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ORIGINAL ARTICLE

Phenolic compounds and antioxidant activities of chestnut (*Castanea sativa* Mill.) fruits

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Abstract

Introduction Anatolia is one of the original centers of European chestnut production. Therefore, chestnut fruits were procured from four regions, 16 provinces in which chestnuts are grown in Turkey and examined in terms of phenolic content, antioxidant activity and some phenolic compounds. *Objectives* Chestnut has become increasingly important because of positive health effects. We aimed to determine whether chestnut is a natural antioxidant source and to learn the phenolic profiles of chestnuts of some provinces of Turkey. *Methods* Total phenolic contents, total antioxidant capacities and specific phenolic compounds of chestnuts were determined. Fifteen antioxidant standards were used in high-performance liquid chromatography with diode-array etection for phenolic compounds' analyses. *Results* The results show that the total phenolic contents varied between 5 mg GAE g^{-1} DM and 32.82 mg GAE g^{-1} DM. All chestnut samples had no significant differences statistically in terms of total antioxidant capacity. Among the specific phenolic analytes, myricetin, kaempferol, fumaric acid and quercetin were not found in any chestnut. In all chestnut samples, vanillic acid was determined in relatively high amounts. *Conclusion* It could be concluded that total phenolic contents and specific phenolic compounds varied between the provinces; the total antioxidant capacities were nearly the same statistically in Turkey.

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Introduction

Among nuts, chestnut is an important plant because of its uses for different purposes (Koyuncu *et al*., 2004). Chestnut belongs to the family Fagaceae, which includes *Aesculus hippocastanum* (horse chestnut), *Betula pendula* (birch), *Fagus sylvatica* (beech) and *Quercus* species (oaks) that are other ecologically and economically important tree species (De Vasconcelos *et al*., 2007). *Castanea* (North America), *Castanea mollissima* (Chinese), *Castanea sativa* (European) and *Castanea crenata* (Japanese) are four economically important species of chestnut (Tan *et al*., 2007). *C. sativa* is the most consumed among the 12 world chestnuts species (Zivkovic *et al*., 2009).

While the chestnut is produced in especially natural habitats, in some countries it is cultured. The important countries in terms of chestnut production are Italy, France, Spain and Portugal in Europe; China, Japan and Korea in Asia. Also, chestnuts are cultured in Greece, Bulgaria, Rumania, Hungary, Yugoslavia, Czech Republic, Slovakia and Switzerland. In the oriental world, chestnut is one of the popular nuts. Chestnut production is particularly important to China. Turkey has a big share in the world chestnut production and is the second main producer of

chestnut in Europe. Anatolia is also one of the original centers of European chestnut production (Koyuncu *et al.*, 2004; Kwon *et al.*, 2004; Tan *et al.*, 2007; Zivkovic *et al.*, 2009).

Stability during storage is a major problem for chestnut. In order to mitigate this problem, different methods such as cold storage, frozen storage and drying are used depending on the technical resources, food consumption and food processing methods. In some countries, chestnuts are often stored after drying to convert into a flour and processed into different foods including snacks, flakes, confectionery, pasta, purees and creams (Koyuncu *et al.*, 2004; Tan *et al.*, 2007; Zivkovic *et al.*, 2009).

From a nutritional point of view, chestnuts are different from other tree nuts. They are important source of starch (up to 70%) but contain low amounts of protein (2–4%) and fat (2–5%). Also, some minerals and vitamins, together with appreciable amounts of fiber, are present in chestnuts (Kunsch *et al.*, 2001; Zivkovic *et al.*, 2009). Carbohydrates are the major components of chestnut. Although starch is the main carbohydrate in chestnut, sucrose is an important carbohydrate because it constitutes one third of the total sugar of a chestnut. Also the sucrose amount is one of the most important parameters for the assessment of the commercial quality of chestnut (Bernardez *et al.*, 2004). Chestnuts have become increasingly important with respect to human health. The reasons are that they are an alternative gluten-free flour source and also a rich source of other beneficial compounds (De Vasconcelos *et al.*, 2007).

The chemical composition of chestnut shows variability depending upon the following:

- Some data refer to chestnuts while others refer to marrons, which are products with different morphological traits and technological characteristics.
- Some varieties (e.g. Marrone Fiorentino) have different ecotypes with different chemical characteristics linked to the ecological surroundings.
- Different clones of the same variety may exhibit different chemical composition, which gives rise to a dramatic variation due to harvesting year, and the interaction between year and cultivar is also significant (Neri *et al.*, 2010).

In today's world, macro- and micronutrient content of foods are inadequate to characterize them because other components that prevent or cure various diseases are known such as antioxidant compounds. Also, together with developing scientific technology, our awareness of the relationship between diet and disease has been increased. Functional foods and their role in bioregulating functions, the maintenance and improvement of health and wellness, have a growing interest (Coskun, 2005). Epidemiological studies have shown that many polyphenol compounds present in plants, such as fruits, vegetables, nuts, including chestnuts, wine and tea, are partly responsible for their beneficial health effects and show antioxidant properties. Thus, phenolic compounds can stop or prevent the free radical reactions that cause many diseases such as cancer, heart disease and lung diseases that account for a major portion of deaths today (Abe *et al.*, 2010; Nizamlioglu & Nas, 2010).

Phenolic compounds are secondary metabolites found in large amounts in plants. The phenolic compounds may be divided into two chemical classes: (a) phenolic acids and (b) flavonoids. Due to large differences in the structure of plants, there are potentially thousands of different phenolic compounds in plants and their products (Nizamlioglu & Nas, 2010). In our study, gallic acid, ferulic acid, rutin, myricetin, syringic acid, caffeic acid, chlorogenic acid, quercetin, p-coumaric acid, kaempferol, catechin, fumaric acid, vanillic acid, naringin and ellagic acid are analyzed in chestnut fruits by high-performance liquid chromatography with diode-array detection (HPLC-DAD).

Materials and methods

Standards and reagents

Standards are: anhydrous gallic acid (Sigma, G7384, purity: ≥ %98, Balcatta, Western Australia), ferulic acid (Fluka, 42280, purity: ≥98, Buchs, Switzerland), rutin (Sigma, R5143, purity: %95), myricetin (Sigma, M6760, purity: ≥96), syringic acid (Sigma, S6881, purity: ≥97), caffeic acid (Sigma, C0625, purity: ≥95), chlorogenic acid (Sigma, C3878, purity: ≥95), quercetin hydrate (Sigma, 337951, purity: ≥95), p-coumaric acid (Sigma, C9008, purity: ≥98), kaempferol (Sigma, K0133, purity: ≥90), catechin hydrate (Fluka, 22110, purity: ≥96), fumaric acid (Fluka, 47910, purity: ≥99), vanillic acid (Fluka, 94770, purity: ≥97), naringin (Sigma, N1376, purity: ≥90), ellagic acid (Sigma, E2250, purity: ≥95).

Chemicals are: Folin–Ciocalteu (FC) phenol reagent (Sigma-Aldrich, E9252, Taufkirchen, Germany), acetic acid (Panreac, 361008, Barcelona, Spain), sodium carbonate (J.T. Baker, 2024, Center Valley, PA, USA), DPPH (2,2 diphenyl,1,picrylhydrazyl) (Sigma, D9132), TPTZ (2,4,6-tripyridyl-s-triazine) (Sigma, 93285), hydrochloric acid (J. T. Baker, 6081, Center Valley, PA, USA), ferric (II)

sulfate (Sigma-Fluka-Reidel, KIM-DST/01CP), ferric (III) chloride (Merck, M1039431000, Darmstadt, Germany).

Samples and sample preparation

Chestnuts were procured from 16 provinces chosen to represent the regions where they are grown in Turkey. The chosen locations were. in the Aegean Region, Izmir, Manisa, Denizli, Kutahya, Aydin, Mugla; in the Marmara Region, Kocaeli, Balikesir, Bursa; in the Black Sea Region, Zonguldak, Samsun, Kastamonu, Sinop, Bartin, Duzce; and in the Mediterranean Region, Isparta. Chestnuts were stored unshelled in perforated and zip lock bags in refrigerator (+4 °C). To prevent the accumulation of moisture on chestnuts, perforated bags were preferred. The shells and pellicles were manually removed. Then, chestnut fruits were ground in a mortar with the aim of providing uniformity and increasing the extraction efficiency.

The extraction liquid was 80% aqueous methanol. Two g of chestnut fine powder was extracted with 50 mL extraction liquid at 70 °C for 30 min in Erlenmeyer flask. The contents of the Erlenmeyer flasks were mixed every 5 min to optimize extraction. The samples were centrifuged (6000 *g*, 15 min), and the supernatant was used for analysis (De Vasconcelos *et al.*, 2007).

Moisture contents

The moisture content of chestnut samples was estimated by AOAC, 925.40 (Moisture in Nuts) (Horwitz & Latimer, 2010).

Determination of total phenolic contents

The FC method described by Singleton & Rossi (1965) and Singleton *et al.* (1999) was used with some modifications to estimate total phenolic content. Gallic acid was used to calibrate the method. FC reagent, 7% Na_2CO_3 solution and gallic acid in 80% methanol (standard solutions) were the solutions used in this analysis. Standard solutions were prepared at concentrations of 10, 20, 30, 40 and 50 mg kg^{-1}.

Sample extract (50 μL) was mixed with 250 μL of FC's phenol reagent. This mixture was kept in the dark at room conditions for 5 min. Then, 750 μL of 7% sodium carbonate solution was added to the mixture and it was adjusted to 5 mL with distilled water. The reaction was kept in the dark for 120 min. The same procedures were applied to standard solutions. Absorbances of samples and standard solutions were read at 765 nm (Varian Cary 50 Bio UV-Vis spectrophotometer, the path length of cuvette was 10 mm). Linearity of the calibration was very good, $R^2 = 0.9915$. The results were expressed as mg of gallic acid equivalents per g of dry matter (mg GAE g^{-1} DM).

Determination of total antioxidant activities

The ferric reducing antioxidant power (FRAP) method described by Guo *et al.* (2003), with some modifications, was used to estimate antioxidant activities of chestnut samples. The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. The FRAP reagent contained 2.5 mL of 10 mmol L^{-1} TPTZ (2,4,6-tripyridy-s-triazine) solution in 40 mmol L^{-1} HCl plus 2.5 mL of 20 mmol L^{-1} $FeCl_3$ and 25 mL of 0.3 mol L^{-1} acetate buffer, pH 3.6, and was prepared freshly. The acetate buffer (pH 3.6) was prepared by weighing 0.775 g sodium acetate trihydrate ($C_2H_3NaO_2.3H_2O$), adding 4 mL acetic acid and adjusting to 250 mL with distilled water. Forty μL of sample supernatant was mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent. The absorbance of reaction mixture was measured spectrophotometrically at 593 nm after incubation at 37 °C for 30 min. As standard solution, iron sulphate ($FeSO_4$) in 5% HCl at different concentrations (0.2–3 mmol L^{-1}) was used. As blank solutions, 5% HCl for standards and 80% methanol for samples were used. The final result was expressed as the concentration of antioxidants having a ferric-reducing ability equivalent to that of 1 mmol L^{-1} $FeSO_4$.

HPLC analysis of phenolic compounds

The phenolic structures of the samples were determined as qualitative and quantitative by using HPLC-DAD. Caffeic acid, vanillic acid, naringin, syringic acid, ferulic acid, ellagic acid, myricetin, kaempferol, catechin, chlorogenic acid, p-coumaric acid, quercetin, rutin, fumaric acid and gallic acid were used as antioxidant standards. Syringic and caffeic acids were not chromatographically resolved with this method, thus amounts of syringic and caffeic acids were indicated together. The method described by Li *et al.* (2006) was used with some modifications. An Agilent Technology 1200 series HPLC system equipped with a pump, a degasser, a thermostatic autosampler and a photodiode array detector (DAD) was used for the analysis of phenolic compounds in chestnut fruits. The separation was carried out in a μ-Bondapak C18 (3.9 × 300 mm, 10 μm) Agilent Tech

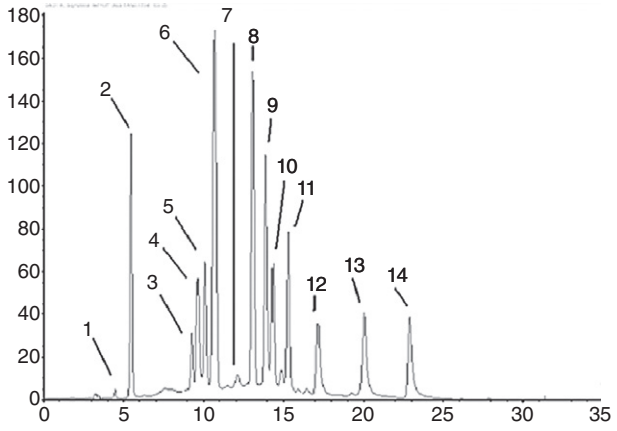

Figure 1 The HPLC chromatogram of standard mixture (280 nm). 1: fumaric acid, 2: gallic acid, 3: catechin, 4: chlorogenic acid, 5: vanillic acid, 6: syringic + caffeic acid, 7: p-coumaric acid, 8: rutin, 9: ferulic acid, 10: ellagic acid, 11: naringin, 12: myricetin, 13: quercetin, 14: kaempferol.

column. Sample extracts and standards were filtered using a 0.45 μm Agilent microfilter. The binary mobile phase consisted of acetonitrile (solvent A) and water containing 2% acetic acid (solvent B). The system was run with a gradient program: 95% B to 60% B in 23 min, 60% B to 45% B in 5 min and 45% B to 95% B in 7 min. The flow rate was kept constant at 0.5 mL min^{-1} for a total run time of 35 min. The sample injection volume was 10 μL. Peaks of interest were monitored at 280, 277 and 254 nm. The HPLC chromatogram of standard mixture at 280 nm was showed in Figure 1.

Statistical methods

The Windows SPSS 15.0 was used for the statistical analysis. One-way ANOVA and Duncan tests were applied and comparisons were carried out at 99% confidence interval.

Results and discussion

Moisture contents

The moisture contents of chestnut fruits from 16 provinces in Turkey were determined and analyses were made in duplicate. The results are given with standard deviations in Table 1.

The moisture contents of raw chestnut fruits were between 35.6% and 60.1% in previous studies (De Vasconcelos *et al.*, 2007, 2010; Neri *et al.*, 2010). In this study, of the 16 provinces, the Izmir chestnut had the highest moisture content with 44.99% (±3.13), and Zonguldak had the lowest value with 26.14% (±4.73). According to statistical analyses, the moisture contents of chestnuts had significant differences.

Table 1 The moisture contents of chestnut samples[1]

Sample	Moisture (%)
Denizli	29.53 ± 3.03^{fg}
Manisa	39.94 ± 3.19^{bcd}
Izmir	44.99 ± 3.13^{abc}
Kutahya	32.77 ± 2.54^{defg}
Aydin	29.66 ± 8.77^{efg}
Mugla	48.40 ± 0.63^{a}
Zonguldak	26.14 ± 4.73^{g}
Samsun	32.26 ± 1.44^{defg}
Kastamonu	36.94 ± 0.37^{bcde}
Sinop	43.77 ± 3.09^{ab}
Bartin	34.78 ± 0.19^{def}
Duzce	29.26 ± 0.08^{efg}
Kocaeli	34.02 ± 2.69^{defg}
Balikesir	29.28 ± 2.70^{fg}
Bursa	35.52 ± 0.04^{cde}
Isparta	42.65 ± 2.12^{abc}

[1]Each chestnut was analyzed in two replications ($n = 2$).
[a,b,c,d,e,f,g]Means in the same column with unlike superscripts differ significantly ($p < 0.01$).

Total phenolic contents

In previous studies, the total phenolic contents of raw chestnuts were reported to be 112.06 μg GAE g^{-1} DM for three Italian sweet chestnut ecotypes (Neri *et al.*, 2010) and 15.80 mg GAE g^{-1} DM for chestnut samples that were grown in Bragança, North East Portugal (De Vasconcelos *et al.*, 2007). In our study, total phenolic contents varied between 5 mg GAE g^{-1} DM Bartin and 32.82 mg GAE g^{-1} DM Mugla. Total phenolic contents of all chestnut samples are given in Table 2 along with total antioxidant capacities. There was no correlation between the results of phenolic contents and total antioxidant activities of chestnuts.

Denizli, Manisa, Izmir, Kutahya, Aydin and Mugla chestnuts from the Aegean Region, showed statistically significant differences. In this region, Mugla had the highest total phenolic content with 32.82 mg GAE g^{-1} DM, and Manisa had the lowest value with 6.50 mg GAE g^{-1} DM.

From the Black Sea Region, Zonguldak, Samsun, Kastamonu, Sinop, Bartin and Duzce, the phenolic contents of chestnuts varied between 24.29 mg GAE g^{-1} DM and 5.00 mg GAE g^{-1} DM. Kastamonu had the highest and Bartin had the lowest values. The chestnuts of this region had significant differences statistically.

Table 2 Total phenolic contents and total antioxidant activities of all chestnut samples[1]

Sample	Total phenolic content (mg GAE g^{-1} DM)	Total antioxidant capacity (mM $FeSO_4$ g^{-1} DM)
Mugla	32.82 ± 9.19[a]	10.89 ± 10.79[a]
Balikesir	31.25 ± 3.4[ab]	11.81 ± 1.93[a]
Kutahya	27.31 ± 2.98 [bc]	9.08 ± 0.58[a]
Kastamonu	24.29 ± 1.74 [bc]	9.61 ± 0.25[a]
Denizli	22.96 ± 1.31[c]	13.83 ± 5.72[a]
Bursa	21.42 ± 1.76[cd]	11.45 ± 3.46[a]
Samsun	20.11 ± 0.09[cd]	9.99 ± 2.78[a]
Aydin	14.73 ± 1.43[de]	11.95 ± 0.90[a]
Sinop	14.45 ± 0.11[de]	12.74 ± 4.89[a]
Duzce	9.13 ± 5.43[ef]	14.15 ± 8.59[a]
Izmir	9.00 ± 3.99[ef]	12.18 ± 3.03[a]
Isparta	7.15 ± 1.16[f]	9.38 ± 0.58[a]
Zonguldak	6.88 ± 0.20[f]	12.34 ± 2.63[a]
Manisa	6.50 ± 0.03[f]	10.83 ± 2.29[a]
Kocaeli	5.64 ± 1.025[f]	11.07 ± 0.78[a]
Bartin	5.00 ± 0.18[f]	13.91 ± 1.33[a]

[1]Statistical analyses were applied on each column separately; each chestnut was analyzed in two replications ($n = 2$).
The values in this table do not differ significantly ($p < 0.01$).

Chestnuts from the Marmara Region (Kocaeli, Balikesir and Bursa) also showed statistically significant differences in GAE content. The total phenolic content of Balikesir chestnut had the highest value with 31.25 mg GAE g^{-1} DM; Kocaeli chestnut had the lowest total phenolic content with 5.64 mg GAE g^{-1} DM.

Isparta was just one province from Mediterranean Region whose chestnut was analyzed. Its total phenolic content was 7.15 mg GAE g^{-1} DM.

The phenolic contents of chestnuts of each region differ considerably in its own provinces. Thus, it could not indicate that chestnuts of any region had the highest or lowest phenolic contents.

Total antioxidant capacities

Total antioxidant capacities of chestnut fruits were expressed as 6.2 µmol Trolox equivalance g^{-1} DM by using the DPPH method (Abe *et al.*, 2010). In a study on the total antioxidant capacities of certain tree nuts, the total antioxidant activity of chestnut was indicated as 0.75 mmol per 100 g by using FRAP antioxidant activity method (Blomhoff *et al.*, 2006). The method and standards used for the calibration in this study have not been used together in previous studies. Therefore, the antioxidant capacity results of our samples could not be compared directly with previous studies. In our study, the total antioxidant capacities varied between 9.08 mM $FeSO_4$ g^{-1} DM Kutahya and 14.15 mM $FeSO_4$ g^{-1} DM Duzce. Chestnut samples from various provinces in Turkey had no statistically significant differences in terms of total antioxidant capacity. Total antioxidant capacities and total phenolic contents of all chestnut samples are given in Table 2.

Total antioxidant capacities of chestnuts of each region differ in its own provinces considerably. Therefore, it could not indicate whether chestnuts of any region had the highest or lowest antioxidant activity.

HPLC analyses of phenolic compounds

Myricetin, kaempferol, fumaric acid and quercetin were not found in any of the chestnuts analyzed in this study. In all chestnut samples, vanillic acid was the most prominent phenolic compounds. The amounts of phenolic compounds found in chestnut samples are given in Table 3. Statistical analyses were applied on every phenolic compound separately and the results of the statistical analyses are also shown in the tables. The results of analyses were expressed as mg per kg. Between chestnuts of Aegean Region (Denizli, Manisa, Izmir, Kutahya, Aydin and Mugla), amounts of gallic acid, vanillic acid, rutin, ellagic acid, p-coumaric acid and naringin showed significant differences; the remaining phenolic compounds were in similar amounts. For chestnuts of the Marmara Region (Kocaeli, Balikesir and Bursa), the amounts of vanillic acid, chlorogenic acid and naringin showed significant differences. Among the chestnuts from the Black Sea Region (Zonguldak, Samsun, Kastamonu, Sinop, Bartin and Duzce), levels of vanillic acid, rutin, catechin and chlorogenic acid showed significant differences. From the Mediterranean Region, just the Isparta chestnut had been analyzed. The HPLC chromatogram of Aydın chestnut extract is given in Figure 2.

Conclusions

Chestnut samples procured from 16 provinces in Turkey were analyzed in terms of total phenol content, total antioxidant activity and specific phenolic compounds. According to the results for total phenolic contents, the samples showed statistically significant differences. Total antioxidant activities of these chestnuts had no significant differences statistically. Total antioxidant activity is mainly contributed by phenolic compounds; however, some other compounds contribute it. Thus, although total phenolic contents varied between the provinces, the total antioxidant capacities were nearly the same statistically. According to the total phenolic

Table 3 The amounts of phenolic compounds of all chestnut samples[1] (mg per kg)

Region	Sample	Gallic acid	Syringic + caffeic acid	Vanillic acid	Rutin	Ellagic acid	Catechin	Chlorogenic acid	p-Coumaric acid	Ferulic acid	Naringin
Black Sea	Samsun	85.89 ± 7.42^{a}	0.00^{a}	0.00^{c}	9.03 ± 0.37^{b}	0.00^{a}	35.09 ± 2.37^{c}	22.02 ± 0.93^{b}	8.56 ± 0.95^{a}	5.71 ± 1.66^{a}	0.00^{a}
	Kastamonu	116.31 ± 9.15^{a}	0.00^{a}	276.77 ± 9.16^{bc}	21.25 ± 2.16^{ab}	11.60 ± 2.19^{a}	59.40 ± 6.38^{c}	5.72 ± 0.26^{c}	0.00^{a}	19.24 ± 5.42^{a}	0.00^{a}
	Sinop	159.63 ± 6.33^{a}	0.00^{a}	745.85 ± 11.28^{ab}	17.77 ± 2.92^{ab}	29.45 ± 4.73^{a}	135.16 ± 10.25^{b}	0.00^{c}	7.85 ± 2.36^{a}	7.59 ± 1.59^{a}	0.00^{a}
	Bartin	137.18 ± 3.47^{a}	0.00^{a}	501.12 ± 9.12^{bc}	11.93 ± 5.29^{b}	13.31 ± 3.29^{a}	125.32 ± 8.14^{b}	0.00^{c}	0.00^{a}	23.19 ± 2.95^{a}	0.00^{a}
	Duzce	114.63+3.48^{a}	18.21 ± 0.39^{a}	449.63 ± 8.49^{bc}	27.70 ± 4.49^{ab}	15.53 ± 0.31^{a}	124.88 ± 2.99^{b}	38.07 ± 1.45^{a}	16.02 ± 4.57^{a}	19.31 ± 0.46^{a}	0.00^{a}
	Zonguldak	179.79 ± 4.36^{a}	13.79 ± 0.21^{a}	1241.70 ± 12.48^{a}	35.50 ± 6.43^{a}	14.18 ± 0.94^{a}	230.02 ± 14.39^{a}	39.20 ± 5.23^{a}	11.95 ± 1.29^{a}	10.47 ± 0.38^{a}	0.00^{a}
Marmara	Kocaeli	264.69 ± 7.65^{a}	5.62 ± 0.32^{a}	563.28 ± 8.20^{ab}	34.45 ± 3.49^{a}	16.09 ± 3.21^{a}	43.24 ± 8.21^{a}	30.92 ± 3.12^{b}	9.51 ± 0.58^{a}	16.13 ± 4.23^{a}	21.95 ± 4.23^{b}
	Balikesir	245.63 ± 8.40^{a}	11.21 ± 0.47^{a}	972.78 ± 4.94^{a}	32.55 ± 7.67^{a}	28.30 ± 3.18^{a}	47.31 ± 2.21^{a}	170.90 ± 16.12^{a}	25.39 ± 2.54^{a}	8.68 ± 0.47^{a}	0.00^{c}
	Bursa	263.29 ± 7.89^{a}	23.51 ± 1.24^{a}	417.42 ± 13.27^{b}	18.66 ± 1.55^{a}	28.55 ± 1.56^{a}	168.46 ± 6.77^{a}	57.48 ± 2.17^{b}	20.50 ± 7.23^{a}	8.34 ± 2.19^{a}	36.34 ± 1.42^{a}
Aegean	Denizli	269.11 ± 5.67^{a}	17.58 ± 2.17^{a}	549.04 ± 12.36^{ab}	17.14 ± 3.28^{b}	25.69 ± 4.27^{c}	221.78 ± 7.36^{a}	0.00^{a}	18.22 ± 3.15^{abc}	0.00^{a}	35.32 ± 3.49^{a}
	Manisa	276.88 ± 4.65^{a}	3.52 ± 2.03^{a}	1241.19 ± 14.23^{a}	16.27 ± 0.98^{b}	48.74 ± 6.19^{a}	95.98 ± 9.28^{a}	42.83 ± 6.43^{a}	21.12 ± 2.38^{ab}	0.00^{a}	0.00^{b}
	Izmir	0.00^{c}	14.51 ± 0.89^{a}	662.51 ± 12.22^{ab}	16.63 ± 2.63^{b}	36.68 ± 1.59^{b}	236.24 ± 6.65^{a}	26.17 ± 4.47^{a}	28.27 ± 0.47^{a}	0.00^{a}	38.61 ± 1.52^{a}
	Kutahya	128.26 ± 3.45^{b}	0.00^{a}	218.58 ± 7.51^{b}	7.24 ± 1.68^{b}	0.00^{d}	52.26 ± 2.11^{a}	12.54 ± 0.39^{a}	9.02 ± 1.85^{bc}	0.00^{a}	10.77 ± 1.46^{b}
	Mugla	198.58 ± 4.67^{ab}	15.31 ± 0.76^{a}	456.18 ± 7.39^{b}	13.79 ± 2.49^{b}	35.51 ± 5.22^{bc}	214.20 ± 4.18^{a}	0.00^{a}	8.72 ± 0.99^{bc}	8.92 ± 2.15^{a}	0.00^{b}
	Aydin	126.26 ± 2.12^{b}	16.26 ± 0.19^{a}	486.44 ± 3.49^{ab}	30.47 ± 5.93^{a}	0.00^{d}	48.96 ± 5.47^{a}	14.97 ± 1.26^{a}	0.00^{c}	12.37 ± 3.56^{a}	0.00^{b}
Mediterranean	Isparta	191.19 ± 2.36	34.26 ± 1.29	674.82 ± 8.21	13.71 ± 3.29	39.53 ± 3.51	167.50 ± 3.25	0.00	57.17 ± 2.33	14.98 ± 2.17	0.00

[1]Statistical analyses were applied on each region and phenolic compound separately; each chestnut was analyzed in two replications (n = 2).
^{a,b,c,d}Means in the same column with unlike superscripts differ significantly ($p < 0.01$).

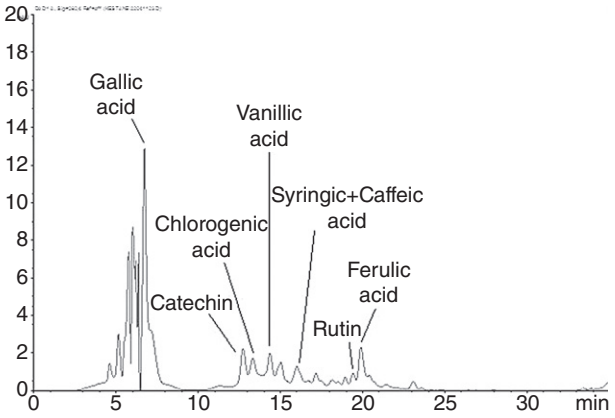

Figure 2 The HPLC chromatogram of aydin chestnut (280 nm).

content analyses, the Bartin chestnut had the lowest total phenolic content and Mugla chestnut had the highest total phenolic content. In terms of total antioxidant capacities, the Kutahya chestnut had the lowest value and Duzce chestnut had the highest value. With regard to the HPLC analyses of phenolic compounds, myricetin, kaempferol, fumaric acid and quercetin were not found in any chestnuts. In all chestnut samples, vanillic acid was determined in high amounts.

Chestnut becomes increasingly important because of positive health effects. It is an alternative gluten-free flour source and a good source of essential dietary nutrients and minerals. The low crude fat content, in combination with the high polyunsaturated fatty acids in this fat, makes chestnuts a very healthy food. The free sugars and high starch content also make chestnuts an energetically valuable food crop. In addition, it is also natural antioxidant source as the results of our study show.

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