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Fatty Acid, Total Phenol and Tocopherol Profiles of Some Walnut Cultivars: A Comparative Study

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Abstract

As a member of nuts, walnut is consumed from snacks to salads and desserts to entrees and an importantpart of human diet for centuries. Walnut biological and nutritional value is also enhanced by its valuable protein and rich in nutrient composition such as vitamins and minerals. The most important characteristic of walnut oil is the abundance of polyunsaturated fatty acids, which makes it a unique food because of high amount of linoleic acid. Due to having valuable protein, vitamins and minerals it enhances biological and nourishment value, also. Recent epidemiological studies showed that consumption of walnut reduce cardiovascular diseases due to the rich in antioxidant properties, valuable fatty acids and tochopherols contents. In Turkey, walnut production and consumption increases year by year. The kernel of walnut genotypes shows variability in terms of their fat, fatty acid and tocopherol profiles. In this paper, it was aimed to characterize 10 walnut (*Juglans regia* L.) cultivars (Bilecik, Chandler, Hartley, Howard, Maraş 12, Maraş 18, Midland, Pedro, Şen and Serr) based on their fatty acid profiles using GC (Gas Chromatography), tocopherol and its isomers by HPLC (High Performance Liquid Chromatography) and total phenol content with spectrometric methods. Among the walnut cultivars "Hartley" was the highest linoleic acid (64.56%) and "Howard" was the α-linolenic acid 13.26 (%). The highest values of α (38.76 μg/g), $\beta + \gamma$ (312.19 μg/g) and δ-tocopherol (40.77 μg/g) and total phenol (349 mg GAE/100 g ext) content were detected in "Sen" cultivar. Obtained results might be significant for further breeding programme to im-

prove rich in especially γ-tocopherol linoleic acids and total phenolic compounds.

Keywords

Fatty Acid, GC, HPLC, *Juglans regia* L., Tocopherols, Walnut

1. Introduction

As a member of Juglandaceae family, walnut (*Juglans regia* L.) is also known as an English or Persian walnut, one of the oldest cultivated nut in the world and grown universally everywhere almost in all geographical regions in Turkey [1] [2]. *Juglans regia* L., is economically cultivated everywhere especially in the temperate regions of the world. There is a report that walnut is possibly originated from the Iran-Afghanistan places and later introduced to Eastern Europe, China and Russia regions. There is another report on history of walnut cultivation grown Mediterranean region [3]. Anatolia is also known one of the native countries for Persian walnut, also [4]. Recently, average year of walnut production in Turkey approximately has reached 212.000 tons [5]. Walnut consumption trend increases year by year due to the scientific reports on health benefits especially loosen the risk of cardiovascular diseases [6] [7]. Walnuts are also important due to the rich in antioxidant properties which loosen the risk of many diseases by reduction of oxidation process [8] [9] [10] and a source of essential fatty acids and tocopherols [11] [12]. There are also previously published reports on antimicrobial distinctiveness of walnut [13].

Walnuts are highly enriched in health related compounds such as polyunsaturated fatty acids, fiber, copper, magnesium, and vitamin E and mostly consist of monounsaturated (MUFA's) and polyunsaturated fatty acids (PUFA's) which positively influence serum lipids. According to clinical and epidemiological reports omega-3 (PUFA's) might have a significant role in the prevention CHD (Coronary Heart Disease) and play antiarrhythmic, antithrombotic, and hypolipidemic roles [14] [15]. Abbey *et al.*, [16] reported that daily intake 68 g of walnuts per day reduced the total and low-density lipoprotein cholesterol by 5 and 9%, respectively; this act reduced the risk of coronary heart diseases. Few studies previously reported on some fruit quality properties such as fatty acid, total phenol and tocopherol contents of walnuts growing in Turkey [17] [18] [19] [2]. There are a few reports previously published related to fat, fatty acid and tocopherol characteristics of walnut in temperate zone regions of Turkey. However, no detailed study has so far been previously published on fat, fatty acid, total phenol and tocopherol content of commercially grown walnut cultivars in the Mediterranean region of Turkey. The purpose of this study was to compare ten walnut cultivars based on their fat, fatty acid, total phenol and tocopherol profiles using spectrophotometric and chromatographic technique.

2. Materıals and Methods

2.1. Experimental Materials

Commercially ripen stages of walnut cultivars were harvested from Research and Experimental Implementation area of University of Çukurova, Faculty of Agriculture, located in the Adana provinces of Mediterranean region of Turkey in November, 2015. Harvesting were done when the husk changes from solid green to yellowish green. Foreign and local cultivars were used due to the comparing their kernel characteristics in Asana ecological conditions. In the experiment, 9 trees were selected for each genotyp and 25 walnut fruits were randomly harvested from each trees. Triplicate analysis were done using randomly selected walnut and each replicate 75 randomly selected walnut fruits were used. Kernel of walnut fruits were analyzed after drying using incubator.

2.2. Oil Extraction

Walnut fruits were separated their kernel using nutcracker and after the separation immediately homogenized and oil extraction of kernel powder (25 g) were done using automatic Soxhalet equipment Bligh and Dyer [20]. Automatic Soxhlet equipment (Gerhardt Soxtherm) was used for oil extraction with hexane organic solvent. Triplicate analysis were done for each variety. Methylation were done using Boron trifluoride/methanol [21] [22].

2.3. Fatty Acid Analyses

Fatty acid analysis were done using a GC (Gas Chromatography)/FID (Flame Ionization Detector) (Perkin Elmer, Clarus 500, USA) equipped with a and SGE column (100 m × 0.32 mm, ID 0.25 μm). As fort he GC condition; the oven temperature was held at 140˚C for 5 min, and then increased to 200˚C at a rate of 4˚C min^{-1} and to 220˚C at a rate of 1˚C min^{-1}. FAME mix standard including 37 component were used for fatty acid identification and quantification.

2.4. Tocopherol Analyses

Tocopherol analysis were done using 1 g of oil. Analyses of tocopherol isomers such as α-tocopherol, B and γ tocopherol and δ tocopherol were performed by HPLC technique according to developed method by Surai *et al.*, [23] and Surai, [24]. 3 μm RP-C18 reverse phase Spherisorb ODS2 column, (15 cm × 4.6 mm) and methanol/water (97:3 v/v; 1.05 ml/min) was used as mobile phase Calibration were done using external standards of tocopherol isomers. Excitation 325 nm and emission 490 nm in the first 5 minutes were followed by excitation 295 nm and emission 330 nm with Fluorescence Detector [23] [24].

2.5. Total Phenolic Content Analysis

Total phenolic content of walnut genotypes were done by modifying spectrophotometric Folin-Ciocalteu's method developed by Spanos and Wrolstad [25] and 10 ml of methanolic extract for 1 g homogenized walnut kernel were used.

Obtained values expressed as mg gallic acid equivalent in 100 g extract (mgGAE/100gext).

2.6. Statistical Analyses

Triplicate GC/FID, HPLC and spectrometric analyses were performed and a completely randomized design using analysis of one-way analysis of variance (ANOVA).

3. Results and Discussions

The results of α, $\beta + \gamma$ and δ-tocopherol content of various walnut (*Juglans regia* L.) cultivars (Bilecik, Chandler, Harley, Howard, Maraş 12, Maraş 18, Midland, Pedro, Şen and Serr) grown in Adana region of Turkey were shown in Table 1. As seen in Table 1, tocopherol and its isomers such as α, $\gamma + \beta$, and δ tocopherol content of experimental walnut varieties were differed. α-tocopherol content of cultivars varied between 28.33 µg/g (Howard) to 38.76 µg/g (Sen), $\beta + \gamma$ tocopherol content varied between 161.01 µg/g (Howard) to 312.19 µg/g (Sen) and as for the δ-tocopherol content varied between 17.35 µg/g (Serr) to 40.77 µg/g (Sen) oil (Table 1). The lowest α, $\beta + \gamma$ tocopherol content was detected in "Howard" walnut cultivar while the highest in Sen cultivar. According to the previous reports, γ tocopherol content of walnut was about 25 mg/100 g in kernel oil and as major tocopherol isomer whereas β and α-tocopherol are about 1 - 5 mg/100 g [26]. In another previous study, γ tocopherol was detected as the highest tocopherol isomer while α-tocopherol was the lowest and approximately 6% [27]. In another study, the highest content of the tocopherol isomers in the kernel oil was determined as $\gamma + \beta$ tocopherol and γ-tocopherol has been reported to be much more powerful than α-tocopherol in detoxifying lipophilic electrophiles [28]. Amaral *et al.* reported that [11], the tocopherol content of walnut (*Juglans regia* L.) cultivars such as Mayette, Arco, Franquette, Lara, Marbot, Hartley,

Table 1. Tocophorels and its isomers and total phenolic contents of various walnut varieties.

Varieties	α-tocopherol µg/g	γ-tocopherol + B tocopherol µg/g	δ-tocopherol µg/g	Total Phenol mgGAE/100 gext
Bilecik	32.65 ± 0.452	247.51 ± 10.649	25.15 ± 0.883	3170 ± 3.8
Chandler	35.17 ± 2.213	254.18 ± 24.048	24.24 ± 2.552	2650 ± 1.9
Hartley	30.78 ± 1.131	266.38 ± 11.377	27.49 ± 0.615	2680 ± 1.0
Howard	28.33 ± 0.848	161.07 ± 7.488	23.89 ± 5,706	2530 ± 4.1
Maras 12	34.46 ± 2.177	236.13 ± 12.183	29.32 ± 1.541	2440 ± 1.3
Maras 18	28.61 ± 0.381	230.70 ± 7.318	28.56 ± 1.555	2840 ± 1.9
Midland	29.43 ± 1.0394	235.48 ± 1.675	25.57 ± 0.438	3170 ± 3.8
Pedro	28.66 ± 0.077	226.44 ± 7.219	21.67 ± 0.593	2370 ± 1.1
Sen	38.76 ± 1.230	312.19 ± 11.455	40.77 ± 1.378	3490 ± 4.9
Serr	31.48 ± 1.626	191.44 ± 7.014	17.35 ± 0.523	2650 ± 3.6

Mellanaise, Parisienne, and Reg were compared and *γ*-tocopherol was the most abundant tocopherol isomer in all cultivars. The same authors reported that environmental factors play an important role oil tocopherol profile.

The total phenolic content of walnut cultivars varied between 2440 mgGAE/100 g ext to 3490 mgGAE/100 g ext (Table 1). As reported previous studies the average value of total phenolics is lowest in pines (32) while highest in walnuts (1625) mg GAE/100 g which is in accordance with previously published data [29] and [30]. In another study, Abe *et al.* [31] reported that total phenolic content of nuts was highly variable and ranged from 50 mg 100 g^{-1} (FW) (Pinhao seeds raw) to 2499 mg 100 g^{-1} (FW) (Walnut raw). Similar to our study and previous reports, Kornsteiner *et al.* [32] also reported the highest total phenolic values in walnuts (1020 - 2052 mg 100 g^{-1} FW) compared to pecans (1022 - 1444 mg 100 g^{-1} FW) and pistachios (492 to 1442 mg 100 g^{-1} FW), respectively.

Total lipid content and fatty acid profile is one of the most significant parameter distinguishing of the walnut cultivars. The results of total lipid and saturated fatty acid profiles of 10 walnut cultivars are given in Table 2. Maraş 18 were the highest lipid content (70.7%), while Maraş 12 (46.4%) the lowest. Mitrovic *et al.*, [33] studied eight walnuts varieties and selections (Dorka, Ceinovo, Ibar, Vujan, Ovcar, G-139, G-251 and G-286) and the highest value detected as 68.81%. Similarly, Maraş 18 is the highest lipid content in our research. Savage *et al.* [27], studied US commercially grown walnut varieties (Tehama and Vina), three European commercial varieties (Esterhazy, G139, G120), and eight New Zealand selections (Rex, Dublin's Glory, Meyric, Stanley, Mckinster, 150, 151, 153) in an implementation area at Lincoln University. The same author reported that total lipid content of nuts ranged from 64.2% to 68.9%. Beceanu *et al.* [34] implied that generally in walnuts total lipid ranged from 62% to 65%.

A total saturated fatty acids are not statistically different among varieties

Table 2. Lipid and saturated fatty acid content (%).

Varieties	Lipid (%)	Saturated fatty acids (%)				
		Myristic C14:0	Palmitic C16:0	Stearic C18:0	Arachidic C20:0	Σ Saturated FA
Bilecik	63.0 ± 2.65	0.019 ± 0.0029	5.82 ± 0.421	3.82 ± 0.491	0.12 ± 0.098	9.77
Chandler	62.3 ± 2.65	0.017 ± 0.0013	5.92 ± 0.581	3.41 ± 0.305	0.10 ± 0.051	9.44
Hartley	62.8 ± 3.25	0.021 ± 0.0083	6.16 ± 0.317	3.59 ± 0.384	0.12 ± 0.020	9.89
Howard	64.5 ± 2.29	0.021 ± 0.0020	6.13 ± 0.451	3.44 ± 0.169	0.12 ± 0.048	9.71
Maraş-12	46.4 ± 2.23	0.021 ± 0.0079	6.57 ± 0.593	3.70 ± 0.153	0.11 ± 0.096	10.40
Maraş-18	70.7 ± 3.28	0.022 ± 0.0071	6.98 ± 0.353	3.59 ± 0.279	0.10 ± 0.091	10.69
Midland	50.20 ± 1.58	0.023 ± 0.0056	6.41 ± 0.553	3.86 ± 0.331	0.11 ± 0.082	10.41
Pedro	65.9 ± 2.89	0.021 ± 0.0020	6.72 ± 0.612	3.97 ± 0.435	0.13 ± 0.066	10.84
Şen	49.4 ± 1.48	0.029 ± 0.0019	6.58 ± 0.577	3.23 ± 0.183	0.11 ± 0.037	9.94
Serr	62.0 ± 2.25	0.021 ± 0.0020	6.57 ± 0.593	3.88 ± 0.260	0.10 ± 0.052	10.57

(Table 2). As seen in Table 2, myristic (C14:0), palmitic (C16:0) stearic (C18:0) arachidic (C18:0) acids were detected as saturated fatty acids and the most abundant saturated fatty acid detected as palmitic acid. Doğan and Akgul [35], detected fatty acid composition of some walnut (Juglans *regia* L.) cultivars from east Anatolia. In their study, a total oil content of walnut ranged from 65.00% ± 0.06% to 70.00% ± 0.58%. The palmitic acid content of the walnut ranged from 5.61% to 5.82% while a trace amount of myristic acid (<0.1%) detected in the samples. Popa *et al.*, [36] investigated the saturated and unsaturated fatty acid profile of walnut (*Juglans regia* L.) kernel oil by GC/MS according to AOAC standards. As a result of that study, palmitic acid (9.75%) was the major saturated fatty acid, followed by stearic acid (3.48%). In another study previously reported by Beyazit and Sümbül [37] and the authors investigated fatty acid composition of five cultivars ("Şebin", "Şen 1", "Tokat 1", "Kaplan 86" & "KR 2") and three genotypes ("Malatya 1", "77H1" & "65/4") selected form the Eastern Mediterranean region of Turkey. According to their results, palmitic acid values of those genotypes were ranged from 6.98% to 8.77%, and steraic acid ranged from 3.22% to 4.99% in walnut genotypes.

The results of monounsaturated fatty acids and polyunsaturated fatty acids were given in Table 3. Monounsaturated fatty acids (palmitoleic and oleic acid) values varied between 0.11% to 14.36% and 0.13% to 27.57%, respectively. The values are similar to previously published researches when compared to the data in this study. Zwarts *et al.*, [38] reported that two US commercial cultivars (Tehama and Vina), three European commercial cultivars (Esterhazy, 139, G120) and five New Zealand selections (Rex, Dublin's Glory, Meyric, McKinster, and Stanley). The oleic acid content of walnut varieties varied between 12.7% to 20.4% of the total fatty acids, while 18:2 content varied between 57.0% to 62.5% and the 18:3 content varied between 10.7% to 16.2%. Popa *et al.*, [36] reported

Table 3. Unsaturated fatty acid content (%) of walnut cultivar.

Varieties	Unsaturated fatty acids (%)			
	Monounsaturated fatty acids		Polyunsaturated fatty acids	
	Palmitoleic C16:1	Oleic C18:1	Linoleic C18:2	*α*-Linolenic C18:3
Bilecik	0.10 ± 0.086	13.80 ± 0.322	62.92 ± 0.260	13.16 ± 0.391
Chandler	0.12 ± 0.021	14.47 ± 0.173	62.82 ± 0.204	12.92 ± 0.735
Hartley	0.11 ± 0.011	12.95 ± 0.148	64.56 ± 0.569	12.12 ± 0458
Howard	0.11 ± 0.091	14.36 ± 0.510	62.20 ± 0.581	13.26 ± 0.805
Maraş-12	0.13 ± 0.074	21.02 ± 0.354	59.62 ± 0.411	8.55 ± 0.387
Maraş-18	0.13 ± 0.090	27.57 ± 0.683	53.42 ± 0.516	7.83 ± 0.924
Midland	0.12 ± 0.074	17.63 ± 0.259	61.22 ± 0.428	10.33 ± 0.268
Pedro	0.11 ± 0.070	14.77 ± 0.112	61.89 ± 0.164	12.17 ± 0.420
Şen	0.12 ± 0.087	26.86 ± 0.916	53.24 ± 0.433	9.50 ± 0.574
Serr	0.12 ± 0.067	14.94 ± 0.201	61.37 ± 1.540	12.74 ± 0.903

the fatty acids composition in saturated and unsaturated of walnut (*Juglans regia* L) kernel oil that also similar to our study. The same authors detected fatty acid profile by GC-MS according to AOAC standards. Among the detected monounsaturated fatty acids, the oleic acid content of the oil was 13.62% of the total fatty acid and oleic acid is detected as the most abundant among monounsaturated fatty acids.

Maximum and minimum values of polyunsaturated fatty acids linoleic acid and α-linolenic acids are detected as 53.24% to 64.56% and 9.50% to 13.26%, respectively (**Table 3**). In parallel to previous studies, linoleic acid was the major fatty acid reaching a maximum value of 60.30% (cv. Lara) followed by oleic, linolenic and palmitic acid which means the fatty acid quantities could be strongly related with the cultivar [39]. Acording to Doğan and Akgül [35], the oleic acid content of the oils ranged from 22.63% to 27.27% of the total fatty acids while the linoleic acid and linolenic contents ranged from 49.93% to 54.41% and 14.32% to 17.82% respectively. The same authors indicated that the fatty acid of walnut cultivars from East Anatolia were distinctive in terms of their linolenic acid profile. Beceanu *et al.*, [34], reported that 44% - 48% of the fatty acids in the walnut oil are replaced by polyunsaturated fatty acids and changing in the content of the total fat between 62% - 65%. In another previos research, linoleic acid content was detected as %56.57 and linolenic acid 12.09% in walnut oil [32]. Fatty acid profile was a significant parameter distinguishing the walnut cultivars and genotypes. As a result of this study, linoleic acid ranged from 41.55 to 59.89% while linolenic acid ranged from 8.44% to 11.0%. The linolenic acid was detected as the highest. Similar to our results, a study published by Beyazıt and Sümbül [37]. The authors published that "65/4", "KR 2" and "Şebin" genotypes were found to be very promising for oil composition especially fatty acid profiles in the Eastern Mediterranean region of Turkey. The same authors reported that linoleic acid varied between 41.55% and 59.89%, linolenic acid 8.44% and 11% in "Şebin-1", "Tokatşen-1", "Kaplan 86", "Sons", "KR 2", "Malatya 1", "77H1" 65/4 "walnut genotypes" [33].

The values of SFA, USFA, MUFA and PUFA varied between 9.44% to 10.84%, 88.95% to 90.33%, 13.06% to 27.70% and 61.25% to 76.74%, respectively. Maraş-18 has the highest MUFA (%27.7), Bilecik, Chandler and Hartley walnut varieties have similarly high PUFA content (approximately %76). The PUFA values of the walnut grown in New Zeland found to be relatively similar to our results (64.9 - 76.6). In New Zeland, Zwarts *et al.* [38], analyzed the fatty acid content of walnut oil obtained with a cold press and reported that the total oil of walnut was ranged between 62.4% to 68.7%. Acording to another study, the walnut oil contained 21.2 g/100 g MUFA and 69.0 g/100 g PUFA [40]. The fruits of "Imshu", "Campbell CW1" (*Juglans ailanthifolia)* and "Combe" ve "Lake" (*Juglans regia* L.) varieties were analyzed for their composition in fatty acids and tocopherol. The same researches reported that polyunsaturated fatty acids showed the higher value (%73 - %80.98) than the other fatty acids [41].

According to the obtained results, Bilecik, Chandler, Hartley, Howard, Maraş 12, Maraş 18, Midland, Pedro, Şen and Serr walnut cultivars have very important values in terms of tocopherols, total phenol, and fat and fatty acids content which are very importance for human health and nutrition. Finally, the fatty acid results indicated that walnut cultivars from East Anatolia were distinctive in terms of their linolenic acid profile. In our study, "Hartley" was the highest both linoleic acid and γ-tocopherol content (64.56% and 266.38 μg/g), "Howard" has the highest linolenic acid content (13.26%). Obtained results might be significant for further breeding studies in order to improve higher health related compounds such as γ-tocopherol and linoleic acids and total phenolic compounds.

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