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Chemical, mineral, and fatty acid compositions of various types of walnut (*Juglans regia* L.) in Turkey

M. Simsek*

**Department of Horticulture, Faculty of Agriculture, Dicle University, 21100, Diyarbakir, Turkey.*

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The aim of the present study was to determine the chemical, fatty acid, and mineral compositions of 10 types of walnut (*Juglans regia* L.) selected during 2010 and 2012 from the Yeşilli (Mardin) district located in the Southeast Anatolia Region of Turkey. The average values of total oil, protein, carbohydrate, and energy were 61.08–64.8%, 14.85–20.26%, 13.77–17.16%, and 686.2–710.0 Kcal, respectively. In terms of mineral composition, K was the major mineral in all samples, ranging from 534.3 to 778.6 mg 100 g^{-1}. P was the next most abundant mineral, ranging from 346.0 to 584.8 mg 100 g^{-1}, followed by Ca and Mg, ranging from 100.9 to 233.9 mg 100 g^{-1} and from 117.8 to 181.4 mg 100 g^{-1}, respectively. Saturated fatty acids levels were lower than those of other types of fatty acids. Of the identified fatty acids, linoleic acid (50.24–60.60%) was the predominant fatty acid, followed by oleic acid (20.70–28.33%) and linolenic acid (10.93–15.04%) in all types of walnut. Other fatty acids were present at trace levels.

Keywords: Walnut, Chemical composition, Fatty acids, Minerals.

INTRODUCTION

Walnut (*Juglans regia* L.) is the oldest cultivated nut in the world and grows readily over almost all of Turkey [1]. This species grows naturally in almost all districts of Anatolia, which has a suitable climate and good geographic conditions. Particularly, very rich walnut populations grow in valleys and on hillslopes, and many walnut types have been selected from these populations. In Southeast Anatolia, the Yeşilli (Mardin) district features microclimatic conditions favouring the growth of various walnut types and cultivars. From the pre-agricultural era to the present day, walnuts and other nuts have been an important part of the human diet, providing micronutrients, macronutrients, and various bioactive constituents.

Walnut kernels contain approximately 60.00% oil. However, this can vary from 50 to 70%. The amount of kernel oil depends on the walnut type and cultivar, the growth location, environmental conditions, and irrigation regimes [2, 3]. Additionally, walnut kernels contain appreciable amounts of proteins (12–24%), carbohydrates (12.00–18.00%), and minerals (1.7–20%) [4-6]. Kernels have high levels of other beneficial components including minerals such as K (390–700 mg 100 g^{-1}) and P (310–510 mg 100 g^{-1}) and are low in Na (1–15 mg 100 g^{-1}) [7-9]. The fatty acids of kernel oil are primarily unsaturated. Compared with most other nuts, which contain principally monounsaturated fatty acids, walnut kernels are highly enriched in both omega-3 and omega-6 polyunsaturated fatty acids, which are essential dietary fatty acids [4]. It has been suggested that the high polyunsaturated fatty acid content of walnut kernels may reduce the risk of heart disease. The major fatty acids found in walnut kernels are linoleic, linolenic, oleic, palmitic, and stearic acid [10-12]. The fatty acid compositions of walnuts and other nuts are important in terms of their economic and nutritional values. In Anatolia, many studies have been conducted on the chemical and other properties of various walnut types and cultivars [13-30].

The present study was designed to evaluate the nuts of walnut types grown in the Yeşilli district in terms of chemical, fatty acid, and mineral compositions. The results will serve as a resource for breeders, growers, and nutritionists.

EXPERIMENTAL

Materials

Ten walnut types were selected from the Yeşilli (Mardin) district of the Southeast Anatolia region of Turkey during the years 2010 and 2012. All types (YE 3, YE 8, YE 15, YE 18, YE 23, YE 26, YE 30, YE 34, YE 39, and YE 48) were harvested in September of those years for determination of chemical, fatty acid, and mineral compositions. After harvest, all fruits were immediately dried and stored in their shells at room temperature until analysis.

* To whom all correspondence should be sent:
E-mail: mikdat.simsek@dicle.edu.tr

Methods

Chemical Composition

Dry matter content was determined by drying samples overnight in a hot-air oven at 105ºC. Moisture content was determined and calculated using the methods of the Turkish Standard Institute [31]. Total protein level was calculated by multiplying the nitrogen content, determined using the Kjeldahl method, by the coefficient 6.25 [32]. Oil content was determined by extraction of 10 g dried ground kernels (per replicate) with petroleum ether in a Soxhlet apparatus at 45–50°C for 8–9 h [3, 33]. Total ash was determined by drying the samples at 105°C for 1 day in an oven and then transferring the crucible to a muffle furnace. The temperature was gradually raised to 600°C, and the samples were ashed for 10–12 h until they were white in colour [32]. The formula used for calculation of carbohydrate content (%) was 100% – (moisture + protein + oil + ash) (%) [34]. The formula for calculation of energy (Kcal) was 9 × lipid (%) + 4 × (protein + carbohydrate) (%) [35]. All analyses were performed in triplicate on samples from each year. Finally, all data on mineral, chemical, and fatty acid compositions were subjected to analysis of variance using JMP 5.0.1. Means were compared using Tukey's test at the 0.05 alpha level.

Mineral Composition

P concentrations were spectrophotometrically determined using the vanado-molybdophosphoric yellow colour method. To determine the contents of other minerals, 1-g amounts of dried, ground, homogenised kernels were placed in platinum crucibles, partially dissolved in 2 mL HNO_3 (65%), and heated on a hot plate to dryness to prevent dry matter loss and black smoke development during ash formation. Next, each sample was heated in a muffle furnace at 550°C for 6 h. After a 10-min cooling period, the ash was dissolved in 2 mL HNO_3 (65%) and diluted with deionised water to a volume of 25 mL. A Unicam flame atomic absorption instrument was used to determine Na, Mg, K, Ca, Cu, S, Mn, Zn, and Fe levels. The tests were performed in triplicate on samples from each year, and values (mg 100 g^{-1}) are expressed on a dry-matter basis.

Fatty Acid Composition

Fatty acid composition was determined by gas chromatography. Oil samples obtained via Soxhlet extraction were converted to the corresponding methyl esters using the AOCS method [3, 36]. The BF_3/methanol method was used for methylation. Chromatographic analysis of fatty acid methyl esters was performed using a gas chromatograph equipped with a BPX70 Forte capillary column (0.25 μm × 0.32 mm × 60 m), a split injector, and a flame ionisation detector. The column temperature programme was 60°C for 2 min, then a rise at 30°C/min to 150°C, a rise at 1°C/min to 190°C, a rise at 20°C/min to 220°C, and 10 min at 220°C. The injector and detector temperatures were 225°C and 250°C, respectively. The carrier gas was nitrogen at a flow rate of 30 mL/min. The air and hydrogen flow rates were 350 mL/min and 35 mL/min, respectively. The peaks of fatty acids were identified by comparing retention times with those of members of a mixture of isomers of standard methyl esters. All analyses were performed in triplicate on samples from each year.

RESULTS AND DISCUSSION

Chemical Composition

The chemical compositions of all walnut types are shown in Table 1. The average levels of moisture, oil, protein, carbohydrate, and ash were 1.48–3.94%, 61.08–64.89%, 14.85–20.26%, 13.77–17.16%, and 1.20–1.93%, respectively. Oil was the major constituent, and ash and moisture were present at the lowest amounts. The values in this study are similar to those reported elsewhere [4, 37, 38]. The total oil content ranged from 62.3 to 66.5%, and the ash value from 1.8 to 2.1%, respectively, in one work [4], and from 51.6 to 67% and 1 to 2.5% in another [37]. In a further study, the total oil content ranged from 63.54 to 69.25%, ash content from 1.27 to 1.95%, and the moisture level from 2.76 to 4.20% [38]. The oil content measured here was lower than that reported in an earlier work [35], in which the total oil content ranged from 78.83 to 82.40%. Another previous study [3] reported carbohydrate and protein levels of various walnut genotypes in the range 9.05–18.92% and 10.58–18.19%, respectively, whereas in a further work [6], the carbohydrate and protein levels in walnuts of various genotypes were 8.05–13.23% and 15.17–19.24%, respectively. Results obtained are in partial agreement with the data in terms of protein content, but the carbohydrate values were higher than those of earlier work. The kernel energy values of the walnut types shown in Table 1 ranged from 686.2 to 710 Kcal, showing that kernels were rich sources of energy. An earlier study [38] reported that the energy values of walnut kernels from Pakistan were 698.1–732.4 Kcal. In Turkey, the energy values were 682–728 Kcal [3], similar to those reported elsewhere [4, 35, 38]. The

differences in energy levels are attributable to differences in the chemical compositions of various walnut types and cultivars and may vary with the year of harvest, environmental conditions, horticultural practices, and genetics.

Mineral Composition

The mineral compositions of the various walnut types are shown in Table 2. The average values for K, P, S, Ca, Mg, Na, Fe, Mn, Zn, and Cu (mg 100 g^{-1}) were 534.3–778.6, 346–584.8, 153.9–256.9, 100.9–233.9, 117.8–181.4, 8.67–19.29, 3.13–5.37, 2.02–4.50, 1.44 –3.63, and 0.77–2.44, respectively. The order of mineral levels was K > P > S > Ca > Mg > Na > Fe > Mn > Zn > Cu. Earlier [39], it was found that the average values of K, P, Ca, Mg, S, Cu, Fe, Mn, Zn, and Na in various walnut genotypes and cultivars, in mg 100 g^{-1}, were 285.9–482.8, 206–401.5, 85.4–184.3, 85.4–184.3, 130.2–220.7, 0.48–1.81, 1.16–3.96, 1.52–5.03, 1.42–2.79, and 0.84–2.67, respectively. In another study [39], the mineral level order was K > P > S > Ca > Mg > Mn > Fe > Zn > Cu. One work [3] showed that the average values for K, Ca, Mg, Cu, Fe, Mn, Zn, and Na of various walnut genotypes and cultivars, in mg 100 g^{-1}, were 359.7–483, 109.5–336, 126–165, 0.92–1.8, 2.78–4.85, 1.52–4.79, 2.45–4.3, and 2.45–9.99, respectively. In the present work, high levels of major minerals (K, Ca, P, S, and Mg) were found, but the levels of minor minerals (Na, Fe, Mn, Zn, and Cu) were low. The K content of walnut kernels varied considerably and was significantly higher than those in earlier works [3, 39]. The Na values were considerably higher than those in a prior work [39]. Calcium values in this study were also considerably higher than values reported previously [8]. It is well known that the elemental composition of soil greatly influences mineral absorption by the walnut and other nuts. In general, acidic soils enhance Mn and Cu absorption, and chalky soils lower iron absorption. Thus, the elemental compositions of walnut kernels can be influenced by the walnut type and cultivar as well as by differences in environments and growth conditions.

Fatty Acid Composition

The fatty acid compositions of various walnut types are shown in Table 3. The major fatty acids were linoleic acid, followed by oleic and linolenic acid. Linoleic acid was the most abundant fatty acid in all walnut types analysed, ranging from 50.24 to 60.6%. Oleic acid, the second most abundant fatty acid, ranged from 20.7 to 28.33%, followed by linolenic acid (5.04 to 10.93%). Of the remaining fatty acids, only palmitic and stearic acids were present in appreciable amounts, ranging from 1.8 to 5.53% and 1.17 to 2.22%, respectively. The overall fatty acid composition was 4–7.86% saturated fatty acids (SFA), 22.17–29.73% monounsaturated fatty acids (MUFA), and 62.73–71.43% polyunsaturated fatty acids (PUFA). Additionally, the total PUFA/total SFA ratio ranged from 8.14 to 17.11. A previous study [3] determined that the PUFA/MUFA ratio varied from 1.54 to 3.97.

Another report [40] found that this ratio varied from 2.22 to 4.54 in the walnut cultivars Franquette, Chandler, and Criolla. A previous study [27] showed that the fatty acid compositions of walnut genotypes was 5.81–9.23% SFA (a minor constituent), 15.13–29.97% MUFA, and 62.85–78.15% PUFA; these were the principal groups of fatty acids in walnut oils extracted from the genotypes studied. Additionally, one report [27] found that that the major PUFA was linoleic acid in all walnut genotypes, with the amount varying from 50.58 to 66.60%. The other PUFA, linolenic acid, ranged from 9.12 to 16.42%. Oleic acid was the second commonest primary MUFA among the genotypes studied, ranging from 14.88 to 28.71%, followed by palmitoleic (0.14–1.69%) and gadoleic (0.0–0.16%) acids. In general, the results in this study were in agreement with earlier data [3, 10-12, 27, 35]. The fatty acid composition of walnut kernels is affected by walnut type, cultivar, fertilisers applied during growth, geographical location, treatment, and climatic and soil conditions. Additionally, oil composition is affected by the maturity of seed at harvest, seed position on the tree, and seed handling after harvest [41].

Table 1. Chemical composition of walnut (*J. regia* L.) types.

Chemical Properties	Walnut Types and Values									
	YE 3	YE 8	YE 15	YE 18	YE 23	YE 26	YE 30	YE 34	YE 39	YE 48
Dry matter (%)	96.47 b	96.55 b	98.52 a	96.06 b	96.77 b	96.46 b	97.13 b	96.99 b	96.79 b	96.63 b
Moisture	3.52 a	3.45 a	1.48 b	3.94 a	3.23 a	3.54 a	2.87 a	3.01 a	3.21 a	3.37 a
Total Oil (%)	62.77 cd	61.53 d	64.42 abc	64.85 ab	64.31 abc	62.87 bcd	64.82 ab	61.08 d	62.15 d	64.89 a
Protein (%)	15.35 de	18.80 b	17.70 bc	15.21 de	15.47 de	16.18 de	16.62 cd	20.26 a	19.07 ab	14.85 e
Carbohydrate (%)	17.16 a	14.29 b	14.86 b	14.57 b	15.48 ab	15.70 ab	14.18 b	14.18 b	13.77 b	15.23 ab
Ash (%)	1.20 a	1.93 a	1.88 a	1.44 a	1.52 a	1.71 a	1.51 a	1.47 a	1.80 a	1.65 a
Energy	694.7 bcd	686.2 d	710.0 a	702.7 abc	702.6 abc	693.3 bcd	706.6	687.5 d	690.7 cd	704.4 abc

Significantly different means (at the 5% level), determined using JMP 5.0.1 to run Tukey's test, are shown with different letters.

Table 2. Some mineral compositions of the walnut (*J. regia* L.) types (mg100 g^{-1}).

Minerals	Walnut Types and Values									
	YE 3	YE 8	YE 15	YE 18	YE 23	YE 26	YE 30	YE 34	YE 39	YE 48
K	589.7 b	590.7 b	778.6 a	714.0 a	743.7 a	725.0 a	753.0 a	534.3 b	542.7 b	712.7 a
P	367.0 cd	400.0 c	519.7 b	505.9 b	584.8 a	536.7 b	513.3 b	346.0 d	373.5 cd	524.6 b
Mg	117.8 e	147.6 bcd	152.3 b	142.8 bcd	148.7 bc	18.4 a	148.9 bc	131.8 de	135.5 cd	177.3 a
Ca	152.5 d	100.9 f	219.8 ab	102.4 f	197.4 c	233.9 a	216.6 abc	145.2 d	124.0 e	212.2 bc
S	153.9 f	188.7 e	230.9 b	196.2 de	224.4 bc	256.9 a	236.2bc	185.2 e	201.9 cde	216.2 bcd
Cu	1.74 c	1.22 de	0.77 f	1.05 de	1.34 d	2.06 b	1.66 c	0.77 f	1.15 de	2.44 a
Fe	3.36 f	4.31 cd	3.85 de	4.32 cd	4.98 ab	5.13 a	4.61 bc	3.13 f	3.55 ef	5.37 a
Mn	2.83 cd	2.98 cd	2.02 e	2.80 d	2.95 cd	3.92 b	3.31 c	2.03 e	2.71 d	4.50 a
Zn	2.53 c	1.92 de	1.46 f	1.88 e	2.17 d	2.98 b	2.53 c	1.44 f	1.88 e	3.63 a
Na	10.13 f	9.37 fg	12.03 e	8.67 g	9.47 fg	16.06 c	19.29 a	17.51 b	9.19 g	13.76 d

Significantly different means (at the 5% level), determined using JMP 5.0.1 to run Tukey's test, are shown with different letters.

Table 3. Fatty acid composition of walnut (*J. regia* L.) types.

Fatty acids	Walnut Types and Values									
	YE 3	YE 8	YE 15	YE 18	YE 23	YE 26	YE 30	YE 34	YE 39	YE 48
Palmitoleic acid (16:1)	0.63 g	1.37 a	0.76 ef	1.20 b	0.95 cd	0.77 e	0.67 fg	1.02 c	1.23 b	0.88 d
Oleic acid (C18:1)	21.41 de	20.70 e	28.33 a	26.62 ab	23.39 cd	27.61 a	22.03 de	24.94 bc	25.54 b	26.81 ab
Gadoleic acid (C20:1)	0.13 a	0.11 a	0.64 a	0.15 a	0.17 a	0.15 a	0.14 a	0.09 a	0.15 a	0.36 a
Total MUFA	22.17 f	22.18 f	29.73 a	27.97 ab	24.51 de	28.53 ab	22.83 ef	26.05 cd	26.92 bc	28.04 ab
Linoleic acid (C18:2)	60.60 a	57.62 b	50.24 f	54.78 cd	56.16 bc	52.05 ef	57.05 b	56.60 bc	55.74 bc	53.20 de
Linolenic acid (C18:3)	11.49 bc	15.04 a	12.49 b	10.93 c	12.09 b	11.55 bc	14.25 a	11.92 bc	11.96 bc	14.86 a
Total PUFA	71.43 a	70.93 ab	62.73 f	65.71 de	68.25 c	63.61 ef	71.30 a	68.53 bc	67.70 cd	68.06 cd
Myristic acid (C14:0)	0.06 h	0.14 f	0.23 d	0.51 b	0.32 c	0.62 a	0.23 d	0.18 e	0.04 h	0.08 g
Palmitic acid (C16:0)	4.26 a	3.96 a	4.74 a	3.54 ab	4.26 a	4.74 a	3.76 ab	3.40 ab	2.49 bc	1.80 c
Stearic acid (C18:0)	2.22 a	1.77 b	1.64 bc	1.37 cde	1.47 cd	1.21 de	1.37 cde	1.27 de	1.82 b	1.17 e
Arachidic acid (C20:0)	0.08 d	1.23 a	0.69 c	0.98 b	1.29 a	1.29 a	0.74 c	0.62 c	1.08 b	0.95 b
Total SFA	6.52 abc	7.09 ab	7.29 ab	6.39 bc	7.31 ab	7.86 a	6.05 bc	5.46 c	5.44 c	4.00 d
Total PUFA/Total SFA	11.09 bc	10.05 bc	8.62 c	10.31 bc	9.39 bc	8.14 c	11.20 bc	12.61 b	11.26 bc	17.11 a

Significantly different means (at the 5% level), determined using JMP 5.0.1 to run Tukey's test, are shown with different letters.

CONCLUSIONS

The walnut types studied exhibited nutritionally promising levels of major minerals and had higher carbohydrate contents than cultivars earlier described in the literature. In the current study, some walnut types contained higher amounts of oleic, linoleic, and linolenic acids than did others. Walnut kernels have high levels of omega-3 and -6 (essential dietary) fatty acids. Many clinical studies have suggested that omega-3 fatty acids may play significant roles in prevention of coronary heart disease and have shown that inclusion of walnuts in the diet afforded significant protective benefits in terms of both fatal and nonfatal coronary heart disease events [42]. The data confirm that walnut kernels are a rich source of significant nutrients that would be very beneficial to human health.

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ХИМИЧЕН, МИНЕРАЛЕН СЪСТАВ И СЪДЪРЖАНИЕ НА МАСТНИ КИСЕЛИНИ В РАЗЛИЧНИ ВИДОВЕ ЛЕШНИЦИ (*Juglans regia* L.) В ТУРЦИЯ

М. Симсек*

**Департамент по хортикултури, Агрономически факултет, Университет Дикле, Диарбекир, Турция*.

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(Резюме)

Цел на настоящата работа е определянето на химичния, минералния състав и на съдържанието на мастни киселини в 10 вида лешници (*Juglans regia* L.), избрани през 2010 и 2012 г. от района Йешили (Мардин), разположен в югоизточния регион в Мала Азия. Средните стойности на общите масла, протеини въглехидрати и енергийно съдържание са съответно 61.08–64.8%, 14.85–20.26%, 13.77–17.16%, и 686.2–710.0 Kcal. По отношение на минералния състав калият е главният минерален компонент. От минералните съставки калият е главния елемент във всички проби в границите от 534.3 до 778.6 mg 100 g^{-1}. Фосфорът е следващият разпространен елемент в границите от 346.0 до 584.8 mg 100 g^{-1}, следван от калция и магнезия (съответно от 100.9 до 233.9 mg 100 g^{-1} и от 117.8 до 181.4 mg 100 g^{-1}). Нивото на наситените мастни киселини е по-ниско от това на другите мастни киселини. От установените, линоловата (50.24–60.60%) е преобладаващата, следвана от олеиновата (20.70–28.33%) и линоленовата киселина (10.93–15.04%) за всички проби от лешници. Другите мастни киселини са в количества като следи.