Determination of fatty acid, tocopherol content and total phenol of nine selected Chinese walnut genotypes

Ömer Faruk BILGIN[1]
Şule Hilal ATTAR[1]
Aibibula PAIZILA[1]
Feng LIU[2]
Yonghong GONG[2]
Salih KAFKAS[1]
Ebru KAFKAS[1]

[1] Department of Horticulture
Faculty of Agriculture
University of Çukurova
Adana, Turkey

[2] Liaoning Institute of Economic Forestry
31 West Zhonghua Ganjingzi District
Dalian, China

CORRESPONDENCE AUTHOR:
Aibibula Paizila
E-mail: aibibulapaizila@gmail.com
Phone: +90 553 377 4371
Fax: +90 322 338 6030

Received: November 1, 2020
Accepted: February 1, 2021

In this study, total lipid content, fatty acid composition, tocopherol content, total phenol, and DPPH activity of nine selected walnut genotypes from Liaoning province of China were studied. The total lipid content of the walnut genotypes was changed between 47.5 (3-2-16) and 66.8% (Liaoning 7). Oleic acid was the major monounsaturated fatty acid that is 42.86% in the Liaoning 7 genotype, while linoleic acid was the dominant polyunsaturated fatty acid reached a maximum of 63.47% at Han Feng genotype. The total tocopherol content of walnut genotypes ranged from 166.15 (Lipin 1) to 382.40 µg/g oil (E1-44). The most abundant tocopherol isomer was γ-tocopherol and it showed maximum level in the Han Feng genotype (318.42 µg/g). We found α-tocopherol was the minor tocopherol compared to other tocopherol isomers and all walnut genotypes showed antioxidant activity that is varied from 52.22 (3-2-16 selection) to 40.18% (Lipin 1). As a result, this study provided valuable information on the chemical compound of selected Chinese walnut genotypes and insights into selecting

Keywords: Total lipid, fatty acids, tocopherol, total phenol, antioxidant activity

INTRODUCTION

Walnut (*Juglans regia* L.) is one of the most important and common nut species and ranked second after almond in terms of production. Walnut trees can be grown in a wide range from South-eastern Europe to the Caucasus regions due to their high ability to adapt to different climate conditions [1, 2]. Walnut includes high nutritious components including macronutrients (protein, fat, carbohydrate), micronutrients (minerals, vitamins) and other bioactive compounds such as monounsaturated fatty acids [MUFA], polyunsaturated fatty acids [PUFA], and tocopherols [3]. Walnut has significant health benefits when consumed in an adequate amount. It has medicinal importance in medical biochemistry and physiology because of its specific composites [4]. Therefore, the production and consumption of walnut have increased at a fast rate in the last decades [5]. The production of world shell-walnut was approximately 3.7 million metric tons, and the major walnut producing countries are China (1.536 mt), USA (613 kt), Iran (409 kt), and Turkey (215 kt) [5]. Among these countries, China is the leading walnut producer in the world and is responsible for 43.3% of world production in 2018.

Walnut has been used in the human diet since ancient times and it includes approximately 60% oil [6], among which the unsaturated fatty acids is the dominant compound. Walnut contains a high level of polyunsaturated fatty acids (PUFAs) including Omega-6 and Omega-3, which are more beneficial for human health compared to monounsaturated fatty acids (MUFAs) [7, 8].

The highest proportions of the unsaturated fatty acids contain α-linolenic acid (18:3ω3) and linoleic acid (18:2ω6) [9]. Ros et al. [10] reported that consumption of walnut has significantly reduced low-density lipoprotein (LDL) cholesterol concentration and prevented coronary heart disease in humans. Kris-Etherton [11] also demonstrated walnut consumption reduces the two major risk factors (lipoprotein cholesterol and diastolic blood pressure) for cardiovascular disease. Additionally, West et al. [12] reported that high walnut consumption in the diet can significantly reduce stress level in humans.

Walnut is also a natural source of antioxidants that protects the human body from the detrimental effects of free radicals [13, 14]. In previous studies, phenols are reported to have antimicrobial activity by many researchers [15, 16]. Walnut contains a high level of phenols thatan prevent the lipid oxidation process [8]. Because the public's awareness of the harmful artificial antimicrobial components added to food is increasing, people are avoiding to use chemical preservations and trying to find new harmless antimicrobials with a natural origin [17, 18]. In recent years, phenolic compounds and antioxidant research in walnut gained more interests in the international community. Many important chemicals such as antioxidants, fatty acids, and tocopherols showed significant variation between different walnut cultivars [19]. Many studies had focused on the chemical compounds of walnut from different countries and nine walnut cultivars grown in Portugal were analysed in a recent study. Content of the three major tocopherols γ-tocopherol, α-tocopherol, and δ-tocopherol varied from 172.6 to 262.0 mg/kg, 8.7 to 16.6 mg/kg and from 8.2 to 16.9 mg/kg, respectively [19]. Greve et al. [20] studied eight New Zealand walnut selections together with five commercial cultivars and reported that the total lipid content of the walnut genotypes ranged from 64.2 to 68.9%. The oleic acid content of the oils varied from 12.7 to 20.4% of the total fatty acids. The total tocopherol contents of these nuts varied from 268.5 to 436.0 µg/g. Nineteen walnut genotypes were selected from the Kahramanmaraş region of Turkey and analysed for fatty acid and tocopherol content by Beyhan et al. [21]. Results showed that the total fat, stearic acid, linoleic acid, linolenic acid, and oleic acid varied from 51.2 to 82.1%, 2.57 to 3.37%, 53.23 to 63.62%, 10.75 to 15.24% and 14.73 to 24.17%, respectively. Tocopherol content including α, γ, and β + δ tocopherol changed between 23.47 and 38.04 µg/g, 161.09 and 292.56 µg/g, and 16.93 and 32.34 µg/g, respectively. Greve et al. [20] analysed 151 cultivars originated from four different countries and cultivated in the California region. As a result, the polyunsaturated fatty acid content of the walnuts as a percentage of total fatty acid content varied from 47.2% (PI 142323 from France) to 81.0% (Ashley from California).

China is the leading walnut producer in the world. However, there is little research on the biochemical components of Chinese walnut cultivars. Furthermore, we need more research on the nutritional quality and health-promoting constituent of walnuts to enhance our knowledge and encouraging walnut consumption. The aim of this study was the characterisation of promising genotypes grown in China in terms of fatty acids, total antioxidant as well as tocopherol content, and the evaluation of the nutritional value of selected varieties to expand the knowledge of the crop and encourage walnut consumption. The result of this study will provide valuable information on the chemical compound of Chinese walnut genotypes.

EXPERIMENTAL PART

PLANT MATERIAL

Mature fruit samples of nine walnut genotypes (E1-44 Selection, Liao Rui Feng, Liaoning 1 7, Liaoning 1 4, 3-2-16, Lipin 2, Liaoning 1, Lipin 1, Han Feng) were harvested from the Liaoning agricultural research centre, which is located in the Liaoning province of China in September 2019. The nuts were selected randomly from each genotype with three replicates. The samples were immediately carried to the laboratory after harvesting and then dried in an incubator at +30°C for 36 hours. The dried samples were transported to Turkey in vacuum-sealed plastic bags for further analysis.

KERNEL OIL EXTRACTION AND FATTY ACID ANALYSIS

Oil extraction of the samples was performed according to the method of Bligh and Dyer [22]. The oil content was calculated based on the weight difference of tubes before and after the experiment. The oil samples were used for the fatty acid and tocopherol assays. Potassium hydroxide (KOH) in methanol was used for the methylation process. At first, the samples from nine genotypes were grounded separately with mortar, then 20 g of ground samples were used for the oil extraction using hexane solvent in Soxhlet equipment (Gerhardt Soxtherm) and a triplicate analysis was reported for each genotype. The samples were mixed with petroleum benzene and kept for 2 h to accomplish the extraction. The samples were dried until reaching a constant weight.

The total fatty acids were evaluated using Agilent Gas Chromatography accompanying with an auto-sampler (Agilent.7820A). The fatty acid analysis was conducted according to the method by Firestone [23] with some modification by Ichihara et al. [24] by transforming the fatty acid to the corresponding methyl ester forms. The main parameters for GC analysis were described in the Table I

TOCOPHEROLS

A total of 1 g of an oil sample from each walnut genotype was used for the tocopherol (α, β, γ, and δ-tocopherol) analysis using the HPLC system (Agilent 1100). In the analysis, Hexane: Acetic Acid: 2-propanol (1000:5:6) was used as mobile phase, samples were injected into the GL Science InterstilTM NH2 (5 µm 4.6 × 250 mm) column, and the peaks were detected using the UV detector at wavelengths of 298 nm.

Table I

Gas Chromatography (Shimadzu gregg GC-2010)	Parameters
Column	FID (Flame Ionization Detector)
Detector	Fused-silica capillary SGE
	Temperature 280°C Length 30 m Film thickness 0.25 µm Inner Diameter 0.32 mm
Oven	Temperature 140-220°C
Injection volume	1 µl
Injection temperature	220°C
Column temperature	190°C
Carrier Gas	Helium (0.5 ml.min^{-1})

TOTAL PHENOL OF THE WALNUT

Total phenolic compounds were evaluated from oil samples according to the Folin-Ciocalteu reagent method by Spanos and Wrolstad, [25] with slight modifications. Briefly, 50 µl of a sample, 100 µl of diluted Folin-Ciocalteu, and 1.5 ml of double-distilled water were added to the 2 ml tubes, respectively. After 10 min 50 µl 20% sodium carbonate was added to the mixture and kept in a dark room for 2 hours. For control and blank read groups, 50 µl double distilled water and 50 µl 80% methanol was used, respectively, instead of samples. 250 µl from each sample was tested for absorbance at 725 nm in a spectrophotometer (Thermo Multi Scan Go). Gallic acid was used for constructing the standard curve. The results were expressed as mg gallic acid equivalents (GAE) 100 g of the dried walnut kernel.

DPPH SCAVENGING ACTIVITY

DPPH (2,2-diphenyl-1-pikrilhidrazil) scavenging potential of each variety was measured according to the method of Cuvelier and Berset, [26] with few modifications. At first 50 µl of the sample was added to the 2 ml falcon tube then, 1950 µl 0.06 Mm DPPH was added to each tube in a dark room. The mixture was vortexed and stand at room temperature in a dark room for one hour. For control and blank read groups, 50 µl double distilled water and 50 µl 80% methanol was used instead of samples, respectively. Absorbance values of the samples were measured at 515 nm in a spectrophotometer (Thermo Multi Scan Go) and the results were calculated as the following formula:

$$\text{DPPH inhibition} = \left(1 - \frac{\text{A control} - \text{A sample}}{\text{A control}}\right) \times 100$$

STATISTICAL ANALYSIS

All extractions and analysis were performed in triplicate. Results were presented as mean values ± standard deviation (SD). Variances between the mean values were compared by One-way ANOVA in SPSS 26.0 software and significant difference between varieties was assigned by the Duncan test. The difference at p <0.05 was considered significant.

RESULTS AND DISCUSSION

The kernels of nine important selected walnut genotypes were analysed for determining walnut kernel oil compositions such as total lipid, saturated and unsaturated oil. Besides, the nine selected walnut genotypes were analysed in terms of total tocopherol and its isomers compounds such as α, β, γ, and δ-tocopherol.

SATURATED FATTY ACIDS

The values of the total lipid (%) and essential saturated fatty acid profile of nine selected walnuts genotypes are shown in Table II. The total lipid content of kernels of the nine selected genotypes ranged from 44.9 (Liaoning 1) to 66.8% (Liaoning 7) (Tab. II). In previous studies, Yerlikaya et al. [27], stated an oil content of 61.3 to 69.4% in seven walnut genotypes, while Beyhan et al. [21] studied the lipid content of 19 selected walnut genotypes from Turkey and the lipid content of the genotypes differed from 51.2 to 82.1%**.** Akbari et al. [28] reported an lipid content of 63.3 to 78.5% in six different walnut genotypes, while Ünver et al. [29] determined an oil content of 43.2 to 58.7% in some selected walnut genotypes. The lipid and fatty acid proportion of walnuts can change depending on the walnut cultivar, the harvesting year, environmental conditions, temperature variation, rainfall, light [30], and the cultivation method such as pruning, fertilisation, and irrigation [31, 32]. The various lipid content among the genotypes may be caused by the cultivar and regional difference of walnut genotypes.

Saturated fatty acids (SFA) are the less abundant fatty acid form in walnut [30]. Low SFA level in walnut is helpful to maintaining normal blood cholesterol and reducing the risk of diabetes and cardiovascular diseases [33]. In this study, SFA is the less abundant fatty acid and palmitic acid constituted the major-

Table II - Total lipid and saturated fatty acid content (%) of the nine selected walnut genotypes (mean ± SD).

Genotypes	Total lipid (%)	Saturated fatty acids (%)			
		Stearic acid	Myristic acid	Palmitic acid	Arachidic acid
E1-44	53.6±4.3^{d}	0.07±0.08^{e}	0.14±0.13^{e}	6.84±0.22^{b}	0.30±0.16^{e}
Liao rui feng	59.8±0.2^{b}	0.11±0.07^{d}	0.22±0.14^{d}	6.51±0.19^{c}	0.33±0.09^{de}
Liaoning 7	66.8±4.4^{a}	0.18±0.04^{c}	0.14±0.16^{e}	5.83±0.09^{f}	0.40±0.14^{c}
Liaoning 4	56.1±3.1^{c}	0.21±0.07^{c}	0.34±0.20^{c}	6.97±0.04^{a}	0.57±0.24^{a}
3-2-16	47.5±4.0^{ef}	0.10±0.03^{de}	0.15±0.07^{e}	6.56±0.22^{c}	0.28±0.10^{e}
Lipin 2	57.1±1.0^{c}	0.09±0.01^{de}	0.19±0.04^{de}	6.88±0.14^{ab}	0.32±0.04^{de}
Liaoning 1	44.9±6.5^{f}	0.34±0.49^{a}	0.53±0.76^{a}	5.74±0.08^{f}	0.46±0.23^{b}
Lipin 1	59.2±3.6^{b}	0.09±0.09^{de}	0.14±0.10^{e}	6.06±0.13^{e}	0.37±0.05^{cd}
Han Feng	48.2±3.9^{e}	0.27± 0.20^{b}	0.43±0.29^{b}	6.38±0.08^{d}	0.40±0.18^{c}

Different letters for the same parameters indicate significant difference among genotypes ($p < 5$).

Table III - Unsaturated fatty acid content (%) of the nine selected walnut genotypes (mean ± SD).

Genotypes	Unsaturated fatty (%)					
	Polyunsaturated fatty acids (%)			Monounsaturated fatty acids (%)		
	Linoleic acid (%)	a linolenic acid (%)	PUFA (%)	Oleic acid (%)	Palmitoleic acid (%)	MUFA (%)
E1-44	43.91±1.81^{e}	7.15±0.52^{bc}	51.06±1.15^{f}	41.18±1.86^{a}	0.42±0.31^{de}	41.60±1.08^{b}
Liao rui feng	61.21±0.80^{b}	8.93±0.38^{ab}	70.14±0.60^{a}	22.14±0.69^{f}	0.56±0.11^{cd}	22.70±0.40^{g}
Liaoning 7	42.02±0.92^{e}	7.81±0.28^{abc}	49.83±0.60^{f}	42.86±0.55^{a}	0.76±0.14^{ab}	43.62±0.34^{a}
Liaoning 4	52.39±3.22^{d}	8.90±0.66^{ab}	61.29±1.94^{d}	29.75±3.71^{c}	0.87±0.22^{a}	30.62±1.96^{d}
3-2-16	56.30±1.82^{c}	8.77±0.42^{ab}	65.07±1.12^{c}	27.46±1.86^{d}	0.39±0.04^{de}	27.85±0.95^{e}
Lipin 2	61.32±1.28^{b}	9.10±0.34^{a}	70.42±0.82^{a}	21.80±1.43^{fg}	0.31±0.09^{e}	22.11±0.72^{gh}
Liaoning 1	57.79±1.69^{c}	9.49±0.56^{a}	67.28±1.12^{b}	24.97±0.42^{e}	0.69±1.02^{bc}	25.66±0.76^{f}
Lipin 1	50.63±0.44^{d}	6.63±0.10^{c}	57.26±0.27^{e}	35.75±0.67^{b}	0.34±0.14^{e}	36.09±0.40^{c}
Han Feng	63.47±0.55^{a}	8.25±0.63^{abc}	71.72±0.58^{a}	20.14±0.90^{g}	0.67±0.45^{bc}	20.81±0.67^{h}

Different letters for the same parameters indicate significant difference among genotypes ($p < 5$).

ity of SFA percentage (Tab. II and Tab. III). Palmitic acid ratios ranged from 6.97% (Liaoning 4) to 5.74% (Liaoning 1), while arachidic acid, myristic acid, and stearic acid content of the SFA were changed from 0.28 to 0.57%, 0.14 to 0.34%, 0.07 to 0.34%, respectively (Tab. II). Unver et al. [29] also detected that palmitic acid is the major SFA form in walnut and palmitic acid content and ranged from 5.20 to 7.29%. In another study, palmitic acid (C16:0) was also the primary SFA in walnut and its ratio changed between 7.28% to 8.95% [30]. In brief, our findings are consistent with these previous studies.

UNSATURATED FATTY ACIDS

Unsaturated fatty acids (USFA), especially polyunsaturated fatty acids (PUFA), have a fundamental role in healthy human nutrition [33]. Walnut oil content consists of predominantly unsaturated fatty acids. PUFA and monounsaturated fatty acids (MUFA) were also studied in this study. In contrast with other nuts such as almond, pistachio and hazelnut, walnut mainly contains PUFAs [33]. Total PUFA content varied between 49.83% (Liaoning 7) to 71.72% (Han Feng) and the total amount of MUFA changed between 20.81% (Han Feng) to 43.63% (Liaoning 7) in the nine selected walnut genotypes (Tab. III). Amaral et al. [8] reported that PUFA content changed between 70.66 and 74.83%, while MUFA content varied from 15.82 to 18.87%. According to Kafkas et al. [34] MUFA content varied between 13.06% to 27.70%, and PUFA content oscillated from 61.25% to 76.74%. The fatty acid content of walnut cultivars cultivated in an experimental orchard at Lincoln University was examined and PUFA content of the walnut oil ranged between 62.4% to 68.7% [7]. According to another study, MUFA and PUFA contents were 21.2 g/100 g and 69.0 g/100 g in the walnut oil, respectively [35]. Li et al. [36] studied the fatty acid compositions of three heartnut (*Juglans ailanthifolia* var. cordiformis) varieties and stated that PUFAs are the foremost group of fatty acids in heartnut and walnut that changed from 73.07 to 80.98%. In all

these studies PUFA content was much higher than the MUFA content and these results were consistent with our results.

The unsaturated fatty acids (%) were linoleic acid, oleic acid, α-linolenic acid, palmitoleic acid in descending order. The highest linoleic acid content among the genotypes was detected in the Hang Feng genotype (63.47%), while the lowest linoleic acid content was detected in Liaoning 7 genotype (42.02%) (Tab. III). The highest oleic acid content was found in Liaoning 4 (29.75%) genotype and the lowest value was found in Han Feng genotype (20.40%). Çaglarirmak [4] found that oleic acid and linoleic acid content varied between 13.10 to 27.12%, and 39.08 to 49.07%, respectively. Uzunova et al, [37] also reported that linolenic acid changed from 9.66 to 10.77% and the highest value of linoleic acid content was 64.14%. These results were similar to the results of the current study. Aparicio et al. [38] stated that the rate of oleic/linoleic acid was the most significant indicator of stability of walnut oil. In particular, a higher oleic ratio and lower linoleic acid ratio means a long shelf-life. This ratio was used in the characterisation of walnut oil [30] and oil quality of walnut cultivars [38]. In this study, the oleic/linoleic acid ratio ranged from 0.32 in Liao rui Feng to 1.02 in Liaoning 7 (data are not shown). This ratio reported in the walnut oil ranging from 0.2 to 0.32 [30] which indicates Liaoning 7 cultivar is the ideal candidate for walnut oil extraction. Moreover, Zwarts et al. [7] stated that the composition of the fatty acids was essential for rancidity, flavour, as well as the taste of the walnut oil.

TOCOPHEROL CONTENT AND TOTAL PHENOLS

Tocopherols are the major membrane localised antioxidants in humans. Lower tocopherol intake increase the risk of certain types of cancer and atherosclerosis [39]. Walnut oils are an important source of tocopherols which includes four homologues: α, β, γ, δ-tocopherols [40]. Lipin 1 genotype had the lowest total tocopherol content (166.15 μg/g), while the E1-44 genotype had the highest tocopherol content (382.40 μg/g) among all the genotypes (Tab. IV). Abdallah et al. [41] reported that the total tocopherol content of six walnut cultivars were changed between 186.54 to 436.2 mg/kg. In another study, the total tocopherol content of Rex cultivar and Dublin's Glory cultivar (selected from New Zealand) had 290.2 μg/g (the lowest) and 34.8 μg/g (the highest level), respectively. These findings were consistent with the results of this study γ-tocopherol is predominant among all tocopherol forms (Tab. IV). The highest content of this form was found in Han Feng (318.42 μg/g) genotype, while the lowest value was found in Liaoning 7 (165.97 μg/g) genotype. γ-tocopherol is more active in decreasing platelet aggregation and LDL oxidation than α-tocopherol [42]. In addition, β -tocopherol content varied between 41.04 (E1-44) and 2.82 μg/g (3-2-16) (Tab. IV). δ -Tocopherol ranged between 35.7 μg/g in Han Feng and 16.39 μg/g in Liaoning 1. α-tocopherol showed a lower level than the detected other tocopherols (1.10-11.40 μg/g) (Tab. IV). In this study, tocopherols content showed a significant difference among the genotypes which is similar to the previous reports [14], [41]. Abdallah et al. [41] stated that β and γ-tocopherol are predominant among the detected tocopherols in six varieties and α-tocopherol had the lowest value. In another study γ-tocopherol was the main tocopherol (127-267 mg/kg) while β-tocopherols were not identified in walnuts cultivated in Canada [36]. Moreover, Kornsteiner et al. [14] reported that β and γ-tocopherol are the main detected tocopherols (120-320 mg/kg) in cultivated walnuts in Austria, and α- tocopherols were not detected. These differences may result from different tocopherol analysing methods used in experiments. Genotypes with high tocopherol content such as E1-44 and Han Feng can be selected as a candidate for commercial production or breeding studies.

The total phenolics content of the nine selected walnut genotypes was listed in Table IV. The mean value of total phenolics oscillated between 1463.09 (Lipin

Table IV - Total Tocopherol Contents (μg/g oil) and total phenols (mg of Gallic acid/100 g fresh) of the nine selected walnut genotypes (mean ± SD)

Genotypes	α	β	γ	δ	Total	Total Phenols
E1-44	11.44±0.85^{a}	41.04±4.03^{a}	308.97±20.29^{a}	20.93±24.72^{cd}	382.40^{a}	1.533±24.10^{g}
Liao rui feng	6.92±0.45^{b}	38.82±1.89^{a}	264.58±12.41^{b}	27.62±1.57^{b}	337.95^{b}	2.016±14.91^{d}
Liaoning 7	2.81±0.59^{cd}	30.15±0.33^{b}	165.97±6.15^{d}	20.31±1.17^{cde}	219.25^{d}	2.353±75.33^{b}
Liaoning 4	1.99±0.66^{de}	12.82±0.74^{b}	264.82±3.24^{b}	16.53±2.51^{e}	291.68^{c}	2.666±13.33^{a}
3-2-16	2.64±0.82^{cde}	02.82±0.86^{d}	200.07±8.53^{c}	27.70±0.12^{b}	233.24^{d}	2.109±10.62^{c}
Lipin 2	1.10±0.37^{e}	16.10±3.11^{c}	253.30±9.71^{b}	17.46±7.65^{de}	288.35^{c}	1.502±73.22^{h}
Liaoning 1	1.36±0.38^{de}	16.05±3.11^{c}	238.93±9.71^{b}	16.39±0.69^{bc}	276.77^{c}	1.932±35.47^{e}
Lipin 1	1.34±0.37^{b}	13.05±0.96^{b}	238.21±3.58^{e}	24.79±0.76^{cde}	166.15^{e}	1.463±4.89^{i}
Han Feng	3.74±0.28^{c}	10.70±0.44^{b}	318.42±7.53^{a}	35.73±1.26^{a}	368.61^{ab}	1.686±11.54^{f}

Different letters for the same parameters indicate significant difference among genotypes ($p < 5$).

1) and 2666.12 (Liaoning 4) GAE/100 g. Kornsteiner et al. [14] studied the total phenols content of four different nuts as a result the highest phenol content was reported in walnut (1020 - 2052 mg/100 g), followed by pistachios (492 to 1442 GAE/100 g) and pecans (1022 - 1444 GAE/100 g). Moreover, Kafkas et al. [34] reported that the total phenolic content of 10 different walnut cultivars oscillated between 2440 and 3490 mg GAE/100 g. These results agree with our study.

THE DPPH SCAVENGING CAPACITY

DPPH assay has been generally used to determine the free radical- scavenging activity of different plants and pure compounds [15, 38]. DPPH provides fundamental information about the extract's antiradical activity. Inhibition of lipid oxidation by antioxidants is one of the best-known mechanism of radical scavenging [44]. It is known that antioxidant active compounds have helped prevent chronic diseases [45]. In this study, DPPH scavenging capacity oscillated between 52.22 (3-2-16 selection) to 40.18% (Lipin 1) genotype (Fig. 1). Akbari et al. [28] studied the DPPH activity of six Iranian walnut genotypes and the results differed between 53.83 (B1) to 94.07%(BHA). Li et al, [46] stated that DPPH activity of the walnut oil (100 mg/mL) was 0.47 (μM TEAC/g), while DPPH of the walnut extraction (50 μg/mL) was 1026.19 (μM TEAC/g). These differences may result from the used analysing methods.

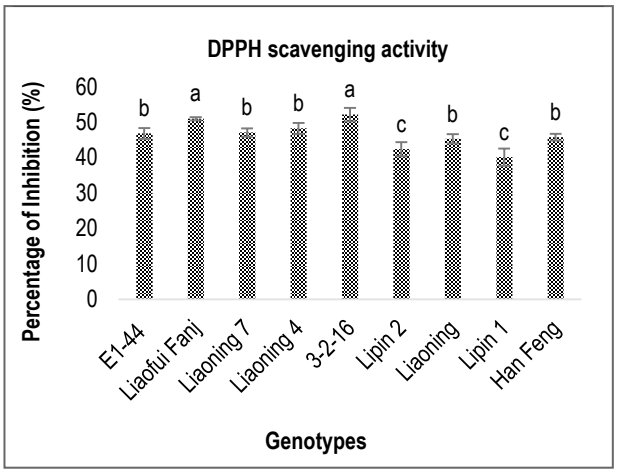

Figure 1 - The antioxidant activity of the nine selected walnut genotypes.

CONCLUSION

Walnut contains many beneficial compounds such as PUFA, MUFA, tocopherols, and antioxidants. Unsaturated fatty acids are not synthesized in the human body, so they are called essential fatty acids. Among the unsaturated fatty acids, PUFAs are proven to be healthier than MUFAs by many types of research. Also, among different tocopherol isomers, γ-tocopherol was suggested to be especially important for human health compared to other tocopherol isoforms. Nine walnut genotypes (E1-44, Liao Rui Feng, Liaoning 7, Liaoning 4, 3-2-16, Lipin 2, Liaoning 1, Lipin 1, Han Feng) were analysed in this study, based on our result in these walnut genotypes. Unsaturated fatty acid content is higher than the saturated fatty acid content. PUFA content is higher than MUFA content. γ-tocopherol is the most abundant tocopherol isomer. All the results above highlighted the importance of walnut as a healthy diet. China is the main walnut producer in the World which is responsible for almost half of the whole world's walnut production and China has many potential superior walnut genotypes. This study revealed useful information on the chemical compound of nine selective walnut genotypes from China. These results can be used to select potential cultivars with high nutrition value and further breeding studies.

Acknowledgments

Gratitude is extended to Liaoning Institute of Economic Forestry, Dalian, China for providing the walnut samples. The analysis was performed at the University of Çukurova, Faculty of Agriculture, Department of Horticulture.

Author contributions

All authors contributed to the study conception and design. Material preparation was carried out by FL and YG. Data collection and analysis were performed by ÖFB, ŞHA, and PA. The first draft of the manuscript was written by ÖFB, PA, SK, and ENK. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This research did not receive any specific donations from funding agencies in the civil commercial, or non-profit sectors.

Data Availability

All data interests and material in this study support published claims of the authors and comply with field standards.

Code availability

No software application and custom code were available in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. There is no conflict of interest.

BIBLIOGRAPHY

[1] M. Sütyemez, N. Kaşka, Bazı yerli ve yabancı ceviz (Juglans regia L.) çeşitlerinin Kahramanmaraş ekolojisine adaptasyonu, KSÜ Fen ve Mühendislik Dergisi 5(1), 148-158, (2002).

[2] A.S. Ikhsan, H. Topçu, M. Sütyemez, S. Kafkas, Novel 307 polymorphic SSR markers from BAC-end sequences in walnut (Juglans regia L.): Effects of motif types and repeat lengths on polymorphism and genetic diversity, Scientia Horticulturae 213, 1-4, (2016). Doi: 10.1016/j.scienta.2016.10.006.

[3] M.L. Martínez, M.A. Mattea, D.M. Maestri, Varietal and crop year effects on lipid composition of walnut (Juglans regia) genotypes, Journal of the American Oil Chemists' Society 83(9), 791-796, (2006).

[4] N. Çaglarirmak, Biochemical and physical properties of some walnut genotypes (Juglans regia L.), Nahrung - Food 47(1), 28-32, (2003). Doi: 10.1002/food.200390004.

[5] FAO, Statistical database: agricultural production of primary crop, p. Accessed 15 Feb 2020, (2020), [Online]. Available: http://www.fao.org/faostat/en/#home,.

[6] R.B.N. Prasad, Walnuts and Pecans, Encyclopaedia of Food Science, Food Technology and Nutrition, Academic Press, London, 4828-4831, (1994).

[7] L. Zwarts, G.P. Savage, D.L. McNeil, Fatty acid content of New Zealand-grown walnuts (Juglans regia L.), International Journal of Food Sciences and Nutrition 50(3), 189-194, (1999). Doi: 10.1080/096374899101229.

[8] J.S. Amaral, S. Casal, J. A. Pereira, R.M. Seabra, B.P.P. Oliveira, Determination of Sterol and Fatty Acid Compositions, Oxidative Stability, and Nutritional Value of Six Walnut (Juglans regia L.) Cultivars Grown in Portugal, Journal of Agricultural and Food Chemistry 51(26), 7698-7702, (2003). Doi: 10.1021/jf030451d.

[9] Y. Kagawa, Standard Tables of Food Composition in Japan, Kagawa Nutrition University Press, Tokyo, (2001).

[10] E. Ros et al., A Walnut Diet Improves Endothelial Function in Hypercholesterolemic Subjects: A Randomized Crossover Trial, Circulation 109(13), 1609-1614, (2004). Doi: 10.1161/01.CIR.0000124477.91474.FF.

[11] P.M. Kris-Etherton, Walnuts decrease risk of cardiovascular disease: A summary of efficacy and biologic mechanisms, Journal of Nutrition 144(4), 547-554, (2014). Doi: 10.3945/jn.113.182907.

[12] S.G. West et al., Effects of diets high in walnuts and flax oil on hemodynamic responses to stress and vascular endothelial function, Journal of the American College of Nutrition 29(6), 595-603, (2010). Doi: 10.1080/07315724.2010.10719898.

[13] T. Fukuda, H. Ito, T. Yoshida, Antioxidative polyphenols from walnuts (Juglans regia L.), Phytochemistry 63(7), 795-801, (2003). Doi: 10.1016/S0031-9422(03)00333-9.

[14] M. Kornsteiner, K.H. Wagner, I. Elmadfa, Tocopherols and total phenolics in 10 different nut types, Food Chemistry 98(2), 381-387, (2006). Doi: 10.1016/j.foodchem.2005.07.033.

[15] J.A. Pereira, I. Oliveira, A. Sousa, I.C.F.R. Ferreira, A. Bento, L. Estevinho, Bioactive properties and chemical composition of six walnut (Juglans regia L.) cultivars, Food and Chemical Toxicology 46(6), 2103-2111, (2008). Doi: 10.1016/j.fct.2008.02.002.

[16] M.V. Hapon, J.J. Boiteux, M.A. Fernández, G. Lucero, M.F. Silva, P.H. Pizzuolo, Efecto de compuestos fenólicos presentes en extractos de plantas argentinas en el crecimiento miceliar del fitopatógeno Botrytis cinerea pers, Phyton 86, 270-277, (2017).

[17] S.H. Häkkinen, A.R. Törrönen, Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: Influence of cultivar, cultivation site and technique, Food Research International 33(6), 517-524, (2000). Doi: 10.1016/S0963-9969(00)00086-7.

[18] Y. Kawai et al., Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries: Implication in the anti-atherosclerotic mechanism of dietary flavonoids, Journal of Biological Chemistry 283(14), 9424-9434, (2008). Doi: 10.1074/jbc.M706571200.

[19] J.S. Amaral, M.R. Alves, R.M. Seabra, B.P.P. Oliveira, 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). Doi: 10.1021/jf050342u.

[20] L.C. Greve et al., Variation in Polyunsaturated Fatty Acids Composition of Persian Walnut, Journal of the American Society for Horticultural Science 117(3), 518-522, (2019). Doi: 10.21273/jashs.117.3.518.

[21] O. Beyhan et al., Fat, fatty acids and tocopherol content of several walnut genotypes, Notulae Botanicae Horti Agrobotanici Cluj-Napoca 45(2), 437-441, (2017). Doi: 10.15835/nbha45210932.

[22] E.G. Bligh, W.J. Dyer, A rapıd method of total lıpıd extractıon and purıfıcatıon, Canadian Journal of Biochemistry and Physiology 37(1), 911-917, (Jan. 1959). Doi: 10.1139/y59-099.

[23] D. Firestone, Official methods and recommen-

ded practices of the american oil chemists' society (Method AOCS Cd 3d–63), IL: American Oil Chemists' Society Champaign, (1999).
[24] K. Ichihara, A. Shibahara, K. Yamamoto, T. Nakayama, An improved method for rapid analysis of the fatty acids of glycerolipids, Lipids 31(5), 535-539, (1996).
[25] G.A. Spanos, R.E. Wrolstad, Influence of Processing and Storage on the Phenolic Composition of Thompson Seedless Grape Juice, Journal of Agricultural and Food Chemistry, (1990). Doi: 10.1021/jf00097a030.
[26] M.E. Cuvelier, C. Berset, 4A Standard Calibration Techniques, The Microflown E-Book 28, 25-30, (1995). Doi: 10.1016/S0023-6438(95)80008-5.
[27] C. Yerlikaya, S. Yucel, Ü. Erturk, M. Korukluoglu, Proximate composition, minerals and fatty acid composition of Juglans Regia L. genotypes and cultivars grown in Turkey, Brazilian Archives of Biology and Technology 55(5), 677-683, (2012). Doi: 10.1590/S1516-89132012000500006.
[28] V. Akbari, R. Heidari, R. Jamei, Fatty acid compositions and nutritional value of six walnut, 1(1), 36-41, (2014).
[29] H. Ünver, E. Sakar, M. Sülüşoğlu, Bestimmung pomologischer und morphologischer Eigenschaften mit Hilfe der Fettsäurezusammensetzung bei Genotypen der Walnuss mit einem günstigen Verhältnis zwischen Nussschale und Nusskern, Erwerbs-Obstbau 58(1), 11-18, (2016). Doi: 10.1007/s10341-015-0249-3.
[30] I. Bouabdallah, I. Bouali, E. Martinez-Force, A. Albouchi, M.C. Perez Camino, S. Boukhchina, Composition of fatty acids, triacylglycerols and polar compounds of different walnut varieties (Juglans regia L.) from Tunisia, Natural Product Research 28(21), 1826-1833, (2014). Doi: 10.1080/14786419.2014.950573.
[31] L.C. Greve et al., Variation in polyunsaturated fatty acids composition of Persian walnut, Journal of the American Society for Horticultural Science 117(3), 518-522, (1992).
[32] J.M. Garcia, I.T. Agar, J. Streif, Lipid characterization in kernels from different walnut cultivars, Turk. J. Agric. For 18(3), 195-198, (1994).
[33] E. Ros, J. Mataix, Fatty acid composition of nuts–implications for cardiovascular health, British Journal of Nutrition 96(S2), S29-S35, (2006).
[34] E. Kafkas et al., Fatty Acid, Total Phenol and Tocopherol Profiles of Some Walnut Cultivars: A Comparative Study, Food and Nutrition Sciences 08(12), 1074-1084, (2017). Doi: 10.4236/fns.2017.812079.
[35] L.S. Maguire, S.M. O'Sullivan, K. Galvin, T.P. O'Connor, N.M. O'Brien, Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut, International Journal of Food Sciences and Nutrition 55(3), 171-178, (2004). Doi: 10.1080/09637480410001725175.
[36] L. Li, R. Tsao, R. Yang, J.K.G. Kramer, M. Hernandez, Fatty acid profiles, tocopherol contents, and antioxidant activities of heartnut (Juglans ailanthifolia Var. cordiformis) and Persian walnut (Juglans regia L), Journal of Agricultural and Food Chemistry 55(4), 1164-169, (2007). Doi: 10.1021/jf062322d.
[37] G. Uzunova, M. Perifanova-Nemska, M. Stojanova, S. Gandev, Chemical composition of walnut oil from fruits on different years old branches, Bulgarian Journal of Agricultural Science 21(3), 494-497, (2015).
[38] R. Aparicio, M.T. Morales, V. Alonso, Authentication of European Virgin Olive Oils by Their Chemical Compounds, Sensory Attributes, and Consumers' Attitudes, Journal of Agricultural and Food Chemistry 45(4), 1076-1083, (1997). Doi: 10.1021/jf960659h.
[39] Y. Wu, W. Yuan, X. Han, J. Hu, L. Yin, Z. Lv, Integrated analysis of fatty acid, sterol and tocopherol components of seed oils obtained from four varieties of industrial and environmental protection crops, Industrial Crops and Products 154, 112655, (2020).
[40] H. Kallio, B. Yang, P. Peippo, R. Tahvonen, R. Pan, Triacylglycerols, Glycerophospholipids, Tocopherols, and Tocotrienols in Berries and Seeds of Two Subspecies (ssp. sinensis and mongolica) of Sea Buckthorn (Hippopha> ë rhamnoides), Journal of Agricultural and Food Chemistry 50(10), 3004-3009, (2002).
[41] I.B. Abdallah et al., 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). Doi: 10.1016/j.foodchem.2014.10.095.
[42] D. Li, T. Saldeen, F. Romeo, J.L. Mehta, Relative effects of α-and γ-tocopherol on low-density lipoprotein oxidation and superoxide dismutase and nitric oxide synthase activity and protein expression in rats, Journal of Cardiovascular Pharmacology and Therapeutics 4(4), 219-226, (1999).
[43] I. Oliveira, A. Sousa, I.C.F.R. Ferreira, A. Bento, L. Estevinho, J. A. Pereira, Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks, Food and Chemical Toxicology 46(7), 2326-2331, (2008). Doi: 10.1016/j.fct.2008.03.017.
[44] L.C. Greve, McGranahan, Fatty acid and to-

copherol contents and oxidative stability of walnut oils, Food Chemistry 55(1), 74-78, (2011). Doi: 10.1007/s11746-999-0204-2.
[45] Y. Cai, Q. Luo, M. Sun, H. Corke, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, Life Sciences 74(17), 2157-2184, (2004). Doi: 10.1016/j.lfs.2003.09.047.
[46] X. Li, Y. Zhao, X. Gong, C. Zhao, X. Zhou, Quality Evaluation of Walnut Oil through HPLC and in Vitro Antioxidant Activity, Journal of Food and Nutrition Research, (2014). Doi: 10.12691/jfnr-2-5-6.