Fatty acid composition, phenolic compound content and antioxidant activity of unique walnut genotypes with red seed coat

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Summary
The objective of the present work was to study total oil, fatty acid, tocopherol, total phenol, phenolic compound content and antioxidant activity of unique walnut genotypes with red-coloured seed coat. Seven red walnut genotypes plus 'Chandler' as a standard cultivar were selected as plant materials for the work. The total oil content of the genotypes ranged from 409.44 $g \cdot kg^{-1}$ to 567.87 $g \cdot kg^{-1}$. Fatty acids from walnut kernel oil were mostly composed of myristic, myristoleic, palmitic, oleic, linoleic and linolenic acids, linoleic acid being dominant. Tocopherol contents varied among the genotypes - α-tocopherol from 1.18 $mg \cdot kg^{-1}$ to 15.25 $mg \cdot kg^{-1}$, β- + γ-tocopherol from 154.76 $mg \cdot kg^{-1}$ to 331.27 $mg \cdot kg^{-1}$ and δ-tocopherol from 3.24 $mg \cdot kg^{-1}$ to 45.86 $mg \cdot kg^{-1}$. Total phenolics content ranged from 118.12 $mg \cdot kg^{-1}$ to 169.6 $mg \cdot kg^{-1}$ (expressed as gallic acid equivalent). Antioxidant capacity was found between 68.2 % and 69.8 %. Contents of phenolics, namely, gallic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid, rutin trihydrate, ellagic acid, quercetin, naringin and juglone varied greatly among genotypes. The results indicate that red walnut variants are a great source of oils, fatty acids, tocopherols and phenolics, having good antioxidant potential as well.

Keywords
red walnut; walnut pellicle; phenolic compounds; ellagic acid; fatty acids; tocopherols

Walnut (*Juglans regia*, L.) is a widely cultivated and consumed tree-nut species with a high economic and nutritional value. The species is commercially cultivated in more than 60 countries including China, Iran, USA, Turkey, France and Brazil. China is the top walnut producer accounting for more than 40 % of the world walnut production [1].

The high nutritional value of walnuts is attributed to oil, fatty acids, proteins, vitamins, minerals and phytochemicals such as phenolics, flavonoids and sterols. Oil can constitute up to 73.9 % (w/w) of the walnut kernel, depending on the cultivar, location and growing conditions [2]. Triacylglycerols are the major component of the oil while diacylglycerols, monoacylglycerols, sterols, sterol esters and phosphatides represent the minor components [3]. Oleic acid (C18:1 ω-9), linoleic acid (C18:2 ω-6) and linolenic acid (C18:3 ω-3) as unsaturated fatty acids, and palmitic acid (C16:0) and stearic acid (C18:0) as saturated fatty acids, are the main fatty acids found in walnut kernels [4]. In addition to the aforementioned fatty acids, walnut kernels may contain up to 13 more fatty acid types [4]. High level of these unsaturated fatty acids is valued by consumers for potential health benefits [5].

Walnut kernels also contain storage proteins, essential amino acids such as arginine and leucine, carbohydrates, pectic compounds, vitamins such as tocopherol and folic acid, and biogenic amines like melatonin and serotonin [6, 7]. Walnut kernels are a good source of macronutrients and micronutrients like potassium, phosphorus, magnesium, sulphur, copper, zinc, manganese and iron as well [7–9].

Walnut kernels are also rich in phytochemicals such as phenolics or flavonoids, which display high

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antioxidant activities [6, 8, 10, 11]. Thus, walnut seeds are considered one the best dietary sources due to the content these compounds with health benefits [12–14]. Walnut kernels were reported to have various beneficial health effects including reducing cardiovascular diseases [15], diabetes [16] and same cancer types [17], alleviating postprandial oxidative stress [18], depleting adiposity and low-grade systemic inflammation [19], and lowering total, low-density lipoprotein cholesterol and triacylglycerol levels while increasing high-density lipoprotein, cholesterol and apolipoprotein A1 levels [12, 20].

Red walnut variants have been recently getting attention by consumers and farmers due to their unique colour. The red colour of the pellicle or testa is attributed to anthocyanins that are detected only in red walnuts [21–23]. We wanted to evaluate nutritional and phytochemical content of red walnuts. Thus, we studied total oil and fatty acid ratio, tocopherol and total phenol content, antioxidant activity and phenolic compounds from 7 red walnut genotypes and from 'Chandler' as a standard cultivar.

MATERIALS AND METHODS

Plant materials

Seven walnut genotypes from walnut orchard genebank of Application and Research Center of Nut Trees (SEKAMER) of Kahramanmaras Sutcu Imam University (Kahramanmaras, Turkey) were selected as experimental materials. 'Chandler' cultivar was included as a control due to its popularity and desired shell and kernel characteristics [3].

Approximately 1 kg nuts were hand-picked in late September and laid in the sun (17–32 °C) for a couple of days followed by oven drying for 28 h at 32 °C until the moisture content decreased to 4 %. Nuts were kept intact for approximately 30 days at 4 °C until the analyses. Prior to each analysis, kernels were manually removed from the shell, the analyses were performed on the kernel with intact pellicle. Some characteristics of the genotypes are shown in Tab. 1. Kernel samples were ground in a mortar with a pestle into powder at room temperature.

Oil extraction

Oil extraction was carried out according the modified method of BLIGH and DYER [24]. The oil from the homogenized walnut kernels (5 g) was extracted by 100 ml petroleum ether (boiling temperature 40–60 °C) for 4 h in a Soxhlet apparatus. The solvent was removed by vacuum distillation until the weight of the residue was constant. The oil ratio was calculated based on the weight difference of tubes before and after the experiment. The extracted oil was later used for fatty acid and tocopherol analyses. Instead of BF_3, methanolic KOH was used for methylation.

Fatty acid profile and content

Fatty acid methyl esters (FAME) were analysed according to AOAC Official Method 966.06 [25]. Fatty acids were analysed by gas chromatography using Clarus 500 (Perkin-Elmer, Waltham, Massachusetts, USA) equipped with flame ionization detector and a fused silica Zebron capillary column (100 m × 0.32 mm inner diameter, 0.25 μm film thickness; Phenomenex, Torrance, California, USA). The oven temperature was initially set at 140 °C for 5 min, then raised to 200 °C at a rate of 4 °C·min^{-1}, and then to 220 °C at a rate of 1 °C·min^{-1}. Injector temperature was 220 °C and detector temperature was 280 °C. Fatty acids were identified and quantified using standard FAME mixture containing 37 components (Supelco, Bellefonte, Pennsylvania, USA)

Tab. 1. Nut and kernel characteristics of walnut genotypes.

Genotypes	Nut weight [g]	Kernel weight [g]	Share of kernel weight in nut weight [%]	Pellicle colour
KSUKZ-1	13.95 ± 0.18	6.90 ± 0.14	49.5 ± 0.4	Dark red
KSUKZ-2	6.35 ± 0.26	3.15 ± 0.23	49.6 ± 0.3	Dark red
KSUKZ-3	10.71 ± 0.29	4.65 ± 0.19	43.4 ± 0.4	Dark red
KSUKZ-4	10.98 ± 0.20	5.65 ± 0.21	51.5 ± 0.4	Dark red
KSUKZ-5	24.69 ± 0.23	9.02 ± 0.17	36.5 ± 0.7	Red
KSUKZ-6	18.82 ± 0.21	8.20 ± 0.21	43.6 ± 0.5	Red
KSUKZ-7	10.94 ± 0.35	5.42 ± 0.29	49.5 ± 0.5	Dark red
'Chandler'	12.86 ± 0.46	6.34 ± 0.37	49.3 ± 0.7	Light yellow

Each value is expressed as mean ± standard deviation (n = 3).

Tocopherol profile and content

A high-performance lipid chromatography (HPLC) technique developed by SURAI (26) was employed to assess α-, β- + γ- and δ-tocopherol content. Hewlett-Packard HP-1100 (Hewlett-Packard, Palo Alto, California, USA) HPLC system was used. A reversed-phase RP-C18 column (Spherisorb ODS2, 15 cm × 4.6 mm; particle size 5 μm, Phenomenex) with a mobile phase of methanol and water mix (97 : 3, v/v; 1.05 ml·min^{-1}) was used. Fluorescence detector was used with excitation at 325 nm and emission wavelength of 490 nm during the first 5 min, which were changed then to excitation at 295 nm and emission wavelength of 330 nm [26]. Tocopherol isomers were used for calibration as external standards (Sigma Aldrich, St. Louis, Missouri, USA).

Total phenolics content

Total phenolics content was determined colorimetrically utilizing the Folin-Ciocalteu method [27]. A 1 ml aliquot of the extract (diluted 1 : 20 with methanol) and 1 ml deionized water were mixed in a 10 ml flask, followed by adding 500 μl Folin-Ciocalteu reagent (Merck, Darmstadt, Germany). After 2 min, 4 ml of 7.5% Na_2CO_3 solution was added to the mixture and it was incubated for 2 h at room temperature. Then, absorbance was measured at 745 nm using UV-VIS spectrophotometer Lambda 5 (Perkin-Elmer). Gallic acid (Sigma Aldrich) was used as a standard and total phenolics content was reported in milligrams of gallic acid equivalents (GAE) per kilogram.

Analysis of antioxidant properties

A spectrophotometric method developed by MENSOR et al. [28] was employed to determine antioxidant activity using elimination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. A volume of 50 μl of methanol-diluted extract with 300 μl ethanol was mixed with 30 μl of 0.5 mmol·l^{-1} methanolic DPPH (Merck). The mixture was shaken and left in to stand in the dark for 30 min at room temperature. Ensuing the desired colour formation (from deep violet to light yellow), the mixture was read at 517 nm using UV-VIS spectrophotometer Lambda 5. The mixture of ethanol (330 μl) and sample (50 μl) served as blank. The control solution was prepared by mixing ethanol (350 μl) and DPPH radical solution (30 μl). The antioxidant activity by scavenging activity was calculated according to MENSOR et al. [28] and expressed in percent.

Phenolics profile and content determination

Phenolics were extracted using a modification of the methods developed by KOSAR et al. [29] and TRANDAFIR et al. [22]. The kernel samples were mixed with acetone and water (1 : 4) and vortex-mixed for 1 min. Trifluoroacetic acid (0.100 ml) was then added to the mixture followed by vortex-mixing for 1 min and by incubation in a hot water bath at 60 °C for 60 min. After cooling, the extracts were filtered through a nylon membrane (pore size 0.45 μm, Merck). Extracts were analysed by HPLC with ultraviolet spectrophotometric detection using LC-20A system (Shimadzu, Tokyo, Japan). A reverse phase column Nucleosil C18 (25 cm × 3.2 mm, particle size 5 μm; Supelco) and a two-solvent system (A: formic acid-water, 2.5 : 97.5, v/v and B: acetonitrile-water, 2.5 : 97.5, v/v) were used. Detection was accomplished at 280–360 nm. Gallic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid, rutin trihydrate, ellagic aside, quercetin, naringenin and juglone (Sigma Aldrich) were used as standards. Content of phenolics was expressed as milligrams per kilogram.

Statistical analysis

General linear model program (PROC GLM) of SAS version 9.3 (Statistical Analyses System Institute, Carry, North Carolina, USA) and Duncan's Multiple Range Test ($P \leq 0.05$) were performed for completely randomized design.

RESULTS AND DISCUSSION

Total oil content

Total oil content, fatty acid profile and content of the red walnut genotypes are shown in Tab. 2. The total oil content ranged from 409.44 g·kg^{-1} (KSUKZ-3) to 567.87 g·kg^{-1} (KSUKZ-5), and the content of 'Chandler' fell in this range with a value of 566.32 g·kg^{-1}. The red walnut genotypes contained less total oil compared to 'Howard' cultivar, which is one of the "parents" of red walnuts [30]. We also found the total oil content of 'Chandler' slightly lower than in a previous study [30]. This difference might be due to environmental conditions, oil synthesis and accumulation, or the less effective oil extraction in our study. No earlier works regarding total oil content for a red walnut variant have reported. However, several studies were published on walnuts with yellow pellicle around the world including Serbia [31], New Zealand [32], Turkey [33] and Argentina [2]. Our data are comparable to those obtained from walnuts with yellow seed coat, indicating that total oil content of the red walnut genotypes is in a similar range as in walnuts with yellow pellicle.

Tab. 2. Total oil and fatty acid content in walnut genotypes.

Genotypes	Total oil [g·kg^{-1}]	Myristic acid (C14:0)	Myristoletic acid (C14:1)	Palmitic acid (C16:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)
		mg·kg^{-1}					
KSUKZ-1	475.15 ± 01.21^{e}	59.98 ± 4.97^{bcd}	65.25 ± 15.05^{a}	6 785.60 ± 342.59^{b}	19 812.99 ± 2157.74^{bc}	60 444.37 ± 9545.25^{a}	11 320.29 ± 1 883.11^{b}
KSUKZ-2	530.56 ± 03.09^{c}	44.44 ± 4.69^{bcd}	91.96 ± 8.01^{a}	6 248.50 ± 119.67^{c}	18 576.28 ± 72.66^{bcd}	57 510.10 ± 3.20^{a}	16 201.51 ± 17.41^{a}
KSUKZ-3	409.44 ± 02.15^{g}	39.02 ± 2.66^{cd}	90.98 ± 23.57^{a}	6 523.20 ± 11.01^{bc}	28 264.08 ± 57.22^{a}	55 584.31 ± 73.39^{a}	8 035.34 ± 17.42^{c}
KSUKZ-4	438.63 ± 0.93^{f}	121.13 ± 1.15^{a}	49.20 ± 3.10^{a}	7 274.93 ± 0.07^{a}	20 291.21 ± 0.20^{bc}	59 771.75 ± 25.05^{a}	10 237.22 ± 0.22^{b}
KSUKZ-5	567.87 ± 02.84^{a}	65.04 ± 4.81^{bc}	100.53 ± 9.65^{a}	5 628.49 ± 26.45^{d}	17 659.23 ± 59.49^{cd}	65 031.03 ± 28.88^{a}	10 597.57 ± 7.24^{b}
KSUKZ-6	536.87 ± 02.55^{b}	43.70 ± 15.69^{bcd}	110.78 ± 48.74^{a}	6 832.42 ± 59.20^{ab}	20 741.56 ± 85.67^{b}	59 892.37 ± 318.09^{a}	11 433.21 ± 87.77^{b}
KSUKZ-7	490.53 ± 03.31^{d}	36.30 ± 0.75^{d}	53.38 ± 37.44^{a}	6 820.95 ± 23.70^{ab}	18 574.79 ± 57.04^{bcd}	62 084.62 ± 35.51^{a}	10 944.87 ± 11.35^{b}
'Chandler'	566.32 ± 03.31^{a}	68.28 ± 13.90^{b}	64.12 ± 15.35^{a}	6 903.05 ± 3.36^{ab}	16 425.30 ± 16.42^{d}	60 511.28 ± 7.71^{a}	14 807.01 ± 49.82^{a}

Each value is expressed as mean ± standard deviation (n = 3). The letters indicate the statistical difference in columns on a significance level of 5 %.

Fatty acid profile

As seen in Tab. 2, linoleic acid was the most abundant fatty acid type followed by oleic, linolenic, palmitic, myristoleic and myristic acid in the red walnut genotypes and alike in 'Chandler'. As expected, polyunsaturated fatty acids content (linoleic and linolenic acid) was greater than that of monounsaturated fatty acids (myristoleic and oleic acid) or saturated fatty acids (myristic and palmitic acid) ratio. On the other hand, within the specific fatty acid contents, the genotypes showed some variations. For example, KSUKZ-4 had the highest myristic acid content while KSUKZ-7 lowest; KSUKZ-4 the highest palmitic acid content while KSUKZ-5 the lowest; KSUKZ-3 the highest oleic acid content while KSUKZ-5 the lowest; and KSUKZ-2 the highest the linolenic acid content while KSUKZ-3 the lowest. As an example, for a walnut with yellow pellicle, 'Chandler' showed a trend very close to the red walnut genotypes, which implies that red walnuts are somewhat similar to yellow walnuts regarding the profile of fatty acids. To our knowledge, there was not any study regarding fatty acid profile for a red walnut variant reported in the literature. However, several works have been published for yellow walnuts in which results comparable to ours were presented [2, 33–35].

Tocopherol profile and content

Tocopherol content of the red walnut genotypes plus 'Chandler' cultivar is presented in Tab. 3. β- + γ-Tocopherol was registered as the most abundant isomer for all red walnut genotypes and 'Chandler', followed by δ-tocopherol and α-tocopherol. α-Tocopherol content varied from 1.18 mg·kg^{-1} (KSUKZ-7) to 15.25 mg·kg^{-1} (KSUKZ-3), β- + γ-tocopherol content from 154.76 mg·kg^{-1} (KSUKZ-5) to 331.27 mg·kg^{-1} (KSUKZ-3) and δ-tocopherol content from 3.24 mg·kg^{-1} (KSUKZ-7) to 45.86 mg·kg^{-1} (KSUKZ-3). KSUKZ-3 genotype had the highest tocopherol contents for all three isomers while KSUKZ-7 the poorest. When compared to 'Chandler' cultivar, the red genotypes showed a mixed pattern with similar, higher and lower values. In this study, 'Chandler' showed lower tocopherol content when compared to previous studies [30, 37]. Similar to total oil content in 'Chandler', this might have been due to environmental conditions, tocopherol synthesis and accumulation, or the less effective extraction in our study. As far as we know, no previous study was reported on tocopherol content of a red walnut variant. Nonetheless, several works on yellow walnuts were published, data in which were similar to ours [2, 31, 33, 36].

Tab. 3. Tocopherol content in walnut genotypes.

Genotypes	α-Tocopherol	β- + γ-Tocopherol	δ-Tocopherol
	[mg·kg^{-1}]		
KSUKZ-1	1.45 ± 0.08^{b}	183.10 ± 7.42^{c}	9.87 ± 0.74^{c}
KSUKZ-2	1.55 ± 0.25^{b}	226.98 ± 2.69^{b}	44.55 ± 0.44^{a}
KSUKZ-3	15.25 ± 0.36^{a}	331.27 ± 2.63^{a}	45.86 ± 0.74^{a}
KSUKZ-4	2.14 ± 0.00^{b}	190.29 ± 1.49^{c}	10.19 ± 0.47^{c}
KSUKZ-5	11.03 ± 2.80^{a}	154.76 ± 0.93^{d}	8.69 ± 0.02^{c}
KSUKZ-6	14.92 ± 2.10^{a}	226.89 ± 2.89^{b}	22.98 ± 0.06^{b}
KSUKZ-7	1.18 ± 0.06^{b}	165.49 ± 1.22^{d}	3.24 ± 3.24^{d}
'Chandler'	1.42 ± 0.18^{b}	183.09 ± 7.30^{c}	12.23 ± 0.95^{c}

Each value is expressed as mean ± standard deviation (n = 3). The letters indicate the statistical difference in columns on a significance level of 5 %.

Total phenolics content

Total phenolics content of the red walnut genotypes ranged from 118.12 mg·kg^{-1} (KSUKZ-3) to 169.63 mg·kg^{-1} (KSUKZ-1) as shown in Tab. 4. The total phenolics content of 'Chandler' (124.23 mg·kg^{-1}) was in the range of other red walnut genotypes. In a study from Romania on six red walnut genotypes, it was reported that their average total phenolics content was 170.95 mg·kg^{-1} [22]. In a study from Slovenia containing data on only one red walnut genotype, it was reported that its total phenolics content was approximately 170 mg·kg^{-1} [23]. In both mentioned studies, the phenolics contents was somewhat higher than we report here. This difference could be due to the genotypes, environmental conditions, phenolic compound synthesis and accumulation or the less effective extraction in our study [22]. However, several works on yellow walnuts were published, data in which were similar to ours regarding phenolics [12, 33, 37].

Antioxidant properties

DPPH free radical-scavenging activity of the red walnut genotypes was determined in the range from 68.2 % (KSUKZ-5) to 69.8 % (KSUKZ-1), while it was 66.7 % for Chandler', and this indicated almost no statistical differences among the genotypes and the cultivar (Tab. 4). On the other hand, Trandafir et al. [22] reported antioxidant activity for six red walnut variants in the range from 1.44 mmol·kg^{-1} to 2.43 mmol·kg^{-1} (expressed as Trolox equivalents) with wider variation among the genotypes compared to our results.

Phenolics profile and content

The determined contents of phenolic compounds of the red walnut genotypes and 'Chandler' cultivar are shown in Tab. 5. With few exceptions, catechin content was the highest in the red walnut genotypes, followed by gallic acid, rutin trihydrate, ellagic acid, caffeic acid, syringic acid, *p*-coumeric acid, naringenin, juglone and quercetin.

Tab. 4. Total phenolics content and antioxidant activity in walnut genotypes.

Genotypes	Total phenolics [mg·kg^{-1}]	Antioxidant activity [%]
KSUKZ-1	169.63 ± 0.32^{a}	69.8 ± 0.1^{a}
KSUKZ-2	147.48 ± 0.86^{c}	68.3 ± 0.2^{ab}
KSUKZ-3	118.12 ± 0.35^{e}	68.7 ± 0.1^{ab}
KSUKZ-4	119.50 ± 0.33^{e}	69.6 ± 0.2^{a}
KSUKZ-5	119.21 ± 0.15^{e}	68.2 ± 0.1^{ab}
KSUKZ-6	118.54 ± 0.85^{e}	68.5 ± 0.3^{ab}
KSUKZ-7	159.93 ± 0.82^{b}	68.8 ± 0.7^{ab}
'Chandler'	124.23 ± 0.08^{d}	66.7 ± 0.3^{ab}

Each value is expressed as mean ± standard deviation (n = 3). The letters indicate the statistical difference in columns on a significance level of 5 %.

The gallic acid content was in the range from 221.56 mg·kg^{-1} (KSUKZ-7) to 465.91 mg·kg^{-1} (KSUKZ-2) for the red genotypes, while it was 197.84 mg·kg^{-1} for 'Chandler', i.e. all our red genotypes had a higher gallic acid content than 'Chandler'. A similar result was published by Bujdosó et al. [38] who reported that gallic acid content in a Romanian red walnut genotype was more than 2-fold higher than that in 'Chandler'.

Catechin content varied greatly from 67.79 mg·kg^{-1} (KSUKZ-6) to 2390.07 mg·kg^{-1} (KSUKZ-3) for our red genotypes whereas it was 538.36 mg·kg^{-1} for 'Chandler'. Catechin content was significantly higher in KSUKZ-2, -3, -4, and -7 genotypes but significantly lower in KSUKZ-1, -5, and -6 genotypes when compared to 'Chandler'. Bujdosó et al. [35] determined a higher content of catechin in a red genotype than in 'Chandler'.

Caffeic acid content ranged from 21.99 mg·kg^{-1} (KSUKZ-6) to 85.07 mg·kg^{-1} (KSUKZ-3) for the red genotypes while it was 35.08 mg·kg^{-1} for 'Chandler'. KSUKZ-1, -3, -4, and -5 genotypes had higher caffeic acid content than 'Chandler' did while KSUKZ-2, -6, and -7 lower.

Syringic acid content was in the range from 3.14 mg·kg^{-1} (KSUKZ-4) to 37.65 mg·kg^{-1} (KSUKZ-2) for the red genotypes whereas it was 23.30 mg·kg^{-1} for 'Chandler'. 'Chandler' mainly contained a higher syringic acid content than the red genotypes with the exception of KSUKZ-2. Similar to our results, Bujdosó et al. [38] determined a lower syringic acid content in a red walnut genotypes than 'Chandler'.

p-Coumaric content varied from 5.35 mg·kg^{-1} (KSUKZ-7) to 43.77 mg·kg^{-1} (KSUKZ-3) for our red genotypes while it was 4.87 mg·kg^{-1} for 'Chandler'. Only KSUKZ-3 and KSUKZ -5 genotypes had a significantly higher content of *p*-coumaric acid content when compared to 'Chandler'.

Rutin trihydrate content was found between 46.94 mg·kg^{-1} (KSUKZ-6) and 222.59 mg·kg^{-1} (KSUKZ-3) whereas it was 60.35 mg·kg^{-1} for 'Chandler'. Majority of the red walnut genotypes contained higher content of rutin trihydrate than 'Chandler', Bujdosó et al. [38] cited the same pattern as well.

Ellagic acid content varied from 29.63 mg·kg^{-1} (KSUKZ-1) to 93.71 mg·kg^{-1} (KSUKZ-6) for the red genotypes while it was 117.64 mg·kg^{-1} for 'Chandler'. Ellagic acid content in 'Chandler' was notably higher than in the red genotypes.

Quercetin content ranged in red genotypes from 1.40 mg·kg^{-1} (KSUKZ-2) to 3.74 mg·kg^{-1} (KSUKZ-7) while it was 8.68 mg·kg^{-1} for 'Chandler'. This means that 'Chandler' contained significantly more quercetin than our red genotypes.

Tab. 5. Phenolic compounds profile and content in walnut genotypes.

Genotypes	Gallic acid	Catechin	Caffeic acid	Syringic acid	*p*-Coumaric acid	Rutin trihydrate	Ellagic acid	Quercetin	Naringenin	Juglone
	[mg·kg^{-1}]									
KSUKZ-1	409.99 ± 3.26^{abc}	116.66 ± 11.24^{d}	41.79 ± 5.49^{bc}	7.86 ± 1.86^{cd}	8.62 ± 2.19^{cd}	104.71 ± 5.41^{bc}	29.63 ± 3.52^{d}	2.16 ± 0.04^{dce}	9.36 ± 3.52^{a}	2.91 ± 1.91^{bc}
KSUKZ-2	465.91 ± 4.37^{a}	1047.9 ± 75.00^{b}	22.94 ± 1.81^{cd}	37.65 ± 4.61^{a}	12.60 ± 0.98^{c}	113.93 ± 8.79^{b}	52.43 ± 1.17^{c}	1.40 ± 0.22^{e}	6.70 ± 0.90^{a}	6.29 ± 0.57^{a}
KSUKZ-3	358.40 ± 11.79^{ab}	2390.07 ± 108.88^{a}	85.07 ± 2.45^{a}	24.88 ± 0.86^{b}	43.77 ± 1.27^{a}	222.59 ± 34.31^{a}	81.42 ± 4.88^{b}	3.45 ± 0.01^{c}	9.32 ± 0.26^{a}	1.64 ± 0.20^{c}
KSUKZ-4	311.92 ± 19.68^{bcd}	1091.84 ± 78.78^{b}	49.19 ± 3.79^{b}	3.14 ± 0.32^{d}	6.28 ± 0.91^{d}	85.97 ± 11.48^{bc}	42.62 ± 0.66^{dc}	1.63 ± 0.42^{de}	10.43 ± 2.07^{a}	4.35 ± 0.08^{ba}
KSUKZ-5	355.03 ± 2.65^{ab}	125.69 ± 2.65^{d}	53.14 ± 2.13^{b}	12.44 ± 0.06^{c}	25.14 ± 1.88^{b}	104.71 ± 5.41^{bc}	29.63 ± 3.52^{d}	2.16 ± 0.04^{dce}	9.36 ± 3.52^{a}	2.91 ± 1.91^{bc}
KSUKZ-6	294.34 ± 4.58^{bcd}	67.79 ± 14.40^{d}	21.99 ± 0.17^{d}	25.59 ± 4.01^{b}	5.84 ± 0.89^{d}	113.93 ± 8.79^{b}	52.43 ± 1.17^{c}	1.40 ± 0.22^{e}	6.70 ± 0.90^{a}	6.29 ± 0.57^{a}
KSUKZ-7	221.56 ± 3.41^{cd}	894.53 ± 69.79^{b}	26.74 ± 0.24^{cd}	4.95 ± 0.10^{cd}	5.35 ± 0.42^{d}	222.59 ± 34.31^{a}	81.42 ± 4.88^{b}	3.45 ± 0.01^{c}	9.32 ± 0.26^{a}	1.64 ± 0.20^{c}
'Chandler'	197.84 ± 6.69^{d}	538.66 ± 19.69^{c}	35.08 ± 3.79^{d}	23.30 ± 2.79^{b}	4.87 ± 0.75^{d}	85.97 ± 11.48^{bc}	42.62 ± 0.66^{dc}	1.63 ± 0.42^{de}	10.43 ± 2.07^{a}	4.35 ± 0.08^{ba}

Each value is expressed as mean ± standard deviation (n = 3). The letters indicate the statistical difference in columns on a significance level of 5 %.

Naringenin content was in the range from 5.91 mg·kg^{-1} (KSUKZ-6) to 10.43 mg·kg^{-1} (KSUKZ-4) for our red genotypes while it was 6.55 mg·kg^{-1} for 'Chandler', with no variation among the genotypes and the reference cultivar.

Juglone content of the red genotypes was found to range from 1.64 mg·kg^{-1} (KSUKZ-3) to 6.29 mg·kg^{-1} (KSUKZ-2) whereas of 'Chandler' it was 1.73 mg·kg^{-1}. The majority of the red genotypes contained a higher content of juglone than 'Chandler', the same pattern being reported by Bujdosó et al. [38].

Literature data are available on the contents of phenolic compounds in walnuts with yellow pellicle [38–42] yet only one literature for red walnut variant [38]. Bujdosó et al. [38] analyzed 9 phenolic compounds in a red walnut variant and ranked them according to their quantity from the highest to the lowest: vanilic acid, cathechin, pyrocathechin, epicathechin, rutin, syringin acid, gallic acid, juglone and cinnamic acid, respectively.

CONCLUSIONS

We found certain red walnut genotypes to be exceptionally rich in phenolic compounds such as gallic acid, catechin, rutin trihydrate, and tocopherol. The rich phytochemical profile together with the red seed coat colour could make the red walnuts variants be attractive to consumers. However, sensory evaluations should be carried out before a definite decision.

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