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Original Article

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Fat, Fatty Acids and Tocopherol Content of Several Walnut Genotypes

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Abstract

There are seed propagated walnut (*Juglans regia* L.) populations with the vast genetic variation in different part of Turkey. There are also lots of monoecious and dichogamous genotypes in Turkey due to continuing sexual propagation. In this study, fruits of 19 selected walnut genotypes grown in Kahramanmaraş region were characterized based on their fat, fatty acid and tocopherol contents. The fatty acids content of genotypes were analyzed using Gas Chromatography. Tocopherol analyses such as alpha (α)-Tocopherol, gamma (γ) and beta (β) + delta (δ) were performed by HPLC technique. According to the total fat and fatty acid results, there were differences among genotypes on most of the fatty acids. Total fat ranged from 51.2 to 82.1%, stearic acid from 2.57 to 3.37%, myristic acid from 0.00 to 0.05%, palmitic acid from 6.42 to 7.92%, arachidic acid from 0.00 to 0.16%, linoleic acid from 53.23 to 63.62%, linolenic acid from 10.75 to 15.24%, oleic acid from 14.73 to 24.17% and palmitoleic acid from 0.00 to 0.16%, respectively. The same genotypes were evaluated based on their tocopherol content and (α)-Tocopherol, gamma (γ) and beta (β) + delta (δ) tocopherol were found between 23.47 and 38.04 µg/g, 161.09 and 292.56 µg/g and 16.93 and 32.34 µg/g, respectively.

Keywords: fat, fatty acids, *Juglans regia*, kernels, tocopherol

Introduction

Fruit trees are familiar to a wide cross-section of human society, both as a common food and for their spiritual importance. Fruits have been used by people for food, either as edible products, or for culinary ingredients. Fruits are natural sources of vitamins, phytochemicals and minerals (Sahin *et al*., 2002; Benjak *et al*., 2005; Celik *et al*., 2007; Ercisli, 2009; Rop *et al*., 2014; Canan *et al*., 2016; Zorenc *et al*., 2016).

Anatolia represents also a germplasm center of walnut and walnut trees are exceptionally abundant within almost all regions in Turkey. Walnut trees are cultivated in Turkey mainly for their nutritious nuts, which are used as a food, in the chocolate industry, for baked foods, as well as in the pharmaceutical and cosmetic industry. The trees are also valuable as timber (Ercisli *et al*., 2012). Turkey is one of the main walnut producers in the world with annually 183.000 tons of walnut production and ranking 4th place after China (1.655.000 tons), Iran (485.000 tons) and USA (418.000 tons) (FAO, 2012). In recent years, the demand for walnut as a species has increased because of its nutritional values. Furthermore, besides its distinctive ability to adapt to different ecological conditions, the incentives for walnut growing around the world resulted in a remarkable increase in this field (Sütyemez and Kaşka, 2002).

The three leading categories necessary for human nutrition are fats, proteins and carbohydrates. The human body heavily relies on a balanced diet rich in basic nutritional elements for leading and persevering a healthy life. It is of utmost importance to identify the composition of walnuts and their nutritional values in order to reveal its position in a balanced diet (Beyhan *et al*., 1995).

Kernel of a walnut fruit can be considered as a type of concentrated food due to its high rate of fat and protein. In other words, as a hard-shelled fruit, walnut is an excellent untouched and wrapped "food store". It abounds in two out of three basic nutritional elements, which are necessary for humans. Furthermore, it can be kept without losing any nutritional value under relatively bad conditions for more than one year (Şen, 2011; Sutyemez and Kaska, 2005).

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When consumed in necessary amounts, walnut can contribute to daily diets thanks to its specific compounds such as polyunsaturated fatty acids, and possesses functional importance for medical biochemistry and physiology. Walnut contains high amounts of fat. Main elements of the fat are triglycerides, which abound in monounsaturated (mainly oleic acid) and polyunsaturated fatty acids (linoleic and α- linoleic acids). In addition, the presence of other bioactive components such as phenols, tocopherols and phytosterols is also known (Martinez *et al*., 2006). It is reported that the consumption of highly concentrated walnut and walnut oil with natural antioxidants can protect human health against certain types of cancer and that it also reduces the risks of cardiovascular diseases (Miraliakbari and Shahidi, 2008; Yang *et al*., 2009). Fatty acids in the walnut oil are mainly unsaturated. When compared to other hard-shelled fruits containing mainly monounsaturated fatty acids (MUFA), walnut is quite rich in terms of polyunsaturated fatty acids (PUFA) such as Omega-6 and Omega-3. Polyunsaturated fatty acids (PUFA) are critical for human nutrition (Amaral *et al*., 2003; Zwarts *et al*., 1999). Walnut has always been consumed throughout the history thanks to its high nutritional value. It is a suitable fruit for healthy diets because it contains fat (50-80%), protein (12-15%), mineral compound (3%), carbohydrate, vitamin as well as amino acids and low sugar level (2.5-4%) (Mitrovic *et al*., 1997). The fat ratio and fatty acid profile of walnut varies between cultivars and genotypes. It is important to identify these differences in locally grown genotypes and to identify which fatty acids give the best nutritional qualities (Greve *et al*., 1992; Zwarts *et al*., 1999).

In this study, some promising selected walnut genotypes in Kahramanmaraş region were examined in terms of fat, fatty acid and tocopherol contents in their fruits. Previously only few studies, based on a very limited number of seed propagated genotypes, have been reported based on their tocopherol and fatty acid composition in detail (Greve *et al*., 1992; Zwarts *et al*., 1999). The current study was carried out to bring new information on some fat, fatty acid and tocopherol contents of 19 walnut genotypes from Turkey.

Materials and Methods

Plant material

Mature walnut fruits of walnut genotypes were harvested from Research and Experimental Implementation area of University of Kahramanmaraş Sütçü İmam, Faculty of Agriculture, located in the Kahramanmaraş provinces of Turkey in November 2015. Fruit samples of 19 walnut genotypes were randomly selected with three replicates. Kernel of walnut fruits were analysed after drying under incubator at 30 °C for 24 hours.

Oil extraction

Oil extraction was performed based on the method of Bligh and Dyer (1959). Oils of 20 g fruits were extracted using hexane solvent for 1h using automatic Soxhlet equipment (Gerhardt Soxtherm) and triplicate analysis were reported for each variety. The residue was dried until constant weight was observed. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAMEs) (AOAC, 1990).

Fatty acids analysis

Fatty acids were analysed using a Clarus 500 Gas Chromatography with an auto sampler (Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector and a fused-silicacapillary SGE column (100 m × 0.32 mm, ID 0.25 μm, BP20 0.25 UM; Perkin Elmer, Austin, TX, USA). The oven temperature was held at 140 °C for 5 min, and then raised to 200 °C at a rate of 4 °C min^{-1} and then to 220 °C at a rate of 1 °C min^{-1}, while the injector and the detector temperatures were set to 220 and 280 °C respectively. The sample volume was 1 μL, and the carrier gas was controlled at 16 psi. The split ratio was 1: 100. Fatty acids were detected by comparing there tentionindices of the FAMEs with a standard 37- component FAME mixture (Supelco, Bellefonte, PA, USA).

Tocopherols

For each replicate, approximately 1 g of fruit from each of the genotypes was weighed. Tocopherol analyses α-Tocopherol, gamma (γ) and beta (β) + delta (δ) were performed by HPLC technique according to the method developed by Surai *et al*., (1996) and Surai (2000). In the analyses, 3 μm C18 recersed-phase column was used (15 cm x 4.6 mm, Spherisorb ODS2, Phase Separation, Clwyd, UK) and mobile phase was methanol/water (97:3 v/v; 1.05 ml/minute). Alpha, beta + delta, and gamma tocopherols were determined using this method. Excitation 325 nm and emission 490 nm retinol in the first 5 minutes were followed by excitation 295 nm and emission 330 nm with fluorescence detector (Surai *et al*., 1996; Surai, 2000).

Results and Discussion

In this study, walnut genotypes grown in Kahramanmaraş region were characterized in terms of total fat, saturated and unsaturated fatty acids and tocopherol contents. The genotypes were analyzed in terms of stearic acid, myristic acid, palmitic acid and arachidic acid, which are saturated fatty acids as well.

The results of total fat ratio of the genotypes grown in Kahramanmaraş were presented in Table 1. Total fat contents of the genotypes ranged from 51.2% to 82.1%. When Table 1 is examined it is seen that the lowest total fat level (51.2%) was observed in genotype 8, it was highest (82.1%) in genotype 9 among the 19 genotypes investigated in the present study. Fat content of our genotypes in general were between limits of the other studies and even higher than the other studies examined before. Portuguese walnuts contained total fat from 62.3 to 72.14% (Pereira *et al*., 2008). In the other study, where the chemical composition (total fat content, fatty acids, triacylglycerols (TAGs) and polar compounds) of six walnuts (*Juglans regia* L.) cultivars (Lauzeronne, Franquette, Hartley, Local pt, Local gd and Parisienne) collected from Mateur (north of Tunisia) was evaluated and the results revealed that Local pt variety had the highest oil amount (62.56%). These differences could be resulted from the harvesting year, environmental conditions, temperature variation, rainfall and light, which can influence the chemical composition of the fruit (Bouabdallah *et al*., 2014).

Table 1 showed the saturated fatty acid contents of the samples. As can be seen, stearic acid concentrations changed between 2.57% and 3.37%, those for myristic acids, palmitic

Table 1. Total fat and saturated fatty acids content (%) of walnut genotypes

Genotypes	Total fat (%)	Saturated fatty acids (%)			
		Stearic acid	Myristic acid	Palmitic acid	Arachidic acid
1	80.3	2.96 ± 0.09	0.02 ± 0.01	7.40 ± 0.08	0.14 ± 0.01
2	66.9	2.97 ± 0.01	0.03 ± 0.00	7.67 ± 0.30	0.14 ± 0.00
3	65.9	2.82 ± 0.04	0.04 ± 0.01	7.92 ± 0.06	0.00 ± 0.01
4	69.7	2.82 ± 0.41	0.04 ± 0.01	7.92 ± 0.01	0.00 ± 0.01
5	58.7	2.97 ± 0.16	0.03 ± 0.01	6.98 ± 0.08	0.00 ± 0.01
6	77.4	2.86 ± 0.02	0.03 ± 0.04	6.77 ± 0.01	0.00 ± 0.04
7	73.9	2.57 ± 0.25	0.00 ± 0.00	7.59 ± 0.05	0.00 ± 0.00
8	51.2	3.37 ± 0.07	0.05 ± 0.02	7.16 ± 0.13	0.15 ± 0.02
9	82.1	3.21 ± 0.02	0.03 ± 0.00	7.24 ± 0.13	0.00 ± 0.00
10	69.7	2.84 ± 0.01	0.03 ± 0.01	7.60 ± 0.06	0.00 ± 0.01
11	55.0	2.87 ± 0.10	0.03 ± 0.01	6.42 ± 0.03	0.00 ± 0.01
12	61.0	2.68 ± 0.05	0.04 ± 0.00	7.18 ± 0.66	0.16 ± 0.00
13	81.8	2.89 ± 0.06	0.03 ± 0.00	6.62 ± 0.06	0.00 ± 0.00
14	57.6	3.14 ± 0.11	0.03 ± 0.00	7.09 ± 0.11	0.00 ± 0.00
15	68.3	3.05 ± 0.28	0.00 ± 0.00	6.77 ± 0.06	0.00 ± 0.00
16	58.7	3.31 ± 0.03	0.04 ± 0.00	6.76 ± 0.05	0.01 ± 0.00
17	66.2	3.02 ± 0.03	0.04 ± 0.01	7.60 ± 0.18	0.14 ± 0.01
18	66.6	2.66 ± 0.01	0.03 ± 0.00	7.41 ± 0.01	0.00 ± 0.00
19	68.2	2.73 ± 0.01	0.03 ± 0.01	6.84 ± 0.04	0.00 ± 0.01

Table 2. Unsaturated fatty acids content (%) of walnut cultivar

Genotypes	Unsaturated fatty acids (%)			
	Polyunsaturated fatty acids		Monounsaturated fatty acids	
	Linoleic acid	Linolenic acid	Oleic acid	Palmitoleic acid
1	59.40 ± 0.05	11.45 ± 0.01	18.36 ± 0.16	0.13 ± 0.00
2	61.94 ± 0.06	11.06 ± 0.70	16.01 ± 0.01	0.15 ± 0.01
3	55.88 ± 0.06	15.24 ± 0.05	17.51 ± 0.01	0.14 ± 0.01
4	55.88 ± 0.72	15.24 ± 0.01	17.51 ± 0.02	0.14 ± 0.00
5	60.45 ± 1.07	12.69 ± 1.34	16.58 ± 5.73	0.14 ± 0.00
6	57.28 ± 0.31	12.57 ± 0.16	19.62 ± 0.01	0.13 ± 0.02
7	56.52 ± 0.02	12.63 ± 0.08	19.59 ± 0.09	0.15 ± 0.00
8	57.22 ± 0.34	11.71 ± 0.11	19.29 ± 0.02	0.14 ± 0.03
9	55.82 ± 0.02	13.44 ± 0.11	19.91 ± 0.03	0.12 ± 0.01
10	57.80 ± 0.37	13.66 ± 0.01	17.91 ± 0.28	0.10 ± 0.00
11	58.52 ± 0.46	12.22 ± 0.04	19.75 ± 0.00	0.13 ± 0.01
12	57.38 ± 0.28	12.89 ± 0.23	19.42 ± 0.12	0.11 ± 0.00
13	63.62 ± 0.21	11.48 ± 0.06	15.07 ± 0.05	0.15 ± 0.01
14	58.78 ± 0.01	10.75 ± 0.04	19.98 ± 0.02	0.15 ± 0.01
15	57.27 ± 1.24	12.06 ± 0.91	20.16 ± 0.25	0.00 ± 0.00
16	62.32 ± 0.10	11.55 ± 0.03	15.39 ± 0.01	0.16 ± 0.01
17	61.45 ± 0.10	12.21 ± 0.02	15.21 ± 6.36	0.14 ± 0.01
18	60.96 ± 0.09	13.13 ± 0.48	14.73 ± 0.06	0.15 ± 0.01
19	53.23 ± 3.60	11.92 ± 0.25	24.17 ± 0.01	0.12 ± 0.04

acids and arachidic acids were 0.00-0.05%, 6.42-7.92% and 0.00-0.16%, respectively. One of recent selection study conducted in Turkey, 67 seed propagated walnut trees were analyzed in terms of fatty acids composition in fruits and palmitic acid, myristic acid and stearic acid contents of the walnuts varied between 5.20-7.29%, 0.01-0.02%, and 1.69-2.55%, respectively (Unver *et al*., 2016). Concerning saturated fatty acids (SFA), palmitic acid (C16:0) was the major fatty acid in walnut oil and its content ranged between 7.28% and 8.95% depending on the varieties. Stearic acid (C18:0) ranged from 3.01% to 3.89% (Bouabdallah *et al*., 2014). These values are consistent with the findings of the present study. As can be understood walnuts are generally poor in saturated fatty acids. Recent studies have emphasized the importance of monounsaturated fatty acids in reducing saturated-fat intake (Mensink and Katan, 1990). Walnuts are rich in polyunsaturated fatty acids and monounsaturated fatty acids. Monounsaturated fatty acids (oleic acids and palmitoleic acids) values ranged from 14.73% to 24.17% and from 0.00% to 0.16%., respectively. The highest value of oleic acid was detected in genotype 19 and the highest value of palmitoleic acid was in genotype 16. However genotype 18 had the lowest oleic acid (14.73%) and genotype 15 had the lowest palmitoleic acid (0.00%) (Table 2).

The chemical composition (total oil content, fatty acids) of six walnuts (*Juglans regia* L.) cultivars ('Lauzeronne', 'Franquette', 'Hartley', 'Local pt', 'Local gd' and 'Parisienne') collected from Mateur (north of Tunisia) was evaluated. Oleic acid contents of these cultivars were found 19.94%, 16.73%, 14.27%, 13.21%, 13.68%, and 16.49% (Bouabdallah *et al*., 2014). In southern Turkey, 10 promising walnut selections were examined fatty acid content and oleic acid was determined to be the second most abundant fatty acid, which changed between 20.7% and 28.33% (Simsek, 2016). In Spain, the proximate and mineral composition, fatty acid profile, total polyphenol, melatonin and serotonin contents were assessed in four walnut (*Juglans regia* L.) cultivars (cv. 'Serr', 'Hartley', 'Chandler' and 'Howard') to decide which cultivar is the most

Table 3. Tocopherol and its isomers contents of various walnut varieties grown in Kahramanmaraş ecological condition

Genotypes	α-tocopherol (ug/g)	Gamma-tocopherol (ug/g)	Beta+Delta-tocopherol (ug/g)
1	30.16 ± 0.41	253.91 ± 3.81	28.63 ± 0.36
2	33.81 ± 0.59	200.17 ± 4.48	25.58 ± 0.52
3	36.00 ± 1.21	187.87 ± 5.68	18.99 ± 0.62
4	24.11 ± 1.28	196.13 ± 6.34	29.32 ± 0.60
5	25.64 ± 0.37	161.09 ± 6.43	17.93 ± 0.75
6	26.40 ± 0.02	175.52 ± 3.36	16.93 ± 0.33
7	31.04 ± 1.19	259.94 ± 6.15	21.86 ± 0.63
8	37.52 ± 4.01	292.56 ± 1.06	31.49 ± 1.35
9	33.24 ± 1.65	230.75 ± 6.26	23.27 ± 0.87
10	36.06 ± 1.30	213.82 ± 6.08	19.72 ± 0.35
11	31.06 ± 1.01	282.25 ± 5.82	32.34 ± 0.49
12	24.13 ± 1.08	219.95 ± 5.63	31.16 ± 0.74
13	30.38 ± 0.06	199.33 ± 0.52	22.66 ± 0.22
14	27.90 ± 0.62	232.89 ± 2.59	22.84 ± 0.24
15	23.47 ± 0.55	190.71 ± 4.67	25.51 ± 0.43
16	30.70 ± 0.01	205.33 ± 4.27	17.69 ± 0.52
17	24.81 ± 1.00	214.70 ± 3.88	24.99 ± 0.83
18	38.04 ± 0.72	215.23 ± 4.94	28.68 ± 0.81
19	29.07 ± 0.94	218.69 ± 7.04	19.65 ± 0.42

suitable considering nutritional and commercial aspects. As a result of the study for 'Serr', 'Hartley', 'Chandler' and 'Howard' cultivars, monosaturated fatty acid concentration was found to be 17.8%, 16.6%, 16.2% and 13.3%, respectively (Tapia *et al*., 2013). Also in our research polyunsaturated fatty acids (PUFAs) were analyzed as linoleic and linolenic acid among to the genotypes. The highest linoleic acid was observed in genotype 13 as 63.62% and also the highest linolenic acid was in 3 and 4 genotype as 15.24% (Table 2). In a recent study, where the fatty acid content and antiradical activity of different walnut (*Juglans regia* L.) genotypes grown in Kolyaei region located in Kermanshah Province (Iran) were investigated and it was reported that linoleic acid and linolenic acid contents of the walnut genotypes ranged from 46.9% to 56.8%, and from 10.8% to 13.9% respectively. Also it is stated that the differences might have resulted from ecological, nutrition and genetically factors. The variation in the fatty acid composition of the nuts from different genotypes may affect the end use of the product (Akbari *et al*., 2015). A study about the determination of physical and biochemical characteristics of 25 seed propagated walnut genotypes grown in Çal (Denizli) region in western Turkey, saturated fatty acids; palmitic acid were between 4.78 and 8.62%, stearic acid 1.95 and 3.53%, unsaturated fatty acids; oleic acid 13.38 and 30.97%, linoleic acid 47.38 and 65.98% and linolenic acid 7.10 and 13.94% (Yarılgac and Yılmaz, 2016).

In the present study, α-Tocopherol, (γ) and (β) + (δ) tocopherol contents of these genotypes were determined and the corresponding results are given in Table 3. The lowest value of α-Tocopherol contents was detected in the genotype 15 with 23.47 ug/g and the highest value was in the genotype 18 with 38.04 ug/g. Gamma (γ) tocopherol is the major isomer of tocopherols present in walnut.

(γ) tocopherol content ranged from 161.09 (genotype 5) to 292.56 (genotype 8) ug/g. (β) + (δ) tocopherol isomers were also analysed in HPLC and the highest value of these tocopherol isomers were found in genotype 11 as 32.34%.

In a research conducted on six walnut (*Juglans regia* L.) varieties, the higher values of γ- tocopherol were detected in 'Lauzeronne' and 'Franquette' varieties (358.95 and 238.45 mg/kg, respectively), and the lowest value was detected in 'Local gd' variety (162.54 mg/kg). δ- Tocopherol was also determined in high levels with values ranging from 16.76 mg/kg (6.19%), in the 'Local pt' variety, to 44.73 mg/kg (15.79%), in the 'Parisienne' variety. While α-tocopherol varied between 1.93 mg/kg (1.03%), in 'Local gd' variety, and 12.81 mg/kg (2.93%) in 'Lauzeronne' variety. β- Tocopherol showed the lower levels of the detected to tocopherols (0.31-3.24 mg/kg) (Abdallah *et al*., 2015). In the other study, it was reported that γ- tocopherol is more potent than α- tocopherol in terms of decreasing platelet aggregation, LDL oxidation, and delaying intra-arterial thrombus formation (Li *et al*., 1999).

Conclusions

Kahramanmaraş is an important region considering walnut production in Turkey. This study highlighted the importance of walnut with several beneficial compounds involving tocopherol isomers and fatty acids. (γ) tocopherol was the predominant tocopherol isomers compound in all genotypes and this isomer is very important because recent studies indicated that γ- tocopherol may be important to human health and it possesses unique features that distinguish it from α- tocopherol. Also according to both our research and recent studies, among fatty acids linoleic acid is the major fatty acids present in walnut, followed by oleic acid and linolenic acid. These unsaturated fatty acids are the most essential fatty acids in terms of health. They are called essential fatty acids because they are not synthesizing in the body and therefore, it is required to be taken them with food. These parameters important for human health and nutrition should be taken into account when obtaining the appropriate field type for the eastern Mediterranean region. Because in recent years the priority in selection of varieties has been predominantly based on fruit quality characteristics rather than fruit yield.

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