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Phenolic Compounds, Antioxidant Activity, Fatty Acids and Volatile Profiles of 18 Different Walnut (*Juglans regia* L.) Cultivars and Genotypes

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Abstract

The study was carried out to determine phenolic compounds, antioxidant activity, total lipids, saturated fatty acids, unsaturated fatty acids, and volatile organic compounds (VOCs) of 18 different walnut (*Juglans regia* L.) cultivars and genotypes grown in Usak province, Turkey. The biochemical compounds were identified for each cultivar and genotype and a comparative evaluation was carried out. The results showed that most of the biochemical compounds are significantly varying among each other ($p < 0.05$). The existence and abundance of the biochemical compounds in the cultivars and genotypes have not only found to be a cause of differentiation, but they have been important parameters for the similarities among the cultivars and genotypes. Total phenolic content and antioxidant activity ranged from 750.67 to 1245.64 mg GAE ml^{-1} and from 42.46 to 56.50% in kernels, respectively. The highest contents of the phenolic compounds were noted from gallic acid (11991 mg kg^{-1}), ellagic acid (1057.16 mg kg^{-1}), catechin (425.4 mg kg^{-1}), and rutin (216.6 mg kg^{-1}), as an average of all cultivars and genotypes. The highest lipid contents were noted from 'Franquette' and 'Fernette' as 64.28% and 63.26%, respectively, while the lowest content was noted from 'Oguzlar 77' (52.52%). A total of 42 VOCs were described from the 18 different walnut cultivars and genotypes. Overall, results showed that the phenolic profiles, fatty acids composition, and VOCs play an important role in similarities and diversities among the cultivars. According to the results, 7 different clusters were developed from the 18 cultivars and genotypes. Herein, 'Fernor', 'Maras 18', 'Arslan Local Type' and 'Cisco' cultivars were found to have the lowest biochemical compositions. The superior cultivars or genotypes, in terms of the abundance of the biochemical composition, were found to be 'Gulizar Hanim Type', 'Lara', 'Pedro', 'Tulane', 'Fernette', 'Balkan' and 'Franquetta', where they were found to develop 3 clusters by 1:3:3, respectively.

Keywords Food composition · Gallic acid · Ellagic acid · Total lipid · Ketones · Aldehydes · Walnut · *Juglans regia* L

Phenolverbindungen, antioxidative Aktivität, Fettsäuren und flüchtige Verbindungen 18 verschiedener Walnusssorten und -genotypen (*Juglans regia* L.)

Schlüsselwörter Walnuss · *Juglans regia* L · Gallussäure · Ellagsäure · Gesamtlipid · Ketone · Aldehyde

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Introduction

As a member of Juglandaceae family, walnut (*Juglans regia* L.) is one of the oldest cultivated and most popular nuts species in the world and is widespread throughout Turkey (Polat et al. 2015). Since the 21th century, numerous studies have been conducted about nuts, in order to determine and discuss the phytochemical and antioxidant properties and their contribution to human nutrition and health. The phytochemicals and phenolic profiles are of particular interest for human health and varying amounts and forms of those compounds provide varying antioxidant potentials to foods. Walnut fruits are unique natural source of basic nutrients. They are characterized by their high nutritional values and extraordinary effects on the human health (Bujdosó et al. 2010; Cosmulescu and Trandafir 2012). They are a valuable nutrient source, which can replace animal protein sources and contain a high content of unsaturated fatty acids which mostly consist of monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). Walnut fruits are also rich in vitamins, including thiamin, vitamin A, B and micro- and macro-elements (Pereira et al. 2008; Tosun et al. 2011). Walnuts do not contain cholesterol and unsaturated fat is high. Fruits are also very rich in terms of linoleic acid and linolenic acid, which are necessary for a healthy life. The clinical and epidemiological studies suggested that walnuts might have a significant role in the prevention of cardiovascular disease, improving human serum lipid profiles, LDL cholesterol and some types of cancer (Banel and Hu 2009; Zhang et al. 2009; Trandafir et al. 2016). These beneficial effects have been found to be affected by their biochemical compounds. Recently, some of the phenolic compounds have been identified and found to have protective effects on human health. Many researchers reported that walnuts are good sources of cancer chemopreventive polyphenolic compounds, ellagic acid and ellagitannins, which are found only in a limited number of fruits and nuts (Fukuda et al. 2003; Colaric et al. 2005; Arcan and Yemenicioğlu 2009). In the literature, walnut phenolic compounds have a stronger antioxidant effect than the nonpolar antioxidants found in the lipid fraction, such as tocopherols and tocotrienols, making them the main compounds responsible for the health protection provided by walnuts (Trandafir et al. 2016). Williamson and Manach (2007) reported that the five main phenolic compounds in walnut kernel are gallic and cinnamic acid, catechin, juglon and routine. Gallic acid is the easiest bioavailable compound for the human body, followed by catechin. Several studies showed that the juglon has anti-cancer activity against human tumor lines and has anti-cancer effect (Bonifazi et al. 2010; Bujdosó et al. 2010). Moreover, rutin has many physiological functions and antioxidant activity (Harborne and Williams 2000). Among other bioactive phenols, vanillic acid, epicatechin, cinnamic acid, which are characteristic for walnut (Bujdosó et al. 2014). The intensity and diversity of the volatile constituents in walnut are very important as the source of flavor for people. The aroma is determined by several compounds with very different chemical natures and includes hydrocarbons, esters, ketones, aldehydes and alcohols. It is usually accepted that the varietal aroma of nuts is mainly localized in the kernel and is significantly vary among cultivars (Lasekan and Abbas 2010). The studies about the volatile compounds in walnuts are very limited in the world. Recently, the development of different and rapid analysis in identification and concentration of volatile aromatic compounds has advanced attention. One such technique is the introduction of solid phase microextraction (SPME) as a unique sample preparation technique presented a relatively easy sampling of the volatiles present in the fruits (Kafkas et al. 2005a; Gozlekci et al. 2011). They are generally the most responsible for defining fruit aromas. In addition, SPME has the advantage of being flexible, simple, fast, precise and solvent-free technique. The chemical content and antioxidant capacity of fruits are affected by various factors. In particular, environmental conditions and genotype have great influence on the biochemical composition of the walnut kernels (Sanchez et al. 2014; Gundesli et al. 2019). In line with this information, the present study was designed to identify the phenolic compounds, antioxidant activity, total lipid, saturated fatty acid, unsaturated fatty acid and volatile organic compounds of 18 different walnut (*Juglans regia* L.) cultivars and genotypes grown in Usak province, Turkey. It was also aimed to determine the similarities and diversities among the cultivars and genotypes which would serve as a resource for breeders, growers and nutritionists.

Materials and Methods

Experimental Materials

Eighteen different cultivars or genotypes were selected from the Uşak city of Turkey located in the Aegean Region. Walnut fruits of the present study were harvested at commercial maturity depending on the cultivars or genotypes. In the trial, different trees were chosen for each cultivar or genotype, and 25 walnut fruits were harvested randomly. Triplicate analysis was made with selected walnuts, and each cultivar or genotype included 75 walnut fruits that were selected randomly. Kernel of walnut fruits was analyzed with an incubator after they dried.

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu reagent using the modified method of Spanos and Wrolstad (1990). 50 µl 80% methanolic extract of dried material was diluted by water and followed by adding 250 µl of Folin-Ciocalteu reagent and 750 µl sodium carbonate (20% w/v). The extracts were centrifuged (centrifuge NF 200, Nuve, Belgium) at 5500 r.p.m. for 15 min and filtered. The solvent was incubated at room temperature in dark for 2 h. The absorbance of all samples was measured at 760 nm using Multiskan TM GO Microplate Spectrophometer. The results were expressed as mg 100 g^{-1}.

Determination of DPPH Radical Scavenging Activity

The ability of hydrogen donating or radical scavenging of sample extracts was measured by using the stable free radical (DPPH) (1,1-dipheny 2-picrylhydrazyl) method according to the method of Brand-Williams et al. (1995), with some modifications. About 1950 µl of the 1 mM solution of methanolic DPPH was mixed with 50 µl extract solution in methanol. The absorbance was measured at 517 nm against the corresponding blank solution as 80% methanol and control were prepared by taking 1950 µl of DPPH by adding 50 µl distilled water instead of sample. Percentage inhibition of radical DPPH was calculated based on control reading by the following equation:

$$\text{DPPH-Percentage \%of reduction power} = ((\text{Ac}-\text{As})/\text{Ac}) \times 100$$

$$\text{DPPH \% inhibition} = (\text{Ac}-(\text{As}-\text{Ab})/\text{Ac}) \times 100$$

Ac: absorbance of control (standard)
As: absorbance of sample
Ab: absorbance of blank

All the samples were directly injected to the reverse phase chromatography column. The sugar standards, glucose, fructose, sucrose, were dissolved in water at a concentration of 30 mg ml^{-1}. All samples and standards were injected three times each and mean values were used.

Determination of Phenolic Compounds

For the extraction and hydrolysis procedure of phenolics, 0.5 g of homogenized and powdered samples were used for boiling refluxed for 1 hr (Maas et al. 1991). After cooling, the mixture was filtered and made up to 10 mL by using distilled water, and was used for HPLC analyses. The liquid chromatographic device (HPLC, SHIMADZU LC-20A) had an in-line degasser, pump, and controller to a photodiode array detector with the automatic injector (20 µL injection volume) attached to a PC with ChemStation chromatography manager software. Separations were carried out with 150 mm × 4.6 mm i. d., 5 µm, reverse-phase Nucleosil C18 analytical column (Supelco, PA) that operated at room temperature with 1 mL min^{-1} flow rate. Detection was made with a sensitivity of 0.1 a.u.f.s. between the wavelengths of 280 and 360 nm. Elution was carried out with a nonlinear gradient of solvent mixture 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B). The composition of B was increased to 13% from 5 in 15 min, to 15% in 5 min, and to 30% in 5 min for 3 min, to 45% in 4 min, for 3 min, to 90% in 5 min for 5 min, and returned to basal values in 5 min. By comparing retention times with authentic standards under analysis conditions and UV spectra with in-house PDA library, components were identified. Between injections 10 min equilibrium time was given. All of the samples were injected to the reverse phase chromatography column after filtration, gallic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid, rutintrihydrate, ellagic acid, quercetin and naringenin were dissolved in methanol at a concentration of 1 mg ml^{-1}, and 5 dilute solutions from them were used for preparing calibration curves. After extracting the recovered amounts of pure substances that were added to frozen strawberries before the trial, recoveries were measured. For HPLC analyses, 3 replicates were used from each sample. All samples and standards were injected three times, and mean values were used.

Determination of Fatty Acids

GC (Gas Chromatography)/FID (Flame Ionization Detector) (Perkin Elmer, Clarus 500, USA) with an SGE column (100 m × 0.32 mm, ID 0.25 µm) was used for fatty acid analysis. The oven temperature was 140 °C for 5 min for the GC condition; and was increased to 200 °C at a rate of 4 °C min^{-1} and to 220 °C at a rate of 1 °C min^{-1}. For identification and quantification of fatty acids, the FAME mix standard with 37 components was used.

Determination of Volatile Compounds

Volatile compounds were analyzed on an HS/GC/MS. The calyx of the fruits separated and homogenized using fruit blender. 10 g homogenate weighed and 0.5 g of NaCl added during 20 min at 30 °C incubation time. The SPME fibers 100 um PDMS (Polydimethylsiloxane; red), 85 um CAR/PDMS (Carboxen/Polydimethylsiloxane; lightblue) were compared (Kafkas et al. 2005b).

The flavor compounds of fig fruits were analyzed using a Perkin Elmer Clarus 600 T Gas chromatography mass spectrometry (GC/MS). HP-Innowax Agilent column

Table 1 Comparison of total phenolic contents (TPC) and antioxidant activity (AA) of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

Cultivars and genotypes	Total phenolic contents (mg $100\,g^{-1}$)	Antioxidant activity	
		% Inhibition	% of DPPH scavenging activity
Chandler	690.88 ± 14.52 n	**72.98 ± 4.70 ab**	**54.07 ± 4.70 a**
Balkan	750.67 ± 3.93 m	68.03 ± 4.28 de	49.12 ± 4.28 cd
Cisco	796.62 ± 16.321	66.60 ± 2.02 de	47.78 ± 2.02 d
Fernor	992.57 ± 10.53 fg	**75.41 ± 0.84 a**	**56.50 ± 0.84 a**
Maras 18	1014.19 ± 30.16de	70.16 ± 0.96 d	51.25 ± 0.96 c
Arslan Local	1100.77 ± 6.11 b	72.40 ± 0.39 b	53.49 ± 0.39 b
Kaman 4	998.65 ± 14.77 fg	69.50 ± 0.81 d	50.59 ± 0.81 c
Yalova 4	958.35 ± 36.92 h	72.19 ± 0.87bc	53.28 ± 0.87 b
Kaman 1	1074.80 ± 16.60 c	68.16 ± 0.06 e	49.25 ± 0.06 d
Sebin	874.04 ± 13.04 j	68.22 ± 4.67 d	49.31 ± 4.67 cd
Lara	894.41 ± 13.38ij	71.98 ± 0.09 c	53.07 ± 0.09bc
Franquetta	1013.3 ± 19.40ef	**74.22 ± 2.53 a**	**55.31 ± 2.53 a**
Haward	900.32 ± 0.86i	**73.81 ± 0.63 a**	**54.90 ± 0.63 a**
Tulane	977.39 ± 36.49fgh	63.80 ± 4.76 def	44.89 ± 4.76 de
Oguzlar 77	871.04 ± 18.13 j	61.37 ± 4.04ef	42.46 ± 4.04 e
Pedro	**1245.64 ± 1.85 a**	68.54 ± 1.75 d	49.63 ± 1.75 c
Fernette	869.78 ± 7.27 j	67.64 ± 1.02 de	48.74 ± 1.02 d
Gulizar Hanim	849.18 ± 11.62 k	70.61 ± 2.20 cd	51.70 ± 2.20 cd

(30 mx 0.25 mm i.d., 0.25 mmm thickness) was used and helium was used as carrier gas. The GC oven temperature was kept at 60 °C and programmed to 260 °C at 5 °C/min and then held constant at 260 °C for 40 min. The injector temperature was at 250 °C. The MS was taken at 70 eV. The mass range was m/z 30–400. A library search was carried out using the commercial Wiley, Nist and Flavor GC-MS Libraries. The mass spectra were also compared with those of reference compounds and confirmed with the aid of retention indices from published sources. Relative percentage amounts of the separated compounds were calculated from total ion chromatograms through the computerized.

Statistical Analysis

Raw data of the phenolic compounds, antioxidant activity, total lipids, saturated fatty acids, unsaturated fatty acids and volatile organic compounds was subjected to the analysis of variance to determine if there is significant difference among the 18 cultivars and genotypes. Thus, mean separations were carried according to Tukey's Honest Significant Difference test ($p \le 0.05$). SPSS software (version 22.0) was used for the statistical analysis. Correlation among the biochemical compounds, hierarchical clustering of the cultivars and genotypes and principal component analysis (PCA) with biplot were then performed with R 3.6.2 statistical program. Library 'corrplot' was used for correlation analysis, 'FactoMineR' library was used for hierarchical clustering and the PCA + biplot analysis was performed with 'factoextra' library.

Results and Discussions

Phenolic Profiles of 18 Walnut Cultivars and Genotypes

According to the results of the present study, the highest total phenolic content (TPC) was measured from the 'Pedro' cultivar and is followed by 'Arslan Local Type' genotype. The whole list can be followed from Table 1. Similar with these two cultivars and genotypes, the 'Kaman 1', 'Maras 15' and 'Franquetta' were found to have more than 1000 mg $100\,g^{-1}$ TPC. Surprisingly, the lowest TPC was observed from the 'Chandler' cultivar. On the other hand, the highest antioxidant activity (AA), as % inhibition, was noted from the 'Haward' cultivar and was followed by 'Franquetta', 'Fernor' and 'Chandler' and no significant difference was observed among these 4 cultivars. At the same time, the 'Oguzlar 77' cultivar with moderate level of TPC was found to have minimum AA as % inhibition. Similar results were then observed for the % of DPPH scavenging activity and the same cultivars were found to have highest values and same cultivar with the lowest value. DPPH assay has been widely used to determine the free radical scavenging activity of various plants and

Table 2 Comparison of phenolic compounds of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

Cultivars and genotypes	Phenolic compounds (mg kg^{-1})									
	Gallic acid	Catechin	Caffeic acid	Syringic acid	*p*-Coumaric acid	Rutintrihydrate	Ellagic acid	Quercetin	Naringenin	Juglon
Chandler	**22,738.5 a**	105.1 hi	8.7 j	12.4 h	11.5 h	127.6 ef	526.8 hi	51.4 fg	104.7 ef	9.6 bc
Balkan	11,338.3 ef	645.2 c	67.6 c	34.5 def	32.3 gh	134.3 e	1174.6 ef	37.6 hi	**654.3 a**	**10.9 a**
Cisco	10,762.1	79.3 i	66.5 c	31.7 ef	55.1 ef	58.7 gh	317.5 i	24.8 ij	180.9 e	6.2 cd
Fernor	11,876.1 def	311.5 g	49.8 ef	8.8 h	61.7 de	77.3 gh	320.8 i	11.8 j	148.1 ef	7.3 bcd
Maras 18	4594.1 h	330.0 fg	31.7 hi	16.3 gh	25.6 gh	84.5 gh	417.3 hi	27.4 ij	77.3 f	6.7 cd
Arslan Local	8325.7 g	115.2 hi	54.3 de	23.6 fg	18.7 gh	113.5 ef	539.6 h	24.8 hi	111.3 ef	7.9 bc
Kaman 4	4873.9 h	152.8 h	32.9 h	**58.0 a**	28.8 gh	125.0 ef	1106.9 f	39.6 gh	343.6 cd	7.5 bcd
Yalova 4	11,823.5 def	368.0 f	36.4 gh	40.4 cde	39.0 fg	101.9 ef	1462.8 bcd	60.2 ef	358.0 cd	9.4 bc
Kaman 1	11,092.8 ef	525.0 de	23.0 i	40.5 cde	14.6 h	50.6 h	745.8 g	34.8 hi	312.5 cd	7.4 bcd
Sebin	10,353.5 fg	490.1 e	42.7 fg	32.2 ef	68.2 cde	273.0 c	1294.9 de	69.4 de	490.9 b	7.3 bcd
Lara	11,371.7 def	670.1 c	81.5 b	**59.9 a**	199.0 b	**441.5 a**	**1705.1 a**	**141.6 a**	363.4 c	8.9 bc
Franquetta	19,462.2 b	681.5 c	**98.5 a**	37.8 de	**254.4 a**	**429.2 a**	1518.7 bc	84.8 bc	473.0 b	7.9 bcd
Haward	17,534.3 bc	71.9 i	31.8 hi	**53.3 ab**	14.5 h	378.1 b	1338.2 cde	73.3 cde	173.2 e	9.2 bc
Tulane	12,828.0 de	549.9 d	60.6 cd	44.0 bcd	86.2 c	397.6 ab	1342.5 cde	**142.1 a**	355.6 cd	9.3 bc
Oguzlar 77	13,718.5 d	373.0 f	10.8 j	10.4 h	16.9 h	184.3 d	1420.2 bcd	84.7 bc	275.6 d	**10.2 ab**
Pedro	11,131.7 ef	733.9 b	50.2 ef	**53.3 ab**	**258.5 a**	286.8 c	1527.0 ab	87.0 b	318.0 cd	8.1 bc
Fernette	16,197.0 c	557.9 d	35.9 gh	36.2 de	81.0 cd	**443.6 a**	1449.6 bcd	80.1 cd	286.8 cd	**10.2 ab**
Gulizar Hanim	5819.4 h	**897.1 a**	53.6 de	50.4 abc	80.4 cd	191.8 d	828.0 g	82.6 bc	182.2 e	**11.1 a**
Average	**11,991.2**	**425.4**	**46.5**	**35.8**	**74.8**	**216.6**	**1057.6**	**64.3**	**289.4**	**8.6**

pure compounds (Sousa et al. 2008). Results of the present study support the well-known information that the AA is not only a result of the TPC and the total flavonoid content also has significant influence on the antioxidant characteristics of the fruits (Gianda 2013). The TPC values of the walnut kernels in the present study were found to be about 10-fold of the walnut green husks values reported by Oliveira et al. (2008) for 'Franquette', 'Mayette', 'Marbot', 'Mellanaise' and 'Parisienne' cultivars. This is in agreement with the some previous studies which reproted that the phenolic profiles of the fruits significantly vary during ripening and diverse among the fruit parts (Bizjak et al. 2013; Zorenc et al. 2016).

A total of 10 phenolic compounds were determined from the walnut kernel (Table 2). Averages of the 18 different cultivars or genotypes suggest that the most abundant phenolic compound in the walnut kernels was gallic acid with 11,991.2 mg kg^{-1}, followed by ellagic acid with 1057.6 mg kg^{-1}. All other 8 phenolic compounds were found to have less than 430 mg kg^{-1} concentration. The least abundant compound was found to be juglon. As a support to the results about the antioxidant activity, the cultivar of 'Chandler' was found to have the highest concentration of gallic acid. However, 'Chandler' cultivar was found to be superior only for gallic acid and the concentrations of other 9 phenolic compounds were less than the other 17 cultivars or genotypes. The 'Lara' cultivar with moderate to high AA, was found to be superior for the second most important phenolic compound (ellagic acid). Ellagic acid was found to be highest at the 'Lara' cultivar. The phenolic profiles of the walnut cultivars and genotypes in the present study were found to be higher than in the reports of Fabani et al. (2013) and Garavand et al. (2015); but similar to the reports of Harnly et al. (2006), Kalogeropoulos et al. (2013) and Slatnar et al. (2015) who reported higher concentrations of gallic acid, ellagic acid and catechin. The main difference between the present study and those of previous studies is juglon. None of those studies did mention juglon, which was detected in the present study but in trace amount. The amounts of the phenolics detected in the current study were also notably higher than the results of Vu et al. (2018) who characterized the phenolic contents of 11 different walnut cultivars in the United States.

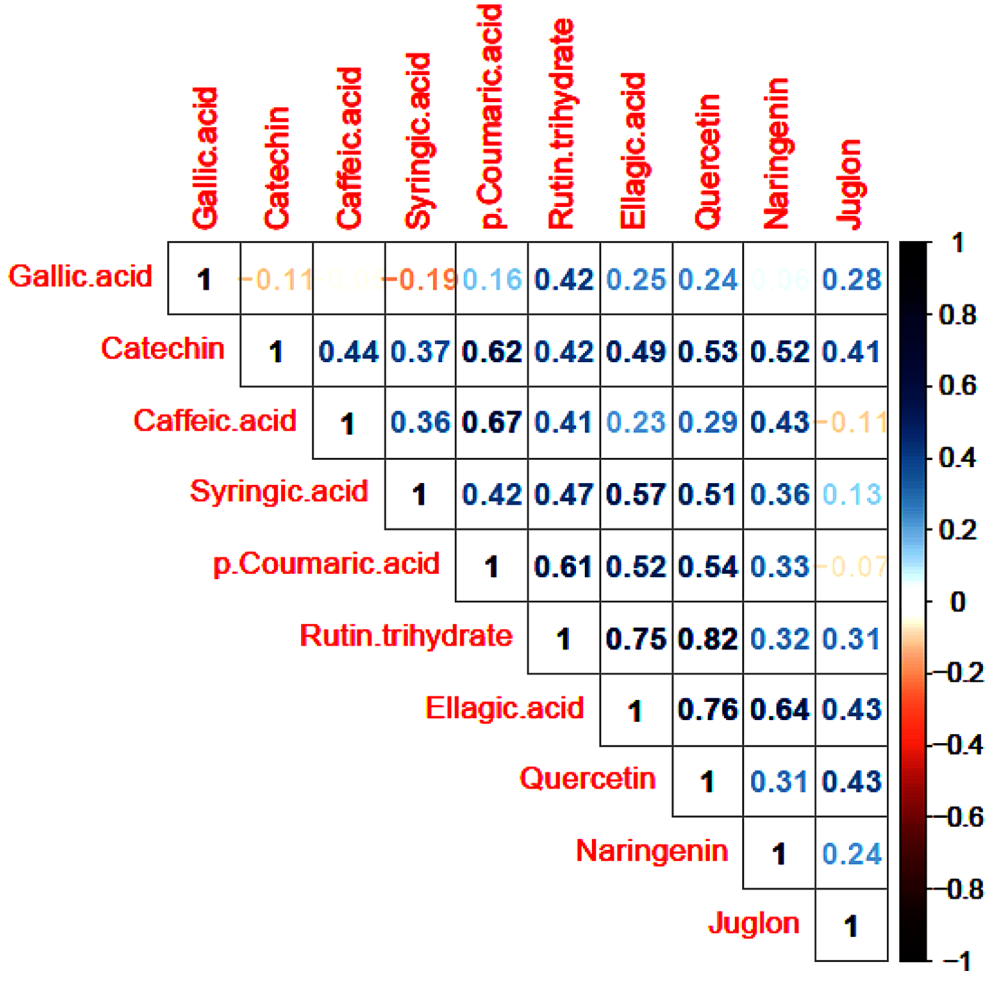


Fig. 1 Correlation among the phenolic compounds of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

The 'Lara' cultivar was also superior to syringic acid, rutintrihydrate and quercetin compounds. Supporting to the results of AA, the 'Franquetta' cultivar with high AA was also found to be superior for three phenolic compounds: caffeic acid, *p*-coumaric acid and rutintrihydrate. Moreover, the 'Pedro' cultivar, which was noted to have the highest TPC, was also found to have the highest *p*-coumaric acid content and one of the highest values for syringic acid. An important result of the present study is that the 'Gulizar Hanim Type' genotype was found to be superior for catechin (the 3rd most abundant phenolic compound in the present study) and for juglon. Finally, the naringenin content was highest at the 'Balkan' cultivar. Results of the present study showed that the concentrations of the phenolic compound significantly vary among the cultivars and genotypes and antioxidant activity of cultivars is highly dependent upon not only the total phenolic contents but upon the phenolic compounds including flavonoids.

According to the results, gallic acid and juglon, which have the highest and lowest concentration in this study, respectively, were found to have negligible correlations with the other phenolic compounds. However, correlation analysis showed that most of other phenolic compounds have moderate to high positive correlation with each other (Fig. 1). The highest correlation was noted between rutintrihydrate and quercetin with $R^2 = 0.82$ and followed by the correlation between ellagic acid and quercetin. Correlations among the phenolic compounds might be a useful tool for the estimation of one phenolic compound with the others and to understand the antioxidant activity of the fruits.

Moreover, hierarchical clustering on principal components (HCPC) analysis was performed to reduce data noise and provide better understanding of the data. Results suggested that the 18 cultivars or genotypes of the present study can be grouped into 3 clusters. The use of regular clustering figures can sometimes cause mis-understanding of the data in which they do not provide close look to the data points on the cluster map. The three-dimensional plot of HCPC (Fig. 2) made it possible to determine whether the data points of the same cluster are close to each other or not. Herein, the 'Chandler' cultivar seems a little far from other cultivar. This can be explained with the high concentration of gallic acid, which is the most abundant phenolic compound in the walnut kernels in the present study. However, the results made it possible to separate the cultivars or genotypes in three clusters. Herein, the first cluster includes 'Chandler', 'Cisco', 'Fernor', 'Maras 18' and 'Arslan Local Type'. Comparison of this result with the phenolic compounds showed that the cultivars or genotypes in this group (except 'Chandler') have lower compositions of the phenolic compounds. The second group was found to consist of the cultivars or genotypes of 'Kaman 4', 'Yalova 4', 'Kaman 1', 'Sebin', 'Haward', 'Oguzlar 77',

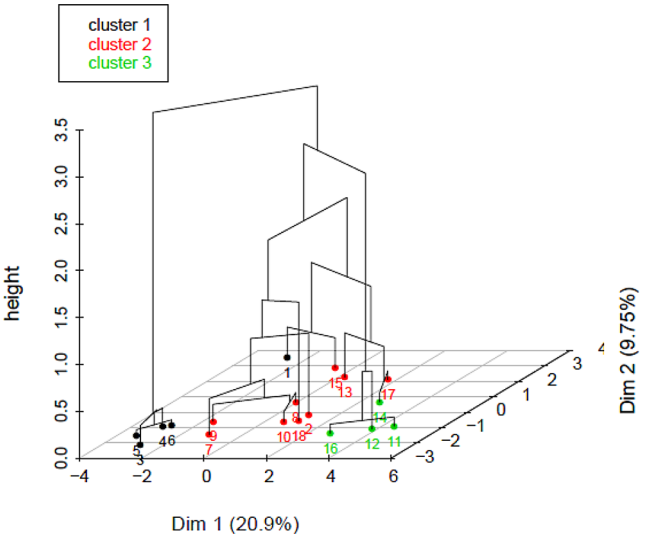


Fig. 2 Hierarchical clustering on the factor map of the phenolic compounds determined from several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey. (1: 'Chandler', 2: 'Balkan', 3: 'Cisco', 4: 'Fernor', 5: 'Maras 18', 6: 'Arslan Local Type', 7: 'Kaman 4', 8: 'Yalova 4', 9: 'Kaman 1', 10: 'Sebin', 11: 'Lara', 12: 'Franquetta', 13: 'Haward', 14: 'Tulane', 15: 'Oguzlar 77', 16: 'Pedro', 17: 'Fernette' and 18: 'Gulizar Hanim Type')

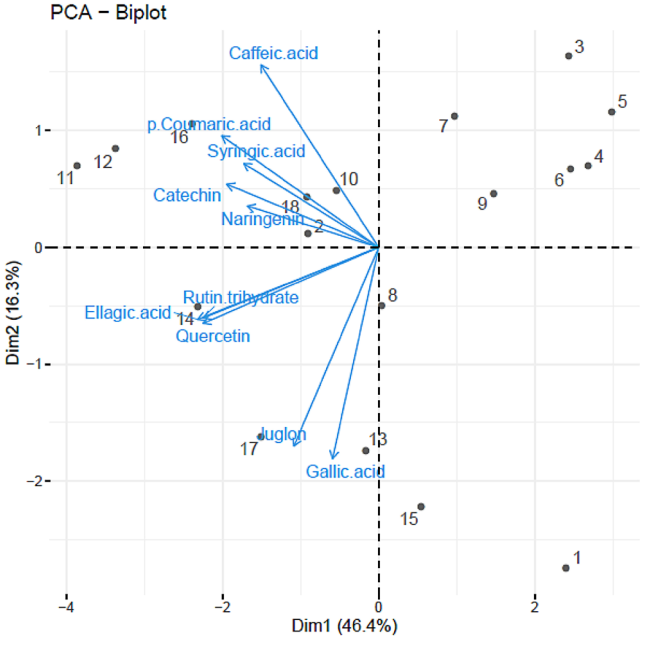


Fig. 3 PCA-biplot analysis for the phenolic compounds of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey. (1: 'Chandler', 2: 'Balkan', 3: 'Cisco', 4: 'Fernor', 5: 'Maras 18', 6: 'Arslan Local Type', 7: 'Kaman 4', 8: 'Yalova 4', 9: 'Kaman 1', 10: 'Sebin', 11: 'Lara', 12: 'Franquetta', 13: 'Haward', 14: 'Tulane', 15: 'Oguzlar 77', 16: 'Pedro', 17: 'Fernette' and 18: 'Gulizar Hanim Type')

Table 3 Comparison of total lipids (%) and saturated fatty acids (%) of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

Cultivars and genotypes	Total lipids (%)	Saturated fatty acids (%)				
		Myristic acid C14:0	Palmitic acid C16:0	Stearic acid C18:0	Arachidic acid C20:0	Σ Saturated FA
Chandler	58.89 b	0.12 e	7.50 e	0.12 f	0.27 e	8.01 f
Balkan	57.91 c	**0.74 a**	**8.88 a**	**0.66 a**	0.54bc	**10.82 a**
Cisco	57.43 c	0.18 de	7.44 f	0.14ef	0.34 d	8.10 f
Fernor	55.41 d	0.30bc	**8.66 ab**	0.15 e	0.25ef	9.36 c
Maras 18	57.17 c	0.19 e	7.09 g	0.13 f	0.30 e	7.71 h
Arslan	50.88 g	0.18 de	7.04 g	0.10 fg	0.35 d	7.67 h
Kaman 4	59.37 b	0.25 cd	8.39 c	0.08 g	0.31 de	9.03 d
Yalova 4	54.50 de	0.10 f	7.12 g	0.07 g	0.29 e	7.58i
Kaman 1	51.54ef	**0.72 a**	7.80 cd	0.46 b	0.78 ab	9.76 b
Sebin	59.41 b	0.47 ab	7.88 d	0.36 c	**0.99 a**	9.95 ab
Lara	58.69 b	0.33 b	8.30 c	0.27 d	0.17 f	9.07 d
Franquetta	**64.28 a**	0.36 b	**9.06 a**	0.39 c	0.26 e	**10.07 a**
Haward	57.37 c	0.31bc	7.11 g	0.18 e	0.09 g	7.69 h
Tulane	59.23 b	0.26 cd	8.57 b	0.17 e	0.10 g	9.10 d
Oguzlar 77	52.52ef	0.35 b	7.71 e	0.48 b	0.34 d	8.88 e
Pedro	53.88 e	0.31bc	7.37 fg	0.17 e	0.11 f	7.96 fg
Fernette	**63.26 ab**	0.31bc	7.92 d	0.28 d	0.17 f	8.68 e
Gulizar Hanim	55.68 cd	0.34 b	6.72 h	0.49 b	0.36 cd	7.91 g

Table 4 Comparison of unsaturated fatty acids (%) of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

Cultivars and genotypes	Unsaturated fatty acids (%)					
	Polyunsaturated fatty acids (PUFA)			Monounsaturated fatty acids (MUFA)		
	Linoleic acid	Linolenic acid	Total PUFA	Oleic acid	Palmitoleic acid	Total MUFA
Chandler	60.13 b	**14.05 a**	**74.17 ab**	0.21 j	17.60 h	17.82 h
Balkan	59.52bc	10.17 de	69.69 de	0.49 fg	18.99gh	19.48 g
Cisco	59.10 c	8.90 g	68.00 e	0.19 j	23.71 c	23.90 d
Fernor	**62.64 a**	9.43 f	72.07 b	0.31i	18.26 h	18.57 h
Maras 18	55.62 e	9.33 f	64.95 g	0.15jk	27.23 b	27.39 c
Arslan	60.21 b	10.99 d	71.21 c	0.13 k	21.10 d	21.12ef
Kaman 4	53.98 f	8.92 g	62.90 h	0.17 j	**27.90 ab**	28.07bc
Yalova 4	**63.61 a**	11.06 cd	**74.67 a**	0.11 k	17.82 h	17.82 h
Kaman 1	48.78 g	11.72 c	60.50i	1.01 b	**28.73 a**	**29.74 ab**
Sebin	52.19ef	9.99ef	62.18 h	0.64 d	27.48 b	28.12 b
Lara	56.59 d	9.52 f	66.11 f	0.51 f	24.31 c	24.82 d
Franquetta	60.08 b	10.68 d	70.76 c	0.72 c	18.47 h	19.19 g
Haward	59.23bc	12.82 b	72.06 b	0.20 j	20.06 f	20.26 g
Tulane	58.80 c	11.48 c	70.27 cd	0.35 h	20.37 e	20.72 f
Oguzlar 77	57.96 d	11.78 c	69.74 d	0.88 c	20.50 de	21.37 e
Pedro	**60.86 a**	10.39 d	71.25 b	0.40gh	20.38 e	20.78 f
Fernette	60.66 ab	10.14 de	70.80 c	0.55ef	20.13ef	20.68 fg
Gulizar Hanim	52.59ef	7.77 h	60.35i	**3.60 a**	**28.14 a**	**31.74 a**

'Fernette', 'Gulizar Hanim Type' and 'Balkan'. This group was characterised with the moderate amount of phenolic compounds. Finally, the final group was found to be composed of 'Lara', 'Franquetta', 'Tulane' and 'Pedro', and noted to have the highest concentrations of phenolic compounds. In the following sections, the cultivars or genotypes were also compared for volatile profiles and for the combination of phenolic compounds and volatile profiles.

The PCA-biplot analysis supported the results of HCPC (Fig. 3). The loadings (herein phenolic compounds) made it possible for us to understand what produces the differences among the clusters. The angles between the loadings also made it possible to understand how those phenolic correlate with each other. These are similar with the data provided in Fig. 1 and discussed above. The important result herein is that none of the phenolic compounds were found to be effective on the cultivars or genotypes in cluster 1: 'Chandler', 'Cisco', 'Fernor', 'Maras 18' and 'Arslan Local Type' (right up and right down of the Fig. 3). According to the results, the similarity between the 'Haward' and 'Fernette' cultivars is coming from the high concentrations of gallic acid and juglon. Moreover, these two phenolic compounds make it possible to distinguish these cultivars from the others. As discussed above, the rutintrihydrate, ellagic acid and quercetin were found to have high correlation among each other and they were found to distinguish 'Tulane' cultivar from the others. Finally, the moderate to high correlations among caffeic acid, *p*-coumaric acid, syringic acid, catechin and naringenin were found to explain the similarity among 'Balkan', 'Sebin', 'Lara', 'Franquetta', 'Pedro' and 'Gulizar Hanim Type'.

Total Lipids, Saturated and Unsaturated Fatty Acids of 18 Walnut Cultivars and Genotypes

The total lipid and saturated fatty acid composition of the 18 walnut cultivars are given in Table 3. The total lipid composition of the 18 different walnut cultivar or genotypes was found to vary from 50.88% to 64.28%. According to the results obtained, the highest lipid concentration was found at the 'Franquetta' cultivar and followed by 'Fernette'. No significant difference was obtained between these two cultivars. The 'Franquetta' cultivar was also found to be superior for palmitic acid, which is the most abundant saturated fatty acid. Palmitic acid was also found to be high at the 'Balkan' and 'Fernor' cultivars. Moreover the 'Balkan' cultivar was also found to be superior for myristic acid and stearic acid. Therefore, the total saturated fatty acid composition was also highest at the 'Balkan' cultivar and followed by 'Franquetta'.

Unsaturated fatty acids generally predominate over the saturated fatty acids (Ha and Bhagavan 2011). Results of the present study supported this knowledge, and the percentage of unsaturated fatty acids composition was found to be higher than the saturated fatty acids. Unsaturated fatty acids contain one (monounsaturated—MUFA) or more double (polyunsaturated—PUFA) bonds. The percentage of PUFA was found to be higher than the MUFA. The highest predominant cultivar for PUFA was observed to be 'Yalova 4' and the predominant cultivar for MUFA was found to be 'Gulizar Hanim Type' genotype. The PUFAs of the cultivars and genotypes were found to be linoleic acid and linolenic acid, while the MUFAs of the walnut cultivars and genotypes were oleic acid and palmitoleic acid (Table 4). Those results are in accordance with Beck et al. (2014) who reported that walnuts are rich in linolenic acids. The content of linoleic acid of walnut kernels in the present study is in conjunction with the findings of Bayazit and Sümbül (2012), but the palmitoleic acid and linolenic acid contents are higher. The oleic acid composition of the present study is also lower than the results of Yerlikaya et al. (2012) and Şimşek (2016). Overall, the results about the concentrations and changes in the unsaturated fatty acids are all in agreement with the results of Bouabdallah et al. (2014) and Gao et al. (2019). Moreover, similarly in both the present study and the study of Gao et al. (2019), the increase linoleic acid content resulted with a slight decrease in oleic acid, which is mainy found in olive oil (Ballus et al. 2015). This can be attributed to the relative balance between linoleic acid and oleic aid (Shi et al. 2018).

Volatile Organic Compound (VOC) Profiles of 18 Walnut Cultivars and Genotypes

Product flavour is among the most important quality indicators of the fruits and is responsible for the sensory attributes of food. It is the combination of taste and aroma (volatiles) and together with size and colour, plays an important role in the consumers' first indication of the quality (Cardello 1994). Therefore, fruits' aroma is the complex mixture of alcohols, esters, aldehydes, acids and ketones (Nie et al. 2004). Volatile organic compounds (VOCs) are organic compounds with relatively low molecular weight and are easily evaporated at room temperature. VOCs include a variety of chemicals and are known to have positive or negative effects on human health for short- and long-term (Quah 2016). A total of 42 volatile organic compounds were described from the 18 different walnut cultivars and genotypes during the present study. These volatile compounds were belonging to 5 different groups. The names and total VOCs of each group are as: alcohols (#7), esters (#12), aldehydes (#15), acids (#6) and ketones (#2). The whole lists of the volatile organic compounds are given in Appendix 1–5 (see online). The total number of VOCs detected in the present study was found to be slightly higher than the results of San Román et al. (2015), who reported 21 VOCs

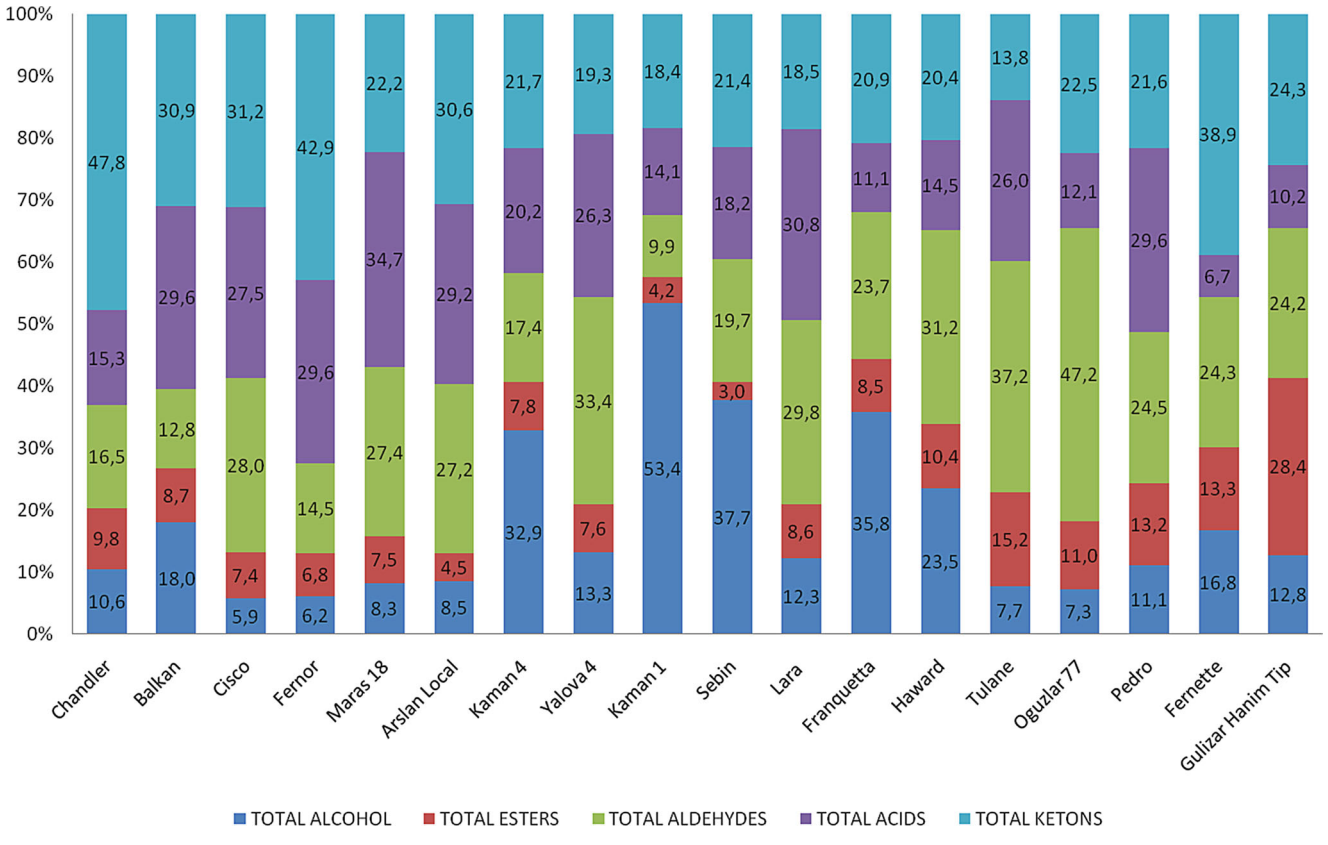


Fig. 4 Comparison of the groups of volatile organic compounds (VOCs) of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

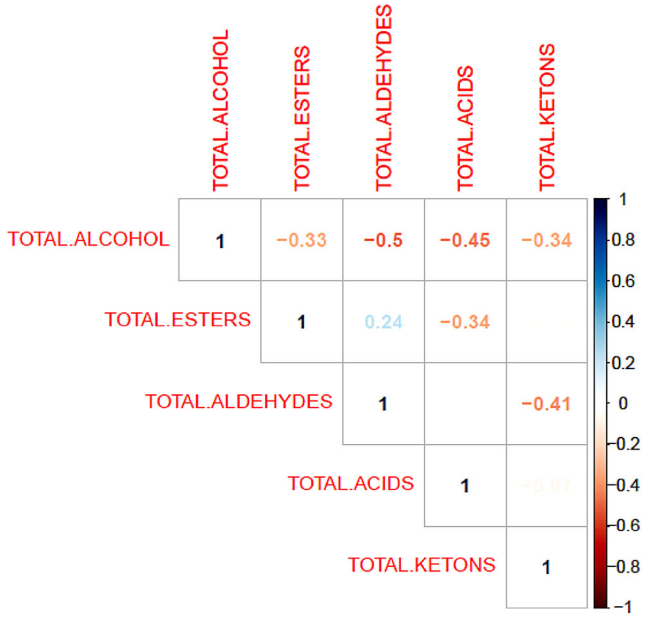


Fig. 5 Correlation among the VOCs groups of several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

(alcohols, ketones, aldehydes, esters, and aliphatic hydrocarbons), but in accordance with the same study in terms of the compounds. Results are also differing from the study of Abdallah et al. (2015) who reported that the main volatiles at walnut kernels are the aldehydes and alcohols. Herein, it was observed that ketones cover the highest percentage of the volatile compounds. Here, it is also important to say that Abdallah et al. (2015) did not mention the ketones in their studies. In other words, rather than ketones, the results of the present study are in agreement with the findings of Abdallah et al. (2015).

Results of the present study showed that the cultivars and genotypes are differing from each other in terms of the contents of VOCs. For example, the 'Chandler' cultivar was predominant with ketones, while the 'Maras 18' cultivar was predominant with acids. Moreover, the highest percentage of alcohol was noted from 'Kaman 1' cultivar. The predominant cultivars for ester and aldehydes were also different. They were noted to be 'Gulizar Hanim Type' and 'Oguzlar 77', respectively (Fig. 4).

According to the results obtained, the percentages of the different groups of VOCs were found to have negligible correlation with each other (Fig. 5).

Similar with the phenolic profiles, the HCPC analysis for VOCs resulted with 3 clusters (Fig. 6). However, the

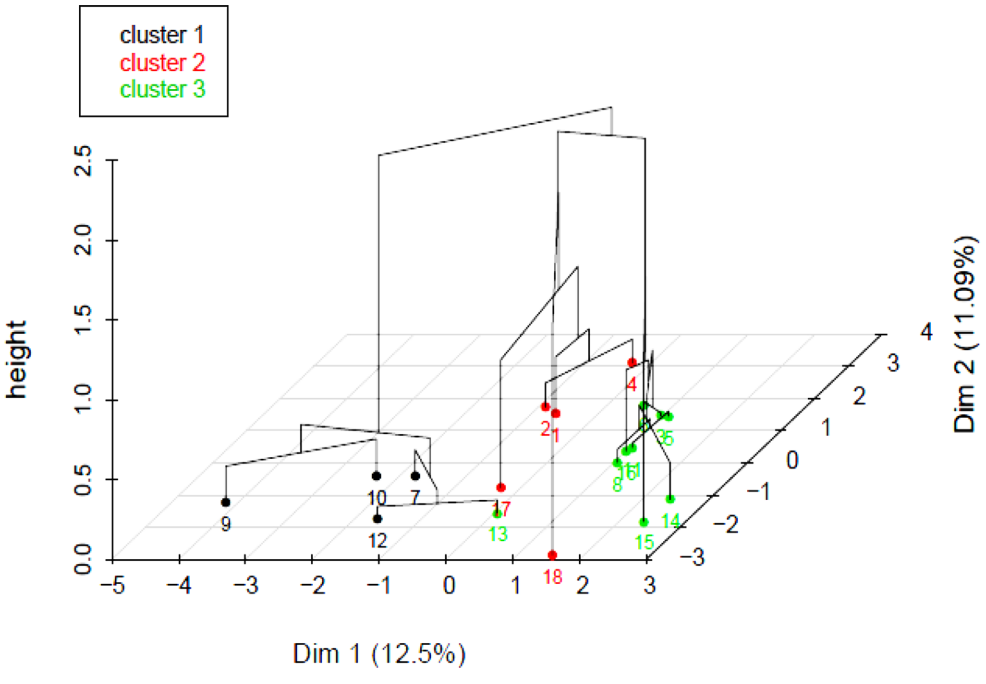


Fig. 6 Hierarchical clustering on the factor map of the volatile groups determined from several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey. (1: 'Chandler', 2: 'Balkan', 3: 'Cisco', 4: 'Fernor', 5: 'Maras 18', 6: 'Arslan Local Type', 7: 'Kaman 4', 8: 'Yalova 4', 9: 'Kaman 1', 10: 'Sebin', 11: 'Lara', 12: 'Franquetta', 13: 'Haward', 14: 'Tulane', 15: 'Oguzlar 77', 16: 'Pedro', 17: 'Fernette' and 18: 'Gulizar Hanim Type')

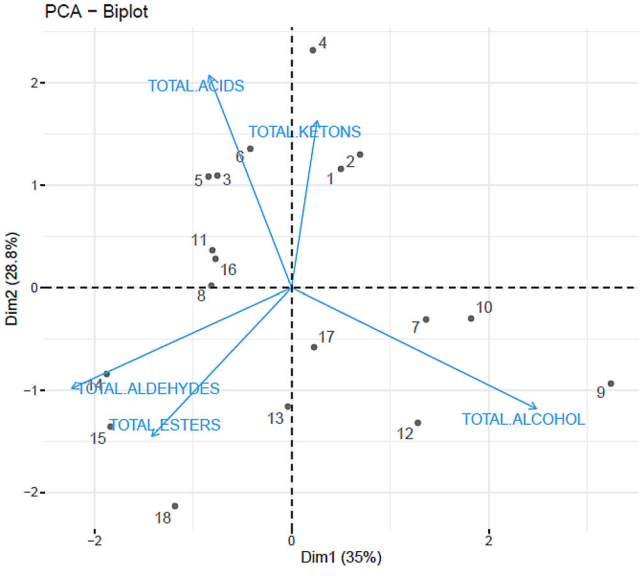


Fig. 7 PCA-biplot analysis for the phenolic compounds of several walnut (*Juglansregia* L.) cultivars and genotypes grown in Turkey. (1: 'Chandler', 2: 'Balkan', 3: 'Cisco', 4: 'Fernor', 5: 'Maras 18', 6: 'Arslan Local Type', 7: 'Kaman 4', 8: 'Yalova 4', 9: 'Kaman 1', 10: 'Sebin', 11: 'Lara', 12: 'Franquetta', 13: 'Haward', 14: 'Tulane', 15: 'Oguzlar 77', 16: 'Pedro', 17: 'Fernette' and 18: 'Gulizar Hanim Type')

cultivars and genotypes in each cluster were found to differ from the clusters of phenolic profiles. The first cluster for VOCs was found to be composed of 'Kaman 4', 'Kaman 1', 'Sebin' and 'Franquetta' cultivars. The important characteristics of the cultivars within this cluster were found to be high contents of alcohols and less percentage of acids and ketones. The second cluster included 'Chandler', 'Balkan', 'Fernor', 'Fernette' and 'Gulizar Hanim Type' cultivars and genotypes. The most common characteristic of the second cluster was found to be higher percentages of acids and ketones. The third cluster included the highest number of cultivars and genotypes, which are: 'Cisco', 'Maras 18', 'Arslan Local Type', 'Yalova 4', 'Lara', 'Haward', 'Tulane', 'Oguzlar 77' and 'Pedro'. This group was characterised with high aldehyde, moderate acid and ketone and low ester and alcohol concentrations.

The PCA-biplot analysis (Fig. 7) supported the HCPC analysis for VOCs (Fig. 6), but also made it possible to identify real similarities and differences among the cultivars and genotypes. The loadings (herein groups of VOCs) made it possible for us to understand what produces the differences among the clusters. The angles between the loadings also made it possible to understand how those volatile groups correlate with each other. It is clear from the Fig. 6 that the common characteristic of the cluster 1 (located on the right down of the Fig. 6) is the high content of alcohol. The second cluster which was developed from the HCPC analysis in Fig. 5 was characterised by its high acids and ketones content. Herein PCA-biplot Analysis made it possible to understand that some cultivars from third cluster ('Cisco', 'Maras 18' and 'Arslan Local Type') together with 'Chandler', 'Balkan' and 'Fernor' are characterised by their high content of total acids and total ketones. According to the results, total aldehydes is the common factor for 'Tulane' and 'Oguzlar 77' cultivars where the high content of total ester is the most common characteristic of 'Oguzlar 77' and 'Gulizar Hanim Type'.

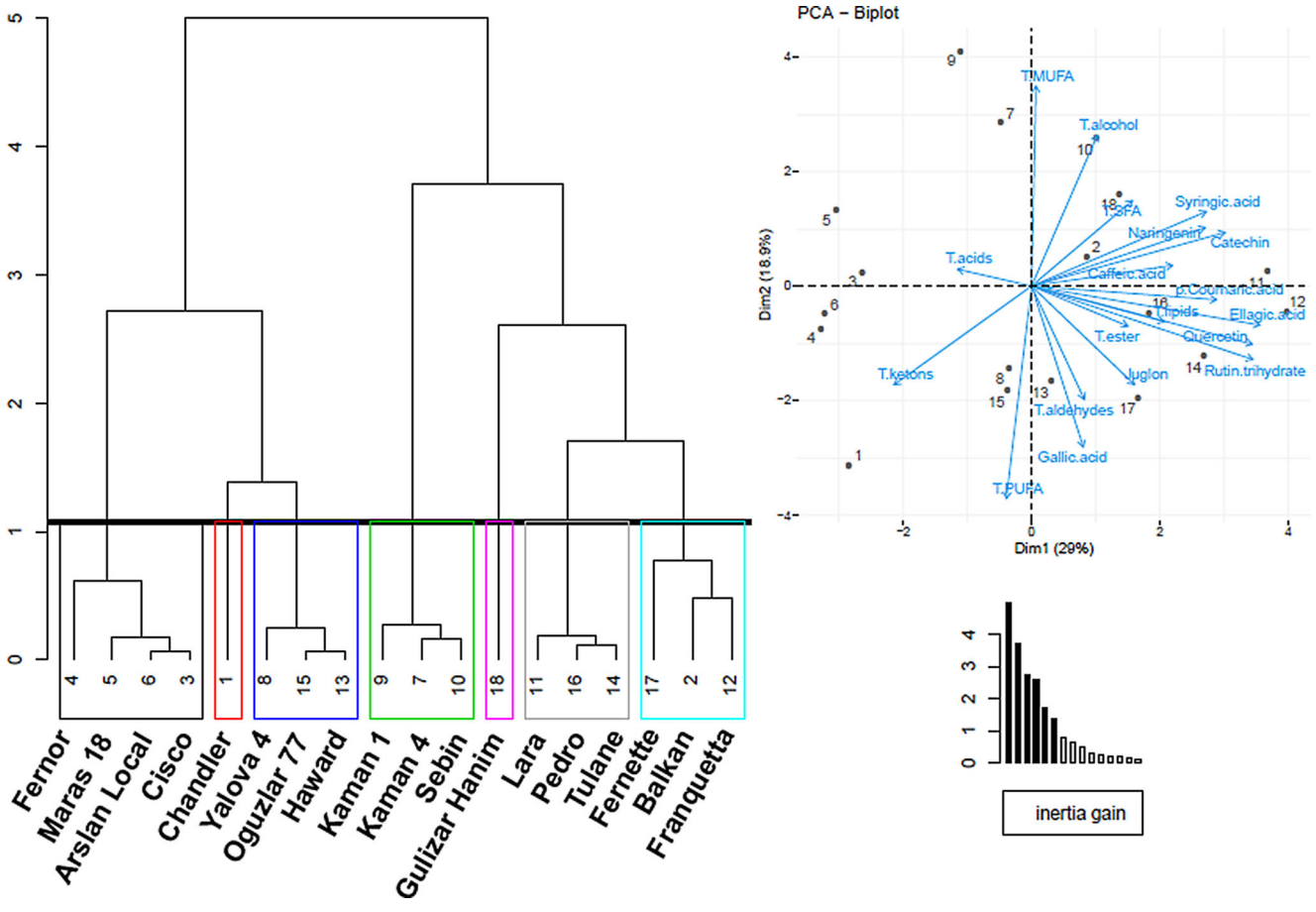


Fig. 8 Hierarchical clustering and PCA-biplot analysis for the combination of phenolic compounds, total lipids, saturated and unsaturated fatty acids and volatile profiles determined from several walnut (*Juglans regia* L.) cultivars and genotypes grown in Turkey

Overall Comparison: Similarities and Diversities of 18 Walnut Cultivars

The overall comparison of the phenolic compounds, total lipids, saturated and unsaturated fatty acids and volatile profiles resulted with 7 different clusters. The first cluster was found to consist of 'Fernor', 'Maras 18', 'Arslan Local Type' and 'Cisco'. As can be followed from the PCA-biplot (Fig. 8) these cultivars were found to have the lowest biochemical composition. The most important characteristics of these 4 cultivars and genotypes were observed to be higher contents of total acids and total ketones. The 'Chandler' cultivar was found to differ from all cultivars and genotypes and was placed in a separate cluster. The important characteristics of the 'Chandler' was found to be high contents of gallic acids, total aldehydes, total ketones and total polyunsaturated fatty acids. The third cluster was found to be the closest group to the 'Chandler' with high contents of gallic acids, total aldehydes and total polyunsaturated fatty acids. The main difference between cluster 3 and 'Chandler' cultivar was found to be the total ketones content. Furthermore, the 'Kaman 1', 'Kaman 4' and 'Sebin' cultivars were then found to have high total monounsaturated fatty acids and total alcohol. The other 7 cultivars ('Gulizar Hanim Type', 'Lara', 'Pedro', 'Tulane', 'Fernette', 'Balkan' and 'Franquetta') were found to have higher concentrations of phenolic compounds and the phenolics were observed to have higher influence on the similarities and diversities among the cultivars. Herein, the 'Gulizar Hanim Type' genotype was found to have high saturated fatty acid, and this characteristic diversified it from the others.

Conclusion

To sum up, results of the present study showed that the walnut cultivars and genotypes have high antioxidant capacity which is result of high phenolic compounds. Results also showed that the 18 different walnut cultivars and genotypes have some important similarities and diversities in terms of phenolic compounds, total lipids, unsaturated and saturated fatty acids and volatile organic compounds. According to the results, the 'Pedro' cultivar is predomi-

nant in terms of total phenolic content and is followed by 'Arslan Local Type' genotype. Moreover, the highest antioxidant activity, both as % inhibition and % of DPPH scavenging activity, was noted from the 'Haward' cultivar and was followed by 'Franquetta', 'Fernor' and 'Chandler', while at the same time. A total of 10 phenolic compounds were determined from the walnut kernel. The most abundant phenolic compound in the walnut kernels was found to be gallic acid with an average of 11,991.2 mg kg^{-1} and was followed by ellagic acid with 1057.6 mg kg^{-1}. 'Chandler' cultivar was found to be superior for gallic acid and the 'Lara' cultivar, with moderate to high antioxidant activity, was found to be superior for the second most important phenolic compound (ellagic acid). The total lipid composition of the 18 different walnut cultivars and genotypes was found to vary from 50.88% to 64.28%. The highest lipid concentration was noted from the 'Franquetta' cultivar and followed by 'Fernette'. The 'Franquetta' cultivar was also found to be superior for the palmitic acid, which is the most abundant saturated fatty acid. The predominant cultivar for PUFA was observed to be 'Yalova 4' and the predominant cultivar for MUFA was found to be 'Gulizar Hanim Type' genotype. A total of 42 volatile organic compounds were described from the 18 different walnut cultivars and genotypes during the present study. These volatile organic compounds were belonging to 5 different groups: alcohols (#7), esters (#12), aldehydes (#15), acids (#6) and ketones (#2). Results showed that the VOCs play an important role for similarities and diversities among the cultivars. Overall all, the 18 cultivars and genotypes were found to develop 7 different groups according to phenolic compounds, total lipids, saturated and unsaturated fatty acids and volatile profiles. Herein, 'Fernor', 'Maras 18', 'Arslan Local Type' and 'Cisco' cultivars were found to have the lowest biochemical compositions. The superior cultivars or genotypes, in terms of the abundance of the biochemical composition, were found to be 'Gulizar Hanim Type', 'Lara', 'Pedro', 'Tulane', 'Fernette', 'Balkan' and 'Franquetta', where they were found to develop 3 clusters by 1:3:3, respectively.

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Conflict of interest The Authors declare that there is no conflict of interest.

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