Determination of Fatty Acids, α-Tocopherol, β-Caroten, Minerals, and Some Pomological Properties of Walnut Genotypes Selected from Aras Valley (Eastern Turkey)

Gülsoy, Ersin*[+]; Pehluvan, Mücahit
Department of Horticulture, Faculty of Agriculture, Igdir University, 76000, TURKEY

Şimşek, Mikdat
Department of Horticulture, Faculty of Agriculture, Dicle University, 21280, TURKEY

ABSTRACT: *Fatty Acids, α-tocopherol, β-caroten, minerals, and some pomological properties of walnut genotypes from four locations (Iğdır, Tuzluca, Karakoyunlu, Kağızman) of Aras Valley (Eastern Turkey) were examined. In this study, the differences among the genotypes were determined in terms of some fruit characteristics, oil and protein contents, fatty acid composition, selenium content, α-tocopherol content, β-carotene content and some macro- and micro- element contents. The genotypes different in the values of nut weight from 8.89 to16.22 g, kernel weight from 4.72 to 9.64 g, kernel ratio from 36.74 to 59.59%, and shell thickness 1.04-3.60 mm, respectively. The contents of fat and moisture of the selected genotypes were in the range of 59.18 to 68.12% and 10.49 to 23.31%, respectively. The contents of most common fatty acids determined in the genotypes were linoleic acid (58.15-64.07%), oleic acid (12.93-17.49%), linolenic acid (9.37-13.61%), palmitic acid (5.60-8.62%) and stearic acid (4.68-6.69%), whereas the contents of remaining fatty acids were rarely detected in trace amounts. In the genotypes, the amount of α-tocopherol was in the range of 8.75 to 35.11 mg/kg, β-carotene was in the range of 0.03 to 0.12 mg /kg and selenium was in the range of 15.89 to 68.19 ng/g. The genotypes were found to have 1.09 to 2.47% N, 230.36 to 451.48 mg/100g P, 350.74 to 666.20 mg/100g K, 2.30 to 3.86 mg/100g Cu, 1.71 to 3.91 mg/100g Zn, 7.16 to 18.82 mg/100g Fe, 144.0 to 452.08 mg/100g Ca, 110.25 to 342.44 mg/100g Mg, 1.73 to 9.67 mg/100g Mn and 7.11 to 25.51 mg/100g Na. The present results revealed that the high nutritional values of walnut genotypes selected from the Aras Valley(Eastern Turkey) could have health benefits effects on human nutrition.*

KEYWORDS: *Walnut; Fatty Acids; α-tocopherol; β-carotene; Selenium; Macro-micro elements.*

** To whom correspondence should be addressed.*
+ E-mail: ersin.gulsoy@igdir.edu.tr
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INTRODUCTION

Turkey, one of the motherlands of walnut, is among the oldest walnut producing countries having a deep-rooted fruit growing culture in the world. Among the cultivars that have spread throughout the world, the cultivar having the highest commercial value is *Juglans regia L.* walnuts grown in Anatolia [1]. A great portion of the walnut production of Turkey are met by the seedlings, and the number of walnut orchards is also increasing in recent years [2]. Among these trees, there are very valuable cultivars and genotypes in terms of their nutritional value. It is also suggested that while these cultivars with high nutritional contents could be used as a parent in breeding programs or used directly in cultivation [3].

Walnut is a nut type that has a high nutritional value. Walnut contains relatively high amounts of fat and protein [4,5]. Additionally, it contains important phytochemical substances with antioxidant properties (melatonin, ellagic acid, vitamin E, carotenoids and polyphenols). These substances are reported to have important functionalities such as delaying and reducing the development risks of neurological diseases such as aging, cancer, inflammation, Parkinson's disease, and Alzheimer's disease [6,7]. Vitamin E mainly consists of four tocopherol forms such as α, β, γ and δ [8]. It inhibits the formation of free radicals, prevents the damage to the mucus and skin cells by means of its antioxidant action and also reduces the oxidative stress and inflammation caused by free radicals [5]. It also reduces the risk of heart diseases by increasing High-Density Lipoprotein (HDL) cholesterol and decreasing Low-Density Lipoprotein (LDL) cholesterol [9].

Walnut contains an abundant amount of selenium in addition to minerals such as phosphorus, potassium, magnesium, iron, and calcium [10]. In a study conducted in Brazil, six walnut cultivars were found to have high amounts of selenium, and it was observed that formation of tumor was experimentally prevented when these walnut cultivars were included in the mouse diet [11]. In a recent animal study, it was found that walnut consumption reduced the risk of Alzheimer's disease and slowed down its progression [12]. Therefore, the nutritionists suggested the incoorporation of human daily diet with walnut fruits [13].

The Aras Valley is located on the migration routes from Asia to Anatolia and is considered as a transition zone of fruit genetic resources across the Euroasia. There are many cultivars of fruits that are known by local names in the region where the fruit genetic resources are rich. The presence of various distinctive ecological regions in the valley also provides rich biodiversity of walnut genetic resources.

The aim of the study was to determine the high quality walnut genotypes by selection and to detect the nutritional properties (i.e., fatty acids, α-tocopherol, β-carotene, and selenium) as well as pomological characteristics of 20 walnuts selected from the 500 regional walnut genotypes.

EXPERIMENTAL SECTION

Material

The study area, Aras Valley, is located in the North-East Agro-Ecological zone in the Eastern Anatolia Region of Turkey. This zone with high elevation area is the coldest part of Turkey, hilly and mountainous area. The valley has a a micro-climatic environment, suitable to grow many fruit species. In the region the walnut cultivars are generally produced from the wild seddlings. Therefore, the trees of walnut are not uniform and are not in the range of any and standard walnut cultivars. Aras Valley is a wide geographical region. Thus, the study area was separeted into four locations according to their ecological and walnut genetic diversity differences: Aralık, Iğdır, Tuzluca and Kağızman in terms of. Altitute in the region varies from 850 and 1400 m with an average annual temperature ranging from 7.6 to 18.3°C [14].

Twenty promising walnut genotypes (5 genotypes from each of 4 locations) was preselected from 500 genotypes of Aras Valley (Aralık, Iğdır, Tuzluca, and Kağızman) by their pomological traits: nut weight, kernel weight, kernel percentage, and shell thickness. The walnut fruit samples were collected in the harvest year of 2013. The samples were stored in shell in paper bags at ambient temperature in room conditions for 1 to 2 months until the chemical analysis.

Method

Pomological analyzes

Pomological properties of nut weight (g), length (mm), width (mm), form index, kernel weight (g), shell thickness (mm) and kernel percentage (%) were determined in 20 walnut fruits of each genotype. The fruit

weight with shell and kernel weight were measured with a digital laboratory balance (precision of 0.01 g). The fruit width with shell, fruit length with shell, fruit height with shell and shell thickness were measured with a digital caliper.

Proximate analyzes

Analyses of moisture, ash and protein contents of walnut samples were performed according to the methods given by the Turkish Standard Institute (TSE) based on AOAC methods [15]. Briefly, moisture content was calculated by calculating weight loss after heating in an oven at 105 ºC [15]. Ash was determined in the crucible to a muffle furnace at 900 ºC for 8 h [15]. The nitrogen content of walnut samples was determined using the Kjeldahl method and converted to protein content by using the conversion factor 6.25 [15].

Total oil and fatty acid composition

Walnut kernels were ground, and their total oil was extracted using a Soxhlet extractor with n-hexane used as a solvent. After 6 h of extraction, samples were evaporated under vacuum, weighted and their oil yield was determined. [16].

Fatty acid contents of walnut kernels were determined by a standard AOAC procedure [15]. After 0.1 g oil sample was dissolved in 2 ml of heptane and added onto 0.2 mL 2 M methanolic KOH solution for the preparation of the Fatty Acid Methyl Esters (FAME) The solution was shaken vigorously for 30 seconds. The upper phase was allowed to stand until clear. Then, the heptane solution was injected into GC. The analysis of FAME was performed on an Agilent 6890 series gas-chromatography equipped with flame-ionization detector and a 60 m capillary column (ID = 0.25 mm) coated with 0.25 µm of 50%-cyanopropylmethylpolysiloxane (J&W Scientific, Folsom, CA, USA). Helium was used as a carrier gas at a flow rate of 30 mL/min and a split ratio of 1:50. Injector temperature was 260 °C, detector temperature was 280 °C and the oven temperature was programmed at 120 ºC for a hold of 1 min and increased to 170 ºC at a rate of 6.5 ºC/min and increased to 215 ºC at a rate of 2.15 ºC/min hold at the final temparture for 12 min. Fatty acid methyl esters were identified by comparison of their retention time and equivalent chain length regarding standard FAMEs (Supelco. 47885-U). Fatty acid methyl esters of kernel samples were quantified based on their percentage area. The percentage composition of the oils was calculated from GC peak areas. All the samples were analyzed in triplicate [17].

α-Tocopherol (Vitamin E) content

A 5 g of homogenized walnut samples were weighed into the amber vial. The samples were saponified using butyl hydroxy toluene (about 2 g) and 5 ml potassium hydroxide (50%), and α-tocopherol extracted with 80 mL of diethyl ether, three times. All the extracts were merged and washed with distilled water before being concentrated and made to volume with ether in a 250 mL volumetric flask. After the ether phase was evaporated in the rotary evaporator, the remaining samples were dissolved in hexane and transferred to a volumetric flask. Afterwards the residue was filtered from inside of 0.45 µm and enjected to HPLC apparatus.

Chromatagraphic analysis for alpha tocopherol was carried out using HPLC-FLD (Agilent Technologies series 1200 system, Agilent Technologies) equipped with an automatic injector, on an Uptisphere 120 A° NH_2 column (Phenomenex LiChrospher 5 µm Sil 60A 250x4.6 mm) and maintained at 30 °C. The injection volume was 20 µL. The mobile phase was a mixture of n-hexane and 1,4-dioxane (97:3). Elution was performed at a solvent flow rate of 1 mL/min for 15 minutes. The detection conditions for tocopherol were 293 nm wavelength for excitation and 326 nm wavelength for emission. Alpha tocopherol was identified in chromatograms according to retention times and spectral data by comparison with standards [18,19].

β-Carotene content

β-carotene was extracted according to the method described by *Konings and Roomans* [20]. For each genotype, 5 g sample was weighted and homogenized in 50 mL methanol and tetrahydrofuran (1:1). Methanol and tetrahydrofuran were used in the preparation of the β-carotene standard solution. About 5 g of sample was homogenized in 50 mL Methanol/Tetrahydrofuran (1:1). The residue was washed twice with acetone until it becomes colorless. The extract was transferred quantitatively to 100 mL volumetric flask. Afterwards the residue was filtered from inside of 0.45 µm and enjected to HPLC. β-carotene analysis of walnut samples using

a stainless steel column (250x4.6m, ID) packed with Vydac 201 TP, 5 µm particle size and a mobile phase consisting of Methanol/Tetrahydrofuran (95:5 v/v) have been described. The flow rate was at 0.8 mL/min the chromatogram was recorded at 450 wavelengths. An injection volume of sample was 20 µL and analysis time was 20 [20].

Analyses of Selenium and Other Elements

Approximately 5 g of sample was ground in a coffe-mill and digested with concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) mixture (2:3 v/v) using a microwave. Content of total Se, P, K, Ca, Mg, Na, Fe, Cu, Mn and Zn in walnut kernels were determined by using ICP-MS (Agilent 7500cx, Agilent Technologies, CA, United States). All the samples were analyzed in triplicate [21].

Statistical Analyses

The effect of various genotypes was statistically determined by the analysis of variance and the differences between the genotypes in any measured parameter was separated by the Student *t* test at $P < 0.05$, using JMP 5.1 software package (JMP, a Business Unit of SAS, Cary, NC, 2003).

RESULTS AND DISCUSSION

Fruit pomological properties of selected walnut genotypes are shown (Table I). The average fruit weight varied from 8.89 g (TUZ 3) to 16.22 g (KAG 2). Kernel's weight varied from 4.52 g (TUZ 59) to 9.64 g (KAG 2). Kernel ratio ranged from 36.74 % (TUZ 59) to 59.59 % (KK-12) and was higher than 50 % in 7 out of 20 selected genotypes. Fruit length varied from 33.30 to 43.29 mm. The average fruit width was between 30.29 and 35.26 mm, and fruit height ranged between 29.67 and 35.49 mm. Shell thicknesses were between 1.04 (KK 5) and 3.60 mm (KAG 33). The results of fruit weight, kernel weight, kernel ratio, fruit length, width, height, and shell thickness were comparable to the findings of *Şimşek and Osmanoğlu* [22] in Turkey and *Ali et al.* [23] in Pakistan. Very large fruits were not observed in the study population and the selected genotypes were in a small and medium size. Due to seedling population, this was not suprising in the current investigation in Aras Valley of Eastern Turkey. However, these genotypes should be tested in adaptation studies in other regions in terms of their pomological characteristics to determine any significant changes somewhere else.

Total oil, protein, ash and moisture contents of selected walnut genotypes in Aras Valley are given in Table 2. Variations in the contents of oil, protein, ash, and moisture among the different genotypes were in the range of 59.18 to 68.12 %, 10.49 to 23.31 %, 0.97 to 4.00 % and 3.18 to 6.00 %, respectively. These results were similar to those reported previously *Simşek* [24], *Bada et al.* [25] and *Gharibzahedi* et al. [26].

The contents of five major and six minor, in total eleven fatty acids of walnut oil were detected and presented in this study (Table 3). The most common fatty acids of the walnut oils were linoleic, oleic, linolenic, palmitic and stearic acids with the contents of 58.15 to 64.07 %, 12.93 to 17.49 %, 9.37 to 13.61 %, 5.60 to 8.62 % and 4.68 to 6.49 %, respectively. The remaining six other minor fatty acids were namely lauric, miristic, palmitoleic, margaric, arachidic and eicosenoic acids with trace quantitiy levels

In general, the fatty acid compositions of walnut genotypes were in agreement with fatty acid composition reported by *Bada et al.* [25]: 59.81-64.77% for linoleic, 11.70-18.90% for oleic, 11.11-15.65% for linolenic and 6.11-7.49% for palmitic acid in Spain. Some fatty acid compositions were also similar to those reported for Portuguese [8], Serbia [27], Iran [28] and Turkey [24,29]. However, our findings were different from the results of *Ozrenk et al.* [30] in Turkey and *Pereira et al.* [31] in Portuguese. These composition differences and similarities may be due to a diversified ecological condition of soil, climate, location, genetics, and routine agronomic practices.

Selenium, β-caroten and α-tocopherol content of selected walnuts genotypes are given in Table 4. Selenium (Se) plays an important role in antioxidant activity in human health. As an essential trace element; low status of Se in humans may contribute to increased risk of various diseases, such as cancer and heart disease [32]. At the present work, Se content of 20 selected walnut types ranged between 15.89 (KK 45) and 68.19 (KK 3) ng/g. The average value of Se in kernels was 43.06 ng/g (Table 4). These results were in the range of limits reported by USDA standarts for commercial cultivars. According to USDA, Se content of walnut was recommended as 49 ng/g [3]. Previously, Se content of

Table 1: Some pomological triats of selected walnut types.

Genotypes	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit height (mm)	Kernel weight (g)	Shell thickness (mm)	Kernel ratio (%)
IĞDIR-5	11.70fg	39.41e	31.40d-g	33.22h	6.27f	1.43ı	53.62c
IĞDIR-22	14.42c	41.49c	35.27a	34.50c	6.89e	2.36e	47.78g
IĞDIR-26	11.75fg	36.20h	31.83c-f	31.18k	7.92d	1.44ı	52.30d
IĞDIR-66	15.74b	43.29a	32.73bc	34.35cd	6.78e	1.99fg	43.05j
IĞDIR-94	12.01ef	39.74d	31.96c-f	35.49a	6.76e	1.76h	49.33f
KK-3	10.67h	34.15kl	30.71f-h	31.03k	6.20f	1.21j	50.72e
KK-5	9.71j	34.20kl	30.39gh	31.07k	5.71g	1.04j	58.78b
KK-9	12.25e	33.30m	30.68f-h	33.47gh	5.25hı	2.16f	42.82j
KK-12	13.26d	41.88b	35.26a	33.72fg	8.46c	2.15f	59.59a
KK-45	12.21e	37.99g	32.55b-d	35.27ab	9.11b	2.52de	42.74j
TUZ-3	8.89k	40.35d	30.29gh	31.04k	4.63k	2.50de	52.09d
TUZ-22	9.02k	30.38n	28.65ı	31.67j	5.00j	2.08f	44.81h
TUZ-36	11.42g	38.75f	32.97bc	34.10de	6.23f	1.77h	47.99g
TUZ-59	10.43hı	34.38kl	31.84c-f	33.93ef	4.52k	2.68cd	41.38k
TUZ-82	10.14ı	35.44ı	31.22e-h	29.67l	4.72l	2.83c	36.74m
KAĞ-2	16.22a	35.02j	31.94c-f	35.12b	9.64a	1.68h	59.44ab
KAĞ-30	10.08ıj	34.42j	34.63b	33.62g	5.23hı	1.83gh	51.86d
KAĞ-33	12.22e	36.17h	29.94hı	32.12ı	5.37h	3.60a	43.96ı
KAĞ-51	13.49d	35.26ıj	32.36b-e	32.06ı	6.23f	3.16b	43.89ı
KAĞ-58	12.21e	34.02l	32.54b-d	34.97b	5.14ıj	3.05b	40.18l
AVERAGE	11.95	36.79	31.96	33.03	6.30	2.18	48.15

Mean values followed by a different letter within the same coloum are significantly different at P<0.0.

walnut was reported as 28 ng/g in Turkey [33], 19.2 ng/g in Greece [33] and 7 and 11 ng/g in France and in California [10] respectively. Our results (15.89-68.19 ng/g) were higher than those reported by *Lavedrine et al.* [10]; *Demirel et al.* [32] and *Pappa et al*. [34] These differences may be due to the different cultivars grown under various ecological conditions.

As a carotenoid, β-carotene is a pigment of the plants and one of the main safe dietary sources of vitamin A. It is essential for normal growth and development, immune system function, healthy skin and epithelia, and vision. It has antioxidant properties that may help to neutralize free radicals–reactive oxygen molecules potentially damaging lipids in cell membranes, proteins and DNA [35]. Vitamin A insufficiency causes ocular disorders in the form of night blindness and (in more serious cases) xerophthalmia, as well as epithelial and immune system defects [36,37]. β-carotene content of selected walnut types were determined to be in the range of 0.03 and 0.12 mg/kg in the current study (Table 4). There were no carotenoids detected in the tested nuts with the exception of pistachios in 10 different nut types studied by *Kornsteiner et al.* [38].

Abdallah et al. [39] reported that β-carotene content of some standart walnut cultivar ranged from 0.22 to 0.58 mg/kg. *Stuetz et al.* [40] found that the concentrations of

Table 2: Total oil, protein, ash and moisture of selected walnut types.

Genotypes	Total oil (%)	Protein(%)	Ash (%)	Moisture(%)
IĞDIR-5	68.12a	15.72e	4.00a	3.40h-j
IĞDIR-22	63.96g	23.31a	2.00c	3.55g-j
IĞDIR-26	64.89e	19.22b	0.99d	3.89f-h
IĞDIR-66	67.71a	11.48j	0.99d	3.44h-j
IĞDIR-94	65.76d	11.46j	1.98c	3.25j
KK-3	62.27j	16.47d	0.99d	4.05d-f
KK-5	62.99h	13.83g	3.00b	3.68f-ı
KK-9	61.20k	15.96e	1.96c	3.81f-ı
KK-12	62.37ıj	15.89e	0.98d	3.39ıj
KK-45	63.13h	15.28f	1.00d	4.91b
TUZ-3	64.92e	17.15c	1.00d	4.76bc
TUZ-22	67.22b	12.84ı	2.97b	3.18j
TUZ-36	66.67c	14.01g	1.98c	6.00a
TUZ-59	65.01e	13.64gh	0.99d	5.69a
TUZ-82	65.14e	16.47d	3.92a	4.15de
KAĞ-2	60.38l	16.81cd	1.98d	4.87h
KAĞ-30	62.38ıj	13.38h	1.96c	4.65bc
KAĞ-33	62.74hı	10.49k	1.98c	4.06d-f
KAĞ-51	59.18m	19.16b	0.97d	4.10de
KAĞ-58	64.39f	16.78cd	2.97b	4.36cd
AVERAGE	63.99	15.60	1.98	4.20

Mean values followed by a different letter within the same coloum are significantly different at P<0.05.

carotenoids measured in the different nut varieties were highest in pistachios (β-carotene of about 2 mg/kg), and in walnuts (0.19 mg/kg). The content of β-carotene in the walnuts in our study was lower than the β-carotene values reported by *Abdallah et al*. [39] and *Stuetz et al.* [40].

Tocopherols are a class of organic chemical compounds and many of which have vitamin E activity. They are well-known fat-soluble antioxidant compounds [30]. When Table 4 was examined, α-tocopherol content of 20 selected walnut genotypes varried from 8.75 (KK 45) to 35.11 (KAG 30) mg/kg and average value of α-tocopherol in kernels was 24.16 mg/kg. In previous studies, α-tocopherol content of walnuts reported to be vary from 8.9 to 16.57 mg/kg in Morocco [41], 5.06 to 7.92 mg/kg in Iran [28] ,18.00 to 26.00 mg/kg in Serbia [27] and 1.80 to 6.80 mg/kg in Turkey [30]. The contents of *α*-tocopherol of walnuts grown in Aras Valley were higher than these values mentioned above. This may be due to different ecological conditions of walnut population.

In the selected walnut genoypes, the mineral concentrations of kernels are given Table 5. In the kernels, mineral concentration ranged from 1.09 to 2.47 % for N, 230.36 to 451.48 mg/100g for P, 350.74 to 666.20 mg/100g for K, 144.0 to 452.08 mg/100g for Ca, 110.25 to 342.44 mg/100g for Mg, 7.16 to 18.82 mg/100g

Table 3: Some fatty acid composition of selected walnut types.

Genotypes	Lauric acid (C12:0)	Miristic acid (C14:0)	Palmitic acid (C16:0)	Palmitoleic acid (C16:1)	Margaric acid (C17:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	Arachidic acid (C20:0)	Eicosenoic acid
IĞDIR-5	0.026ab	0.053de	6.93bc	0.016e	0.045b-e	5.83bc	15.57ef	58.15j	13.24b	0.043de	0.09e
IĞDIR-22	0.021ab	0.054c-e	7.06bc	0.071b-d	0.029c-e	5.93b	17.49a	58.49ıj	10.58ı	0.063c-e	0.22bc
IĞDIR-26	0.014b	0.098a	5.93de-g	0.069b-d	0.016e	5.63b-e	15.53ef	63.07b	9.37k	0.068cd	0.21b-d
IĞDIR-66	0.014b	0.095ab	5.91e-g	0.072a-d	0.016e	5.60b-e	15.18fg	61.72c	11.11gh	0.078c	0.21b-d
IĞDIR-94	0.013b	0.085a-c	5.91e-g	0.071b-d	0.016e	5.35def	15.18fg	60.38fg	12.70c	0.087bc	0.19b-d
KK-3	0.014b	0.075a-d	6.56c-e	0.051c-e	0.039b-e	5.59b-e	16.76bc	59.47h	11.05gh	0.130a	0.25ab
KK-5	0.012b	0.057c-e	6.04d-g	0.093a-c	0.020de	5.23ef	12.93j	63.32b	12.10d	0.062c-e	0.14de
KK-9	0.039a	0.041ef	7.02bc	0.072a-d	0.041b-e	5.47c-e	16.11d	59.39h	11.51e	0.071cd	0.25ab
KK-12	0.013b	0.064b-e	5.68fg	0.092a-c	0.017e	4.68g	13.98ı	64.07a	11.20f-h	0.060c-e	0.14de
KK-45	0.013b	0.078a-d	5.60g	0.070b-d	0.016e	5.04fg	15.19fg	60.73ef	12.98bc	0.086bc	0.19b-d
TUZ-3	0.038a	0.052de	6.68b-d	0.046de	0.054b-e	5.65b-e	15.03gh	61.08de	11.03h	0.044de	0.31a
TUZ-22	0.015b	0.081a-d	6.46c-e	0.092a-c	0.066bc	5.54b-e	16.88bc	59.24h	11.41ef	0.031e	0.19b-d
TUZ-36	0.015a	0.08a-d	6.42c-f	0.092a-c	0.073b	5.50c-e	16.00d	60.26fg	11.34e-g	0.031e	0.19b-d
TUZ-59	0.026ab	0.054c-e	7.43ab	0.015e	0.041b-e	5.71b-d	14.70h	58.29ıj	13.61a	0.031e	0.09e
TUZ-82	0.015b	0.016f	6.07d-g	0.117a	0.056b-d	5.74b-d	16.95b	60.18g	10.57ı	0.121a	0.15c-e
KAĞ-2	0.038a	0.049de	8.05a	0.046de	0.051b-e	5.74b-d	16.53c	59.55h	9.59k	0.044de	0.31a
KAĞ-30	0.015b	0.068a-e	5.99d-g	0.050c-e	0.039b-e	6.49a	15.57ef	58.69ı	12.74c	0.085bc	0.25ab
KAĞ-33	0.016b	0.05de	6.44c-e	0.071b-d	0.029c-e	5.79bc	17.07b	60.22g	10.03j	0.063c-e	0.21b-d
KAĞ-51	0.031ab	0.042ef	8.62a	0.057c-e	0.180a	4.97fg	16.89bc	58.46ıj	10.46ı	0.068cd	0.23b
KAĞ-58	0.014b	0.016f	7.32ab	0.160ab	0.060bc	5.03fg	15.91de	61.36cd	9.92j	0.119ab	0.15c-e
AVERAGE	0.021	0.060	6.65	0.073	0.05	5.53	15.72	60.38	11.34	0.070	0.20

Mean values followed by a different letter within the same coloum are significantly different at P<0.05.

for Fe, 7.11 to 25.51 mg/100g for Na, 1.73 to 9.67 mg/100g for Mn, 2.30 to 3.86 mg/100g for Cu and 1.71 to 3.91 mg/100g for Zn. The mineral element concentrations were in the following ascending order: K>P>Ca>Mg>Fe>Na>Mn>Cu>Zn. The highest content of P, K and Zn obtained from TUZ- 82 genotypes, while TUZ-3 genotype gave the highest content of Fe and Mn. The results concerning mineral elements in walnut genotypes were comparable to findings of *Zhai et al.* [42] for Ca, Fe Mn and Cu, to the results of *Simşek* [24] for P,

Table 4: Selenium, β-carotene and α-tocopherol level of selected walnut types.

Genotypes	Selenium(ng/g)	β-carotene (mg/kg)	α-tocopherol(mg/kg)
IĞDIR-5	53.65e	0.09f	26.49ı
IĞDIR-22	39.88m	0.06o	19.94o
IĞDIR-26	25.84s	0.04s	15.67r
IĞDIR-66	42.73k	0.08h	30.83d
IĞDIR-94	40.58l	0.09e	30.22e
KK-3	63.04b	0.10c	34.41b
KK-5	33.91p	0.05p	20.71n
KK-9	44.13j	0.09d	28.05h
KK-12	18.76t	0.04r	11.93s
KK-45	15.89u	0.03t	8.75t
TUZ-3	51.37g	0.07k	25.38k
TUZ-22	68.19a	0.12a	31.45c
TUZ-36	61.21c	0.09d	28.56g
TUZ-59	46.75h	0.07ı	18.89p
TUZ-82	55.26d	0.07l	22.76l
KAĞ-2	30.45r	0.08g	29.34f
KAĞ-30	53.27f	0.10b	35.11a
KAĞ-33	38.43n	0.06n	22.37m
KAĞ-51	35.18o	0.07j	26.02j
KAĞ-58	44.69ı	0.07m	20.79n
AVERAGE	43.06	0.07	24.16

Mean values followed by a different letter within the same coloum are significantly different at P<0.05.

Mg, Na, Mn and Zn, the results of *Tapia et al*. [43] for K. The values Fe and Cu in this study were higher than those reported by *Cosmulescu et al.* [3] and *Muradoglu et al.* [44]. These differences may be explanied studied different cultivars and ecological conditions.

CONCLUSSIONS

In general, the selected walnut genotypes had small and medium size fruits. Kernel ratio was higher than 50% in 7 out of 20 selected genotypes. However, the genotypes were found to be rich in α-tocopherol, Se and some other minerals. Moreover, the fatty acid, total oil, protein, ash, moisture composition were similar to those reported for some standard walnut cultivars. Therefore, the studied genotypes can be recommended for human consumption with respect to their beneficial health effects. The phytochemical substances of walnut genotypes and cultivars can be delay and reduce the development of neurological diseases such as aging, cancer, inflammation, Parkinson's disease, and Alzheimer's disease. From the perfective of human nutrition, the selected walnut genotypes collected in Aras

Table 5: Mineral composition of selected walnut types (mg/100g).

Genotypes	P	K	Cu	Zn	Fe	Ca	Mg	Mn	Na
IĞDIR-5	375.98f	461.77j	3.86a	3.56b-e	17.35c	324.50b	280.43c	4.83cd	21.79b
IĞDIR-22	418.24b	541.86e	3.41bc	3.63a-e	16.29d	144.00r	342.44a	5.18bc	14.49e
IĞDIR-26	232.83s	350.74t	2.30g	1.72ı	10.03j	304.62e	159.61m	3.02hı	8.88j
IĞDIR-66	362.96h	423.73m	3.35bc	3.79a-c	18.02b	240.89ı	232.20g	5.42b	11.97g
IĞDIR-94	309.49n	471.22g	3.28b-d	3.52c-e	11.62h	238.71j	276.29d	2.83ı-k	8.73j
KK-3	353.66j	410.77o	3.16c-f	3.10fg	14.04h	452.08a	227.45h	4.70d	13.55f
KK-5	253.51p	389.03p	2.53g	1.91ı	11.55h	208.82m	148.63n	4.56de	13.57f
KK-9	350.19k	440.75k	3.24cd	2.80gh	16.20de	322.75c	224.13ı	4.00f	14.59e
KK-12	246.76r	368.92s	2.97ef	3.36rf	7.16l	184.71o	138.72o	3.42gh	7.47kl
KK-45	313.58m	589.40c	2.92f	2.84gh	8.12k	200.00n	247.37f	2.54jk	10.13h
TUZ-3	347.80k	416.35n	3.15c-f	3.71a-d	18.82a	208.50m	226.81h	9.67a	7.19l
TUZ-22	367.61g	579.76d	3.23c-e	3.65a-e	15.77e	307.23d	216.51j	3.87fg	25.51a
TUZ-36	414.16c	650.80b	3.27b-d	2.77h	9.53j	249.50h	290.29b	3.16hı	19.93c
TUZ-59	263.35o	472.19g	2.30g	1.71ı	10.04j	295.84f	110.25p	2.95ı-k	7.11l
TUZ-82	451.48a	666.20a	3.17c-f	3.91a	17.70bc	305.29e	281.40c	4.21ef	17.21d
KAĞ-2	230.36s	372.86r	3.21c-e	2.78h	12.71g	173.76p	169.36l	2.51k	11.83g
KAĞ-30	382.10e	435.09l	3.36bc	3.04gh	11.21hı	229.51k	178.70k	2.53jk	9.45ı
KAĞ-33	357.60ı	486.70f	3.22c-e	3.43de	11.25hı	200.20n	268.07e	2.98h-j	9.51ı
KAĞ-51	406.12d	467.86h	3.08d-f	3.84ab	10.94ı	260.00g	232.09g	2.48k	7.72k
KAĞ-58	343.47l	463.74ı	3.51b	2.74h	11.56h	224.06l	230.84g	1.73l	10.49h
AVERAGE	339.23	476.21	3.12	3.07	13.00	257.78	224.28	4.00	12.90

Mean values followed by a different letter within the same coloum are significantly different at P<0.05.

Valley still holds great potential in the future breeding walnuts programmes and be evaluated as genitors.

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REFERENCES

[1] Şen S.M., "Ceviz Yetiştiriciliği", Eser Matbaası, Samsun (1986).

[2] Çiftçi K., İzmir ve Manisa İllerinde Ceviz Yetiştiriciliğinin Sosyo-Ekonomik Yönü ve Sorunları Üzerine Bir Araştırma, *Ege Üniversitesi Fen Bilimleri Enstitüsü. Yüksek Lisans Tezi*. 89 s. İzmir (2004).

[3] Cosmulescu S., Bacıu A., Achim G., Botu M. Trandafır I., Mineral Composition of Fruits in Different Walnut (*Juglans regia L.)* Cultivars, *Not. Bot. Hort. Agrobot. Cluj.*, **37** (2): 156-160 (2009).

[4] Tapsell L., Batterham M., Tan S.Y., Warensjö E., The Effect of a Calorie Controlled Diet Containing Walnuts on Substrate Oxidation During 8-Hours in a Room Calorimeter. *Journal of the American College of Nutrition*, **28**(5): 611-617 (2009).

[5] Şen S.M., Karadeniz T., The Nutritional Value of Walnut, *Journal of Hygienic Engineering and Design*, **11**: 68-71 (2015).

[6] Reiter R.J., Manchester L.C., Tan D.X., Melatonin iİn Walnuts: Influence on Levels of Melatonin and Total Antioxidant Capacity of Blood, *Nutrition*, **21**(9): 920-924 (2005).

[7] Carey A.N., Poulose S.M., Shukitt-Hale B., The Beneficial Effects of Tree Nuts on the Aging Brain, *Nutrition and Aging*, **1**(1): 55-67 (2012).

[8] Amaral J.S., Alves M.R., Seabra R.M., Oliveira B P., Vitamin E Composition of Walnuts (*Juglans regia L.*): A 3-Year Comparative Study of Different Cultivars, *Journal of Agricultural and Food Chemistry*, **53**(13): 5467-5472 (2005).

[9] Davis L., Stonehouse W., Loots D.T., Mukuddem-Petersen J., Van der Westhuizen F., Hanekom S.J., Jerling J.C., The Effects of High Walnut and Cashew Nut Diets on the Antioxidant Status of Subjects with Metabolic Syndrome, *European Journal of Nutrition*, **46**: 155-164(2007).

[10] Lavedrine F., Ravel A., VilletA., Ducros V., Alary J., Mineral Composition of Two Walnut Cultivars Originating in France and California, *Food Chemistry*, **68**(3): 347-351 (2000).

[11] Ip C., Lisk D.J., Bioactivity of Selenium from Brazil Nut for Cancer Prevention and Selenoenzyme Maintenance, *Nuts Cancer*, **21**: 203-212 (1994).

[12] Orhan I.E., Suntar I.P., Akkol, E.K., In vitro Neuroprotective Effects of the Leaf and Fruit Extracts of Juglans regia L.(walnut) through Enzymes Linked to Alzheimer's Disease and Antioxidant Activity. *International Journal of Food Sciences and Nutrition*, **62**(8): 781-786 (2011).

[13] Vinson J.A., Cai Y., Nuts, Especially Walnuts, Have Both Antioxidant Quantity and Efficacy and Exhibit Significant Potential Health Benefits, *Food & Function*, **3**(2): 134-40 (2011).

[14] TSMS., Turkish State Meteorological Service (2017).

[15] AOAC., Official Methods of Analysis, 15th AOAC International, *Washington, DC* (1990).

[16] James C.S., Analytical Chemistry of Foods. *Chapman and Hall.1st ed*. London (1995).

[17] Dieffenbacher A., Pocklington W.D., "IUPAC Standart Methods for The Analysis of Oils, Fats and Derivatives", 6th ed. (Fifth Edition Method II.D.19) PP. 96-102, Pergamon Press Oxford (1992).

[18] Manz U., Philipp K., Method for the Routine Determination of Tochopherols in Animal Feed and Human Foodstuffs with the Aid of HPLC, *International Journal for Vitamin and Nutrition Research*, **51**: 342–348 (1981).

[19] Eittenmiller R.R., Lin Ye W.O., Landen Jr., "Vitamin Analysis for Food the Health and Food Sciences", CRC Press, New York, Second Edition, 119-191 (2008).

[20] Konings E.J.M., Roomans, H.H.S., Evaluation and Validation of an LC Method for the Analysis of Carotenoids in Vegetables and Fruit, *Food Chemistry*, **59**(3): 599-603 (1997).

[21] Cubbada F., Inductively Coupled Plasma-Mass Spectrometry for the Determination of Elements and Elemental Species in Food: A Review, *Journal of AOAC International*, **87**: 173-204 (2004).

[22] Şimşek M., Osmanoğlu A., Mazıdağı (Mardin) Yöresindeki Doğal Cevizlerin *(Juglans regia L.)* Seleksiyonu, *YYÜ Tar Bil Derg (YYU J Agr Scı)* **20**(2): 131-137 (2010).

[23] Ali M., Ullah A., Ullah H., Khan F., Ibrahim S.M., Ali L., Ahmad S., Fruit Properties and Nutritional Composition of Some Walnut Cultivars Grown in Pakistan, *Pakistan Journal of Nutrition*, **9**(3): 240-244 (2010).

[24] Simsek M., Chemical, mineral, and fatty acid compositions of various types of walnut (Juglans regia L.) in Turkey, *Bulgarian Chemical Communications*, **48**(1): 66-70 (2016).

[25] Bada J.C., León-Camacho M., Prieto M., Copovi P., Alonso L., Characterization of Walnut Oils *(Juglans regia L.)* from Asturias, Spain, *Journal of the American Oil Chemists' Society*, **87**(12): 1469-1474 (2010).

[26] Gharibzahedi S.M.T., Mousavi S.M., Hamedi M., Khodaiyan F., Determination and Characterization of Kernel Biochemical Composition and Functional Compounds of Persian Walnut Oil, *Journal of Food Science and Technology*, **51**(1): 34-42.(2014).

[27] Rabrenovic B., Dimic E., Maksimovic M., Sobajic S., Gajic- Krstajic L., Determination of Fatty Acid and Tocopherol Compositions and the Oxidative Stability of Walnut (Juglans regia L.) Cultivars Grown in Serbia, *Czech Journal of Food Sciences*, **29**(1): 74-78 (2011).

[28] Tsao L.R.; Yang, R.; Kramer, J.K. Hernandez M., Fatty Acid Profiles, Tocopherol Contents, and Antioxidant Activities of Hearnut *(Juglans ailanthifo var. Cordiformis)* and Persian Walnut *(Juglans regia)*, *Journal Agric Food Chem.*, **55**(4): - (2007).

[29] Özcan M.M., Some Nutritional Characteristics of Fruit and Oil of Walnut (Juglans regia L.) Growing in Turkey, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **28**(1): 57-63 (2009).

[30] Ozrenk K., Javidipour I., Yarilgac T., Balta F., Gündoğdu M., Fatty Acids, Tocopherols, Selenium and Total Carotene of Pistachios *(P. vera L.)* from Diyarbakır (Southestern Turkey) and Walnuts *(J. regia L.)* from Erzincan (Eastern Turkey), *Revista de Agaroquimica y Tecnologia de Alimentos*, **18**(1): 55-62 (2012).

[31] Pereira J.A., Oliveira I., Sousa A., Ferreira I.C., Bento A., Estevinho L., Bioactive Properties and Chemical Composition of Six Walnut (*Juglans regia L.)* Cultivars, *Food and Chemical Toxicology*, **46**(6): 2103-2111 (2008).

[32] Tinggi U., Selenium: Its Role as Antioxidant in Human Health, *Environ Health Prev Med*, **13**: 102–108 (2008).

[33] Demirel S., Tuzen M., Saracoglu S., Soylak M., Evaluation of Various Digestion Procedures for Trace Element Contents of Some Food Materials, *Journal of Hazardous Materials*, **152**(3): 1020–1026 (2008).

[34] Pappa E.C., Pappas A.C., Surai P.F., Selenium Content in Selected Foods from the Greek Market and Estimation of the Daily İntake, *Science of the Total Environment*, **372**(1): 100–108 (2006).

[35] Khatib S., Vaya J., Fig, Carob, Pistachio and Health, In: Watson R and Preedy VR (eds) "Bioactive Foods in Promoting Health: Fruits and Vegetables", Oxford: Elsevier Academic Press, 245–263 (2010).

[36] Bai C., Twyman R.M., Farré G., Sanahuja G., Christou P., Capell T., Zhu C., A Golden era—Pro-Vitamin A Enhancement in Diverse Crops, *In Vitro Cell. Dev. Biol. Plant*, **47**: 205–221 (2011).

[37] Berman J., Zorrilla-López U., Farré G., Zhu C., Sandmann G., Twyman R.M., Capell T., Christou P. Nutritionally Important Carotenoids as Consumer Products, *Phytochem. Rev*., **14**: 727–743 (2015).

[38] Kornsteiner M., Wagner K.H., Elmadfa I., Tocopherols and Total Phenolics in 10 Different nut Types, *Food Chemistry*, **98**(2): 381-387 (2006).

[39] Abdallah I.B., Tlili N., Martinez-Force E., Rubio A.G.P., Perez-Camino M.C., Albouchi A., Boukhchina S., Content of Carotenoids, Tocopherols, Sterols, Triterpenic and Aliphatic Alcohols, and Volatile Compounds in Six Walnuts *(Juglans regia L.)* Varieties, *Food Chemistry*, **173**: 972-978 (2015).

[40] Stuetz W., Schlörmann W., Glei M., B-Vitamins, Carotenoids and α-/γ-Tocopherol in Raw and Roasted Nuts, *Food Chemistry*, **221**: 222-227 (2017).

[41] Kodad O., Estopañán G., Juan T., Sindic M., Genotype and Year Variability of the Chemical Composition of Walnut Oil of Moroccan Seedlings from the High Atlas Mountains, *Grasas y Aceites*, **67**(1): 116- (2016).

[42] Zhai M., Wang Z., Wang D., Xu J., Shı G., Comparative Analysis of Mineral Elements and Essential Amino Acids Compositions in Juglans sigillata and J. Regia Walnuts, *Kernels Not Bot Horti Agrobo*, **42**(1): 36-42 (2014).

[43] Tapia M.I., Sanchez-Margado J.R., Garcia-Parra J., Ramirez R., Hernandez T., Gonzales-Games D., Comparative Study of the Nutritional and Bioactive Compounds Content of Four Walnut (*Juglans regia* l.) Cultivars, *Journal of Food Composition and Analysis*, **31**: 232-237 (2013).

[44] Muradoglu F., Oguz H.I., Yildiz K., Some Chemical Composition of Walnut *(Juglans regia L.)*Selections from Eastern Turkey, *African Journal of Agricultural Research*, **5**(17): 2379-2385 (2010).