also known that oxidation stability is better in oils obtained by cold pressing [22]. Thanks to the cold pressing method applied in this study, the tocopherol and tocotrienol content in the composition of the oil were preserved at the maximum level without being damaged.

The high content of linoleic acid and tocopherol in vegetable oils increases the nutritional value and health benefits of the oil [33]. α-tocopherol is the most biologically important vitamin E analogue and γ-tocopherol has been shown to be more effective in reducing cytokine-induced cellular damage [22]. In our study, the most common fatty acid was found to be linoleic acid, and this pine nut oil was found to be rich in α- and γ-tocopherol. In the light of these informations, it can be easily said that the pine nut oil analyzed in this study has high nutritional value and health benefits. Also, it has high vitamin E content and a big potential to prevent cytokine-induced cellular damage.

β-sitosterol and campesterol in vegetable oils are ingredients that reduce the risk of LDL cholesterol [21]. Based on the fact that the most dominant sterols in pine nut oil examined in this study are β-sitosterol and campesterol, it can be said that this oil has LDL cholesterol inhibitory effect.

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