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Research Article

Characterization of Some Physicochemical Properties of Cold Press Sweet Cherry (*Prunus avium*) Seed Oil

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Abstract

This study aimed to determine physicochemical properties (PV, FFA, total phenolic content, antioxidant activity), fatty acid, sterol, tocopherol and phenolic compound profile of sweet cherry seed oil. Sweet cherry oil was obtained by cold press process. Tocopherol and phenolic compounds were determined by HPLC and fatty acid and sterol composition were analyzed by GC system. PV and FFA values were found as 1.40 meqO_2/kg and 2.71 % respectively. Apparent viscosity value was 0.057 Pa.s. TPC and AC values of the sweet cherry oil were determined as 22.17 mg GAE/g of extract and 1.05 mmol TE/g of extract. UFA content was higher than that of the SFA. SFA and UFA levels were 10.53% and 89.47 % respectively. MUFA (37.44 %) level was lower than PUFA level (52.03 %). Oleic acid and linoleic acid were found to be major fatty acids with a ratio of 36.73 % and 39.45 %, respectively. 8 different sterols were analyzed for sweet cherry seed oil. β-sitosterol was found to be the major sterol with a ratio of 88.93 % followed by campesterol (3.12 %), Δ7-stigmasterol (2.48 %), Δ5-avenasterol (2.12 %) and sitostanol (1.42 %). Other sterols showed small amount (<1%). Total four tocopherols namely, α, β, γ and δ tocopherols were identified for sweet cherry oils. α-tocopherols (96.72 mg /kg) were found as major tocopherol followed by γ-tocopherols (57.40 mg/kg) and δ tocopherols. β-tocopherol showed a small amount. This study suggested that sweet cherry seed oil can be utilized in food industry due to high level of bioactive compounds and low levels of PV and FFA content.

Keywords: Cherry seed oil, cold press oil, phenolic profile, sterol composition, tocopherol

Soğuk Press Kiraz (*Prunus avium*) Tohum Yağının Bazı Fizikokimyasal Özellikleri

Öz

Bu çalışma kiraz çekirdeği yağının fizikokimyasal özellikleri (PV, FFA, toplam fenolik madde ve antioksidan aktivite), yağ asidi, sterol, tokoferol ve fenolik bileşen kompozisyonunu belirlenmesini amaçlamıştır. Bu çalışmada kullanılan kiraz çekirdeği yağı soğuk presleme yöntemiyle eld edilmiştir. Kiraz çekirdeği yağının PV ve FFA değerleri sırasıyla 1.40 meqO_2/kg ve % 2.71 olarak tespit edilmiştir. Kiraz çekirdeği yağının görünür viskozite değeri 0.057 Pa.s olarak tespit edilmiştir. Kiraz çekirdeği yağının TPC ve AC değerleri sırasıyla 22.17 mg GAE/g ekstrakt ve 1.05 mmol TE/g ekstrakt olarak belirlenmiştir. Kiraz çekirdeği yağının doymamış yağ asitleri miktarı doymuş yağ asidi miktarından daha yüksek olarak tespit edilmiştir. Yağın tekli doymamış yağ asitleri miktarı (% 37.44) çoklu doymamış yağ asitleri miktarından (% 52.03) daha az çıkmıştır. Oleik ve linoleik asit major yağ asitleri olarak tespit edilmiş ve değerleri % 36.73 ve % 39.45 olarak bulunmuştur. 8 farklı bitkisel sterol tanımlanmıştır. β-sitosterol % 88.93 oranıyla en yüksek miktarda tespit edilen steroldür. β-sitosterolden sonra kampesterol (% 3.12), Δ7-stigmasterol (% 2.48), Δ5-avenasterol (% 2.12) ve sitostanol (% 1.42) yüksek miktarda tespit edilen sterollerdir. Diğer steroller az miktarda tespit edilmiştir (< % 1). α, β, γ and δ olmak üzere 4 farklı tokoferol kiraz çekirdeği yağının karakterizasyonu için tanımlanmıştır. α- tokoferol (% 96.72) major olarak

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tespit edilmiştir. α-tokoferolü γ-tocopherols (57.40 mg/kg) ve δ tocopherols takip etmiştir. β- düşük oranda tespit edilmiştir. Bu çalışma kiraz çekirdeği yağının yüksek biyoaktif madde içermesi ve düşük PV ve FFA değerlerinden dolayı gıda sanayiinde kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Kiraz çekirdeği yağı, soğuk press yağ, fenolik profili, sterol kompozisyonu, tokoferol

1. Introduction

Vegetable oils are used in the formulation of many food products due to their nutritional properties and affecting the textural and sensory quality parameters of many products. Some edible oils such as cold-pressed and virgin oils can be directly consumed. In addition to seed oils such as soy, sunflower, cotton and canola, palm and olive oil are among the most commonly used fruit oils (Siano et al., 2016). The need for alternative vegetable oil sources is increasing day by day. Cold pressed oil is produced by a mechanical extraction process. Since solvent extraction and refining processes are not used, cold pressed oil is rich in bioactive compounds. The trend in cold pressed oil consumption has been increased due to their bioactive properties (Al Juhaimi & Özcan, 2018).

Cherry (*Prunus avium*) is a fruit that is widely grown especially in Europe. Cherry fruit is subjected to different processes or directly consumed. After its processing and consumption, its seed is released as a waste(Siano et al., 2016). The seed of cherry fruit is very rich in fat and other bioactive substances. In recent years, seed oils of cherry, have been receiving growing interest due to their high concentration of hydrophilic and lipophilic bioactive components, which have important pharmacological properties on human health. The cherry seed oil has the potential to be utilized in food and other industries and to be added to the economy. This waste could be utilized in several industries due to its rich bioactive material and fat content (Bernardo-Gil, Oneto, Antunes, Rodrigues, & Empis, 2001; Siano et al., 2016).

In an evaluation of the cold-pressed oil fatty acid composition are crucial characteristics affecting both the nutritional and stability of the edible oils. Sterol, tocopherol and phenolic composition are key parameters ina evaluating nutritional and medicinal properties of cold pressed oil (Koç et al., 2019).

Some studies were conducted to determine some quality characteristics of cherry and sour cherry seed oil. However, There have been no comprehensive studies related to tocopherol, sterol, phenolic compounds profile of sweet cherry seed oil. The aim of this study is to characterize cold pressed cherry seed oil in terms of physicochemical, fatty acid composition, sterol, tocopherol, and phenolic composition.

2. Material and Method

2.1. Material

Sweet cherry seed oil were obtained oil by the cold-press extraction process. A cold press (Tokul Ltd. Co, Izmir, Turkey) was used for the extraction of sweet cherry seed oil. The press capacity was 6 kg seed production per hour and nozzles sizes were 5 mm. The temperature was lower than 50 °C to preserve oil quality. After pressing, solid impurities were removed from the oil by filtering through the filter paper. After filtration, oil samples were stored in colored bottles at 4°C for further analyses.

2.2. Methods

2.2.1 Physicochemical Analyses of oil samples

The free fatty acidity (FFA) and the peroxide values (PV) of the oils were determined according to the method described by IUPAC 2.201 and 2.50, respectively. The viscosity of the oil sample was determined by a stress and temperature controlled rheometer (Anton Paar, MCR 302, Austria) equipped with a Peltier heating system at 0.5 mm gap level and 25 °C in 100 s^{-1} shear rate interval.

2.2.2. Extraction of phenolic compounds

Methanol was used for the extraction of phenolic compounds from the sweet cherry oil. Firstly, 2 mL hexane was mixed with 4 mL oil and 4 mL of methanol was added to the hexane/oil mix. Then, the obtained solution was incubated at room temperature for 1 h in the shaking water bath (Memmert WB-22) for the extraction of the phenolic compounds. After the extraction process, the extracts were centrifuged (Hettich, Universal 320R, Tuttlingen, Germany) at 2,500 g for 10 min and the methanolic phase was taken. This operation was repeated three times to remove the hexane phase.

2.2.3Total phenolic contents (TPC)

The total phenolic compounds of sweet cherry seed extracts were determined by the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965). Inıtialy, 2.5 mL of 0.2 N Folin–Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3 were mixed respectively with 0.5 mL of the methanolic extract. This mixture was held for 45 min at room temperature in a dark place. At the end of the incubation time, the absorbance was recorded at 760 nm using a UV-vis spectrophotometer (Shimadzu, UV-1800). The TPC was determined as gallic acid equivalent. TPC was calculated from a calibration curve obtained with gallic acid. Total phenolic was expressed as Gallic acid equivalents (mg GAE/g of extract).

2.24. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The antioxidant capacity (AC) values of methanolic extracts were determined using DPPH (1,1-diphenyl-2- picrylhydrazyl) method according to the method described by Singh, Murthy, and Jayaprakasha (2002). After, 0.1 mL extract and 2 ml methanolic DPPH solution were mixed. The mixture was vigorously shaken and incubated at room temperature for 30 min. The absorbance was recorded at 517 nm by a spectrofotorometer (UV–Mini 1240, Schimadzu, Kyoto, Japan). The Trolox equivalent's antioxidant capacity (TEAC) value is expressed as millimole Trolox equivalents per grams of extract of cold press sweet cherry seed oil sample (mmol TE/g of extract).

2.2.5. Fatty acid composition

The sweet cherry seed oil samples were methylated using BF3-methanol according to AOCS (1990). The fatty acid methyl ester was transferred to gas chromatography (with a capillary column, HP-88, 100m x 0.25mm, film thickness: 0.20 mm) and analyzed by gas chromatography (Agilent 6890N) equipped with a flame-ionization detector (FID). The carrier gas was selected as helium, with a flow rate of 0.5 mL/min. The temperatures of the injector and the detector were adjusted at 250 and 280 ºC, respectively. The initial oven temperature of 120 ◦C was for 10 min, raised to 240 ◦C at a rate of 5 ◦C/min. The injection volume was 1 µL. The fatty acid methyl esters of sweet cherry seed oils were identified by comparing the retention time of the samples and appropriate fatty acids methyl esters standards. The percentage of individual fatty acid content, saturated fatty acid (SFA), unsaturated fatty acid (UFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) content are presented.

2.2.6. Individual phenolic compounds

Individual phenolic compounds of methanolic extracts were determined by HPLC coupled to a diode array (HPLC-DAD, Shimadzu Corp., Kyoto, Japan). The methanolic extract was filtered through a 0.45-µm membrane filter and 1 mL of the filtered sample was introduced to HPLC system (LC-20AD pump, SPDM20A DAD detector, SIL-20A HT autosampler, CTO-10ASVP column oven, DGU-20A5R degasser, and CMB-20A communications bus module; (Shimadzu Corp., Kyoto, Japan). Separations were carried out at 40 °C on a reversed-phase column (Intersil® ODS C-18, GL Sciences, Tokyo, Japan) with a 250 mm × 4.6 mm length, 5 µm particle size. The mobile phases were solvent A (distilled water with 0.1% (*v*/*v*) acetic acid) and solvent B (acetonitrile with 0.1% (*v*/*v*) acetic acid). A gradient elution were 10% B (0 to 2 min), 10% to 30% B (2 to 27 min), 30% to 90% B (27 to 50 min) and 90% to 100% B (51 to 60 min) and at 63 min returns to initial conditions. The flow rate was 1 mL/min. Chromatograms were taken at 254-356 nm. Identification and quantitative analysis were conducted based on retention times and standard curves. The result of individual phenolics amounts was expressed as mg/kg for fresh and dried samples.

2.2.7. Sterol Composition

Before sterol composition fallowing pre-treatment was performed; sterol composition, 0.5 g of oil sample was transferred to a test tube and saponified with 5.0 mL saturated methanolic KOH at 80°C for one h. Then, it was extracted with 5 mL of hexane three times; resulted solution was dried with anhydrous sodium sulfate. A 0.5 mL of dried hexane extract was silylated with solution of 0.1 mL bis (trimethylsilyl) trifluoroacetamide/trimethylchlorosilane (4:1 v/v). After pre-treatment, the sterol composition of the oils was determined using GC equipped with FID. Separation of the sterols was conducted by using CP-SIL 24 CB (60m x 0.32 mm x1.00 µm), and the following method parameters were identified for sterol composition analysis. Working conditions were as follows: carrier gas, helium; flow rate was 0.8 mL/min; injector temperature, 280°C; detector temperature, 300°C; oven temperature program, initial temperature was 50°C for 2 min, increased at 60°C/min to 245°C, held for 1 min and then increased at 3°C/min to 275°C, held for 35 min (Kamm et al., 2002).

2.2.8. Tocopherol content

Tocopherol content (mg of α-tocopherol per kg of oil) was determined by the HPLC method of AOAC (2000). The chromatographic separation was carried out using the mobile phase consisted of ethyl acetate: acetic acid: hexane (1:1:98 v/v/v) at a flow of 1.5 mL/min. The fluorescence detector at 290 nm (excitation) and 330 nm (emission) wavelengths were used. The number of tocopherols in the samples was calculated as µg tocopherols in ml oil extract using external calibration curves (0-10 µg ml $r^2 = 0.999$), which were obtained with the α-tocopherol standard.

3. Results and Discussion

3.1. Physicochemical analysis

PV, FFA, apparent viscosity, TPC and AC value of the sweet cherry oil were determined. PV and FFA values were found as 1.40 meqO_2/kg and 2.71 % respectively. Both FFA and PV values were determined within the allowed limit (FAO/WHO, 2015). Apparent viscosity value was 0.057 Pa.s. Apparent viscosity value was similar to other vegetable oil. TPC and AC values of the sweet cherry oil were determined as 22.17 GAE/g of extract and 1.05 mmol TE/g of extract. Sweet cherry oil showed had a low level of PV and

FFA with a high level of TPC and AC and should be utilized as edible oil. TPC and AC value of this study were in conformity with Yılmaz and Gökmen (2013) study.

3.2. Fatty acid composition

Table 1 showed the fatty acid composition of sweet cherry seed oil. As can be seen, UFA content was higher than that of the SFA. SFA and UFA levels were 10.47% and 89.53 % respectively. MUFA (40.50 %) level was close to the PUFA level (52.03%). Oleic acid and linoleic acid were found to be major fatty acids with a ratio of 36.73% and 39.45 %, respectively. Bernardo-Gil et al. (2001); Siano et al. (2016) reported similar results. Doğantürk and Seçilmiş Canbay (2019) reported that oleic acid was in the range of 42.625 to 55.265 g/100 g and linoleic acid with 23.276 g/100 g. The oleic acid value was higher than our study. These differences could be due to different extraction techniques. Their study solvent extraction was conducted. Oleic and linoleic acid content was reported as 37.5 % and 40 % in Comes, Farines, Aumelas, and Soulier (1992) study. Linolenic acid content was 12.40 %. Yılmaz and Gökmen (2013) and Bernardo-Gil et al. (2001) reported similar linolenic acid in sour cherry and sweet cherry oil respectively. Their results were comparable with our results. When considering the saturated fatty acid palmitic acid were major fatty acids. Its levels were 6.79 %, respectively. Other fatty acid levels were lower than 1%.

Fatty acid content is considered one of the most crucial criteria affecting the nutritional value and stability of edible oil. Sweet cherry oil showed a higher level of UFA, indicating that it is rich in unsaturated fatty acid and could be shown positive health effects. Among the UFA, linoleic acid showed a higher level following oleic acid. Linoleic acid has lots of bioactive properties, especially for the cardiovascular system. It is an essential component of the cell membrane and arachidonic acid precursor (Boso, Gago, Santiago, Rodríguez-Canas, & Martínez, 2018). The consumption of linoleic acid and oleic acid instead of saturated fatty acid could reduce low-density lipoprotein levels (Vázquez et al., 2018). Oleic acid showed a positive effect on the brain system. Oleic acid /Linoleic acid ratio of the sweet cherry seed oil was closed to 1, indicating that sweet cherry seed oil shows higher stability compared to other oils having higher linoleic acid content. In conclusion, sweet cherry seed oil could be utilized as alternative edible oils with high nutritional value and desire stability (Boso et al., 2018; Koç et al., 2019).

Table 1. *Fatty acid composition of sweet cherry seed oil*

Fatty acid	Con %
Mrystic acid (C14:0)	0.03±0.00
Palmitic acid (C16:0)	6.79±0.04
Palmitoleic acid (C16:1)	0.40±0.02
Heptadecanoic acid (C17:0)	0.07±0.01
Stearic acid (C18:0)	2.46±0.01
Oleic acid (C18:1)	36.73±0.98
Linoleic acid (C18:2)	39.45±0.65
Arachidic acid (C20:0)	0.92±0.06
Linolenic acid (C18:3)	0.18±0.02
Eleostearic acid (C18:3)	12.40±0.72
Gadoleic acid (C20:1)	0.31±0.02
Behenic acid (C22:0)	0.20±0.00
Lignoceric acid (C24:0)	0.06±0.00
∑SFA	10.53±0.12
∑UFA	89.47±2.41
∑PUFA	52.03±1.39
∑MUFA	37.44±1.02

3.3. Sterol composition

The percentage of individual sterol distribution was presented in Table 2. 14 different sterols were analyzed for sweet cherry seed oil. β-sitosterol was found to be the major sterol with a ratio of 88.93 % followed by campesterol (3.12%), Δ7-stigmasterol (2.48%), Δ5-avenasterol (2.12%) and sitostanol (1.42%). Other sterols showed a small amount (<1%). In a study of seed oils belonging to different cherry species, it was stated that the amount of sterol varies between 233,6 - 419,4 mg / 100 g oil (Górnaś et al., 2016). In the same study, β-sitosterol was the first among sterol types. It was found that the data obtained from our study showed similarity with the data in this study. Straccia et al. (2012) stated that the amount of β-sitosterol in cherry seed oil was 0.569 g / kg and the amount of campesterol was 0.025 g / kg. These values were much lower than ours'. Bernardo-Gil et al. (2001) reported similar results. In their study, β-sitosterol) was reported to be a major sterol with a percentage level of 83.42 % followed by Δ5-avenasterol, stigmasterol, and campesterol. Sterol showed various positive health effect and trends in consumption of plant sterol have increased. In a sımılar to various plant sources, sweet cherry oil showed β-sitosterol content as a major level. High β-sitosterol has antimicrobial, antioxidant, immunomodulatory, angiogenic and antidiabetic properties (Bin Sayeed, Karim, Sharmin, & Morshed, 2016). Sweet cherry oil showed higher β-sitosterol content than grape seed oil (Koç et al., 2019) and sour cherry oil (Górnaś et al., 2016b). This study concluded that sweet cherry oil could be considered a good source of β-sitosterol.

Table 2. *Individual sterol compositions of sweet cherry seed oil*

Sterols	Sweet cherry	
	Ppm	%
Cholesterol	5.78±0.02	0.15
Cholestenol	0.00	0.00
Brasikasterol	0.00	0.00
24-methylene-cholesterol	1.42±0.02	0.04
Campesterol	120.10±0.18	3.12
Campestenol	2.86±0.04	0.07
Stigmasterol	4.04±0.10	0.14
Δ-7-campesterol	3.75±0.04	0.10
Klerosterol	25.78±0.08	0.67
β-sitosterol	3424.22±58.88	88.93
Sitostanol	54.66±0.30	1.42
Δ-5-avenasterol	81.62±0.29	2.12
Δ-5-24-stigmastadienol	15.89±0.20	0.41
Δ-7-stigmasterol	95.35±0.43	2.48
Δ-7-avenasterol	14.87±0.08	0.39
Eritrodiol	0.00	0.00
Uvaol	0.00	0.00
Total sterol	**3850.36±60.66**	100

3.4. Tocopherol and phenolic composition

Tocopherol content was an important criterion affecting the oxidative stability of vegetable oil during storage due to antioxidant properties. Total four tocopherols namely, α, β, γ and δ tocopherols were identified for sweet cherry oils. α-tocopherols (96.72 mg /kg) were found as major tocopherol followed by γ-tocopherols (57.40 mg/kg) and δ-tocopherols. β-tocopherol showed a small amount. Our results were similar to the tocopherol content of sour cherry oil reported by Yılmaz and Gökmen (2013).

Table 3 showed the individual phenolic composition of sweet cherry seed oil. 14 different phenolic compounds were investigated and 8 of them were identified. Phenolic acids levels were higher than other phenolic compounds. Among the phenolic acid, benzoic acid was major phenolic acids and presented as 58.8 mg/kg. p-hydroxybenzoic acid was the second major phenolic acid and its level higher than 1 mg/kg. Quercetin and vanillin were identified as higher than 1 mg/kg. Other phenolic compounds were found at a very low level. The obtained concentration values were found to be lower than other studies (Nyam et al., 2009, Casazza et al., 2010). In a study that determined phenolics in cherry seeds; 5-caffeolkinic acid (105.10 µg/g), procyanidine dimer (67.15 µg/g) and elagic acid pentoside (50.09 µg/g) phenolics have been reported (Senica *et al.*, 2015). The values hereby were found to be higher than the data in our study.

Table 3. *Phenolic composition of sweet cherry seed oil*

Phenolics	**Sweet cherry**
Gallic acid	nd
Catechin	nd
p-hydroxybenzoic acid	1.10±0.01
Syringic acid	nd
Vanillin	1.22±0.01
p-com acid	0.12±0.00
Benzoic acid	58.8±0.81
o-coum	0.05±0.00
Rutin	nd
Cinnamic acid	0.20±0.00
Quercetin	1.05±0.00
Luteolin	nd
Kamferol	nd
Apigenin	0.75±0.03

nd: not detected

4. Conclusions and Recommendations

In this study physicochemical properties, fatty acid, sterol, tocopherol and phenolic compounds profile of cold press sweet cherry oil were analyzed. Sweet cherry oil showed low-level PV and FFA and comparable level of TPC and AC. Sweet cherry seed oil is rich in linoleic and oleic acid. It also contained linolenic acid higher than 10 %. Sweet cherry oils showed high level of β-sitosterol, benzoic acid and α-tocopherols. This study suggested that sweet cherry seed oil can be utilized in food industry due to high level of bioactive compounds and low levels of PV and FFA.

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