

ORIGINAL ARTICLE

# Effect of processing on the phenolic content and antioxidant activity of chestnuts

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## Keywords

antioxidant activity; boiled; chestnut; phenolic compounds; raw; roasted.

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## Abstract

**Introduction** Chestnut samples from three provinces (Aydin, Bursa and Zonguldak) were analysed in terms of phenolic content and antioxidant activities in roasted, boiled and raw forms because of their widespread. **Objectives** The aim was to consider whether roasting and boiling affected total phenol content, total antioxidant activity and phenolic compounds. **Methods** Total phenolic content and total antioxidant activity were determined by the method of Folin–Ciocalteau and the ferric reducing antioxidant power. Fifteen antioxidant standards were used in high-performance liquid chromatography with diode array detection for phenolic compounds. **Results** The results of the analyses showed that the total phenolic contents of roasted chestnuts were higher than boiled ones. There were differences between three provinces. Total antioxidant activities between them did not differ statistically. The results of high-performance liquid chromatography analysis (15 antioxidant standards used that are 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) identified that four antioxidant standards (myricetin, kaempferol, fumaric acid and quercetin) were found in any chestnut sample. **Conclusion** It was concluded that processing affects phenolics positively, especially roasting process.

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## Introduction

Chestnut is an important plant because its fruits are highly regarded and widely consumed throughout Europe, America and Asia (Soylu, 2004; De Vasconcelos *et al.*, 2010a). Chestnut (*Castanea* Miller) belongs to the beech family (Fagaceae) together with the beech (*Fagus*), the oak (*Quercus*) and *Castanopsis*. The 13 *Castanea* species are native to the temperate zone of the Northern Hemisphere. Five species are in East Asia, seven species are in North America and one species is in Europe. *Castanea* (North America), *Castanea mollissima* (Chinese), *C. sativa* (European) and *C. crenata* (Japanese) are economically important species (Soylu, 2004; Tan *et al.*, 2006). Among the 13 world chestnut species, *C. sativa* is the

most consumed because it produces high-quality fruits. Also, it is a significant food source for wildlife (Vidal *et al.*, 2005; Zivkovic *et al.*, 2009).

Chestnuts are produced in natural habitats. In Europe, they are grown in Italy, France, Spain and Portugal. In Asia, China, Japan and Korea are important countries in terms of chestnut production. Chestnuts are also cultured in Greece, Bulgaria, Rumania, Hungary, Yugoslavia, Czech Republic, Slovakia and Switzerland. Anatolia is also one of the original centres of European chestnut production and Turkey has a big share in the world chestnut production (Koyuncu *et al.*, 2004; Zivkovic *et al.*, 2009).

From a nutritional viewpoint, chestnut is different from most other tree nuts. Chestnuts are low in protein (2–4%)

and fat (2–5%) but rich in carbohydrate with starch predominating (up to 70%) and substantial levels of free sugar sucrose (saccharose). Glucose, fructose and maltose are identified in chestnut. These sugars are very important for the commercial quality of chestnut fruit. Also, they have a higher water activity, moisture content, some minerals and vitamins together with appreciable amounts of fibre (Künsch *et al.*, 2001; Overy *et al.*, 2003; Kwon *et al.*, 2004; Zivkovic *et al.*, 2009; De Vasconcelos *et al.*, 2010b). According to results of previous analyses, chestnuts contain important macro-elements (Ca, P, K, Mg and S) with potassium representing the majority of this group and also important microelements (Fe, Cu, Zn and Mn). The content of carotenoids in chestnut fruits were studied and only beta-carotene (pro-vitamin A), lutein and zeaxanthin were detected. The antioxidant vitamin E and vitamin C were also previously reported in chestnut fruits (De Vasconcelos *et al.*, 2010b).

Raw fresh chestnut fruits are rarely consumed. Generally, they are utilized by peeling, roasting and boiling, or are used for manufacturing products such as confectionery, pasta, purees, creams, snacks and flakes (Kwon *et al.*, 2004; Zivkovic *et al.*, 2009; De Vasconcelos *et al.*, 2010a). They are processed to improve the organoleptic properties (aroma, flavour, texture) and digestibility of the fruits. It has been shown that significant changes occur in the macromolecular structure of the starch during processing; processing makes nutrients more bioavailable. Also the shelf life of various chestnut products from industrial processes is longer than raw fruits. It is possible to process chestnuts at home or on an industrial scale. Although all cultivars are collected when they are ready for consumption, certain cultivars are more appreciated because of their organoleptic properties (De Vasconcelos *et al.*, 2010a).

The effects of different types of treatments (drying, boiling, roasting and milling) on the nutritional composition of Italian chestnut fruits were studied. The results indicated that boiled chestnuts gained humidity but lose about 25% of their caloric value and on roasting the available sugars can increase by 25% and the energy levels increase significantly (200 kcal 100 g<sup>-1</sup>). Also, it was indicated that the raw fruits contained a higher content of malic acid compared with cooked chestnuts (Bounous *et al.*, 2000). Künsch *et al.* (2001) found that weight loss due to roasting ranged from 23% to 30% and they concluded that roasted chestnuts contained 260–350 g kg<sup>-1</sup> starch, 50–102 g kg<sup>-1</sup> sucrose, 0.5–4.4 g kg<sup>-1</sup> fructose, trace amounts of glucose and 9–15 g kg<sup>-1</sup> total fatty acids.

According to the results of a study on chemical composition of raw, roasted and boiled chestnuts; the caloric content

of chestnut was increased by roasting and decreased by boiling. Although there were no data on the carbohydrate content of roasted chestnut, it was shown that boiling decreased carbohydrate content. The fibre contents of chestnuts were decreased by both treatments. The fat content of chestnuts was affected considerably by the treatments. Sodium, potassium, phosphorous, calcium and magnesium contents of boiled chestnuts were higher than roasted and raw chestnuts. The treatments did not affect vitamin B contents too much.

Today's world takes a close interest in the role of free radical damage in the aetiology of human diseases. Free radicals are formed during oxidation processes occurring in various products and biological systems. They are known to be responsible for oxidative deterioration, health damage, accelerated aging, molecular transformations and gene mutations in many types of organisms. Despite oxygen being essential for aerobic forms of life, oxygen metabolites are highly toxic. By means of natural antioxidative defence systems in healthy individuals, free radical production is continuously balanced. Because of aging and other factors, reactive oxygen species (ROS) production and elimination become unbalanced; it is called oxidative stress. ROS are liable to many cell disorders and the development of many diseases including atherosclerosis, cardiovascular diseases, chronic inflammation, cataracts and neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. ROS and free radicals cause the deterioration of foods and are also considered as inducers of lipid peroxidation. The endogenous antioxidant defences in organisms become insufficient in time; therefore, other antioxidants from the diet, both from natural and synthetic origin, are essential. Antioxidants can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction. Thus, they appear to be very important in the prevention of many diseases. The potential of toxic and carcinogenic effects of some synthetic antioxidants has intensified research efforts to discover and utilize antioxidants from natural sources such as fruits and vegetables (Barreira *et al.*, 2008; Zhang *et al.*, 2009).

Vitamins and minerals in foods are called nutrient antioxidants. Phytochemicals are identified as non-nutrient antioxidants. There are thousands of phytochemicals that show strong antioxidant effects such as catechin, quercetin, tanin, ellagic acid (Alasalvar & Shahidi, 2009). Phenolics are the main group of phytochemicals and are classified into five groups: (1) polyphenols, (2) stilbenes, (3) coumarins, (4) lignans, (5) tannins (Alasalvar & Shahidi, 2009; Yilmaz, 2010).

Polyphenols have two subgroups: (1) phenolic acids, (2) flavonoids. Also, phenolic acids and flavonoids have some subgroups.

## Materials and methods

### Standards and reagents

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

Chemicals were Folin–Ciocalteau (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), ferric (II) sulfate (Sigma-Fluka-Reidel, KIM-DST/01CP), ferric (III) chloride (Merck, M1039431000, Darmstadt, Germany).

### Samples and sample preparation

Chestnut fruit samples were obtained from the provinces of Aydin (Aegean Region), Bursa (Marmara Region) and Zonguldak (Black Sea Region) in Turkey. They were analysed as raw, roasted and boiled. Chestnuts were stored unshelled in a refrigerator ( $+4\text{ }^{\circ}\text{C}$ ) in perforated and ziplock bags before processing. Perforated bags were preferred for the purpose of preventing the accumulation of moisture on the samples. Unshelled chestnuts were roasted in an oven at  $180\text{ }^{\circ}\text{C}$  for 25 min and boiled in water for 20 min. The shells and pellicles were manually removed. Then, with the aim of providing uniformity and increasing the extraction efficiency, chestnut fruits were ground.

Methanol-water (%80, v/v) were chosen as the extraction liquid. Two grams of fine chestnut powder were extracted with 50 mL extraction liquid at  $70\text{ }^{\circ}\text{C}$  for 30 min in an Erlenmeyer flask. To increase extraction efficiency Erlenmeyer flasks were mixed every 5 min. The samples were centrifuged ( $6000\times g$ , 15 min), and the supernatant were used for the analyses (De Vasconcelos *et al.*, 2007).

### Moisture content

The moisture content of chestnut samples were determined by AOAC method 925.40 – Moisture in Nuts (Horwitz & Latimer, 2010).

### Determination of total phenolic contents

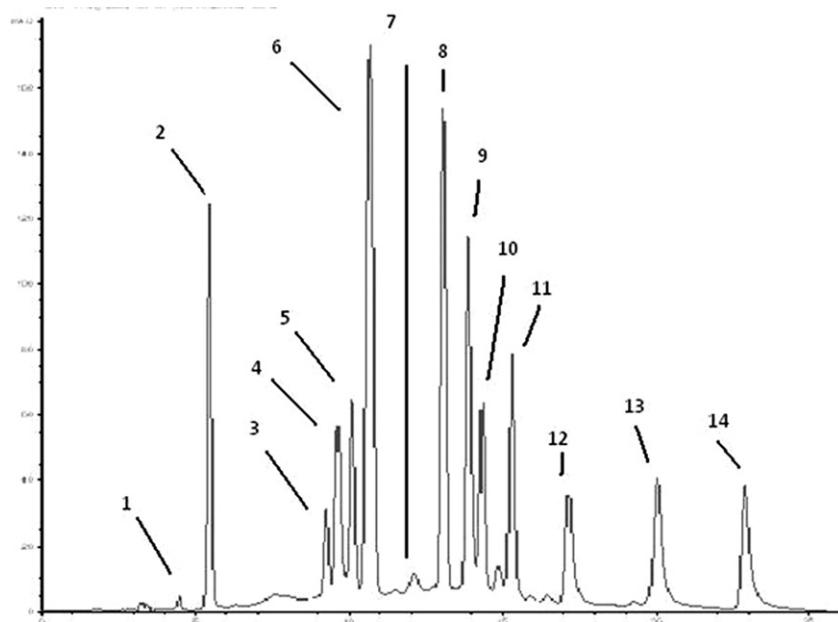
The total phenolic contents of chestnut fruits were determined based on the FC method described by Singleton & Rossi (1965) and Singleton *et al.* (1999) with some modifications. Gallic acid was used to produce the calibration curve. The solutions used in this analysis were the following:

- FC reagent
- 7%  $\text{Na}_2\text{CO}_3$  solution
- Standard solutions: gallic acid in 80% methanol (10–20–30–40–50–100 ppm)

Basically, 50  $\mu\text{L}$  of sample extract was mixed with 250  $\mu\text{L}$  of Folin and Ciocalteu's phenol reagent. This mixture was kept in the dark at room conditions for 5 min. Then, 750  $\mu\text{L}$  of 7% sodium carbonate solution was added to the mixture and it was diluted to 5 mL with distilled water. The reaction was performed in the dark for 120 min. The same procedures were applied to standard solutions. Then the absorbances of samples and standards were read at 765 nm (Varian Cary 50 Bio UV-Vis spectrophotometer; Varian Medical Systems Inc., Palo Alto, CA, USA). The results were expressed as mg of gallic acid equivalents (GAEs).

### Determination of total antioxidant activities

The antioxidant activities of chestnut samples were measured using the ferric reducing antioxidant power (FRAP) method described by Guo *et al.* (2003) with some modifications. The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, coloured form in the presence of antioxidants. The FRAP reagent contained 2.5 mL of a  $10\text{ mmol L}^{-1}$  TPTZ solution in  $40\text{ mmol L}^{-1}$  HCl plus 2.5 mL of  $20\text{ mmol L}^{-1}$   $\text{FeCl}_3$  and 25 mL of  $0.3\text{ mol L}^{-1}$  acetate buffer, pH 3.6 and was prepared freshly. The acetate buffer at pH 3.6 was prepared by weighing 0.775 g sodium acetate trihydrate ( $\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$ ), adding 4 mL acetic acid and adjusting to 250 mL with distilled water. 40  $\mu\text{L}$  sample supernatant were mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent. The absorbance of the reaction mixture at 593 nm was measured spectrophotometrically after incubation at  $37\text{ }^{\circ}\text{C}$  for 30 min. As a standard solution, iron sulphate ( $\text{FeSO}_4$ ) in 5% HCl at different concentrations ( $0.2\text{--}3\text{ mmol L}^{-1}$ ) was used. As



**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.

blank solutions, 5% HCl for standards and 80% methanol for samples were used.

sample injection volume was 10  $\mu$ L. Peaks of interest were monitored at 280, 277 and 254 nm. The HPLC chromatogram of standard mixture is showed in Figure 1.

### HPLC analysis of phenolic compounds

The phenolic structure of the samples in terms of 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 analysed using high-performance liquid chromatography (HPLC) with diode array detection. The method described by Li *et al.* (2006) was used with some modifications. An Agilent Technology 1200 series HPLC (Agilent Technologies Inc., Santa Clara, CA, USA) system equipped with a pump, a degasser, a thermostatic auto-sampler and a photodiode array detector was used for the analysis of phenolic compounds chestnut samples. The separation was carried out in a  $\mu$ -Bondapak C18 (3.9  $\times$  300 mm, 10  $\mu$ m) Agilent Tech column. Sample extracts and standards were filtered by 0.45  $\mu$ m Agilent microfilter. The binary mobile phase consisted of water containing 2% acetic acid (solvent A) and acetonitrile (solvent B). The system was run with a gradient programme: 95% A to 60% A in 23 min, 60% A to 45% A in 5 min and 45% A to 95% A in 7 min. The flow rate was kept constant at 0.5 mL min<sup>-1</sup> for a total run time of 35 min. The

### Statistical methods

For the statistical analysis, the program used was SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Comparisons were carried out at the 99% confidence level by application of analysis of variance and Duncan tests.

## Results and discussion

### Moisture contents

The moisture contents of raw chestnuts in previous studies (De Vasconcelos *et al.*, 2007; De Vasconcelos *et al.*, 2010a; Neri *et al.*, 2010) were between 35.6% and 60.1%. It was noted that weight losses due to roasting ranged from 23% to 30%. Data for the moisture contents of boiled chestnuts is not found in previous literature. In this study, we found that weight losses due to roasting were 4.26%, 4.01% and 3.05% for Aydin, Bursa and Zonguldak chestnuts, respectively. The mean of weight loss was 3.77%. This mean value was lower than indicated in previous studies. The difference could result from variation of species, soil structures, climate or

**Table 1** Moisture contents, total phenolic contents and total antioxidant activities of raw, roasted and boiled chestnuts of Aydin, Bursa and Zonguldak\*

Moisture (%)	Total phenolic content						Total antioxidant activity		
				(mg GAE g <sup>-1</sup> DM)			(mM FeSO <sub>4</sub> g <sup>-1</sup> DM)		
	Aydin	Bursa	Zonguldak	Aydin	Bursa	Zonguldak	Aydin	Bursa	Zonguldak
Raw	29.6 ± 8.77	35.5 ± 0.04	26.1 ± 4.73	14.7 ± 1.43 <sup>a</sup>	21.4 ± 1.76 <sup>a</sup>	6.9 ± 0.20 <sup>b</sup>	11.9 ± 0.90 <sup>a</sup>	11.4 ± 3.46 <sup>a</sup>	12.3 ± 2.63 <sup>a</sup>
Roasted	25.4 ± 0.22	31.5 ± 0.62	23.1 ± 0.9	27.2 ± 3.29 <sup>a</sup>	27.5 ± 2.68 <sup>a</sup>	57.4 ± 1.47 <sup>a</sup>	11.9 ± 2.39 <sup>a</sup>	13.1 ± 2.08 <sup>a</sup>	9.9 ± 0.27 <sup>a</sup>
Boiled	55.7 ± 1.26	50.4 ± 0.3	54.1 ± 0.43	8.8 ± 7.69 <sup>b</sup>	14.2 ± 8.53 <sup>a</sup>	7.3 ± 2.50 <sup>b</sup>	12.0 ± 4.04 <sup>a</sup>	12.7 ± 2.70 <sup>a</sup>	8.5 ± 4.57 <sup>a</sup>

\*Statistical analyses were applied on each analyses and provinces separately. <sup>a,b</sup>Means in the same column with unlike superscripts differ significantly ( $P < 0.01$ ).

growing conditions. Weight increases due to boiling were 26.06%, 14.86%, 27.95% for Aydin, Bursa and Zonguldak chestnuts, respectively. The mean of the weight increases was 22.96%. All results of moisture content are given in Table 1 with total phenolic contents and total antioxidant activities.

### Total phenolic contents

The total phenolic contents of raw chestnuts were indicated as 112.06 µg GAE g<sup>-1</sup> dry matter (DM) (Neri *et al.*, 2010) and 15.80 mg GAE g<sup>-1</sup> DM (De Vasconcelos *et al.*, 2007) in previous studies. In our study, the total phenolic contents of roasted chestnuts and boiled chestnuts of three provinces were evaluated separately. Also raw, roasted and boiled chestnut results for each province were compared. The results are given in Table 1.

For raw chestnuts, Zonguldak had the lowest and Bursa had the highest value for total phenolic content. Although the total phenolic content of roasted Zonguldak chestnut had the highest value and was significantly different from the others, the total phenolic contents of roasted Aydin and Bursa chestnuts were not significantly different. Roasted Zonguldak chestnuts had the highest value (57.43 mg GAE g<sup>-1</sup> DM sample). Roasted Aydin and Bursa chestnuts had close values that were 27.18 and 27.48 mg GAE g<sup>-1</sup> DM sample, respectively. The total phenolic contents of boiled chestnuts were not significantly different. The total phenolic contents of boiled Bursa, Aydin and Zonguldak chestnuts were 14.24, 8.78 and 7.30 mg GAE g<sup>-1</sup> DM sample, respectively. It was observed that the results of boiled chestnuts were lower than roasted chestnuts.

The total phenolic contents of Aydin chestnuts differed significantly. Roasted Aydin chestnut had the highest value (27.17 mg GAE g<sup>-1</sup> DM sample). The total phenolic contents of raw and boiled Aydin chestnuts were 14.73 and 8.78 mg GAE g<sup>-1</sup> DM sample, respectively.

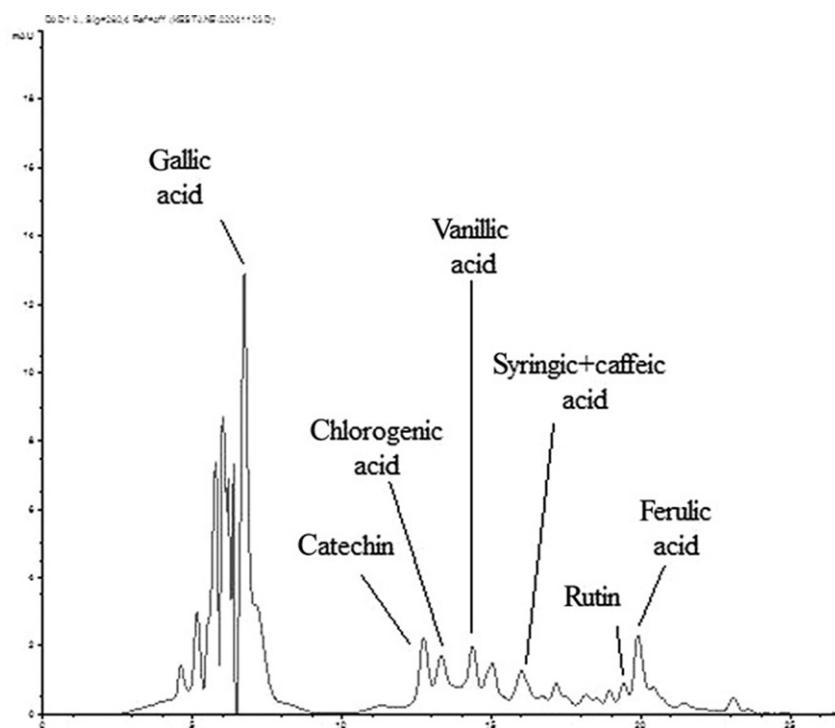
The total phenolic contents of Bursa chestnuts were not significantly different. The total phenolic contents of roasted, raw and boiled Bursa chestnuts were 27.48, 21.42 and 14.24 mg GAE g<sup>-1</sup> DM sample, respectively.

The total phenolic contents of Zonguldak chestnuts were significantly different. Roasted Zonguldak chestnut had the highest value (57.43 mg GAE g<sup>-1</sup> DM sample). The total phenolic contents of raw and boiled Zonguldak chestnuts were 6.88 and 7.3 mg GAE g<sup>-1</sup> DM sample, respectively.

Within all samples, the total phenolic contents of roasted chestnuts were higher than raw and boiled ones; the reason could be associated with bound phenolics. The free forms of phenolic compounds are very rarely present in plants. More often, they occur as esters, glycosides and bound complexes (Nardini & Ghiselli, 2004). The roasting process could convert bound forms to free. Whereas total phenolic content of raw Zonguldak chestnut was very low, roasted Zonguldak chestnut had very high value. This could mean that Zonguldak raw chestnuts had a lot of bound phenolics in their structure and the roasting process converted a lot of bound phenolics to free forms.

### Total antioxidant activity

Chestnut methanolic extracts were analysed by the FRAP antioxidant activity method and ferric sulphate was used for the calibration curve. Thus, the results of the analyses are expressed as mM FeSO<sub>4</sub> g<sup>-1</sup> DM. In one study, total antioxidant activity of chestnuts were identified by a DPPH method and found to be 6.2 µmol Trolox equivalence g<sup>-1</sup> DM (Abe *et al.*, 2010). Blomhoff *et al.* (2006) studied the total antioxidant capacity of some nuts and chose the FRAP method to determine total antioxidant activity. They indicated that the total antioxidant capacity of chestnut was 0.75 mmol/100 g. The method and standard for the calibration curve that we used were not used together in previous studies. Therefore, antioxidant capacity results of our samples can not be



**Figure 2** The HPLC chromatogram of raw Aydin chestnut (280 nm).

compared to previous studies. The results of this study are given in Table 1.

The total antioxidant activities of raw, roasted and boiled chestnuts showed no significant differences; total antioxidant activities of Zonguldak, Aydin and Bursa chestnuts were 12.34, 11.95 and 11.45, respectively; the total antioxidant activities of roasted Bursa, Aydin and Zonguldak chestnuts were 13.07, 11.92 and 9.88 mg GAE g<sup>-1</sup> DM sample; and the total antioxidant activities of boiled Bursa, Aydin and Zonguldak chestnuts were 12.74, 11.97 and 8.46 mg GAE g<sup>-1</sup> DM sample.

It can be concluded that the treatments did not affect the total antioxidant activities for the samples from the different provinces. Although there were significant differences in the total phenolic contents of chestnuts, the total antioxidant activities were nearly same for each group in itself.

### HPLC analyses of phenolic compounds

Myricetin, kaempferol, fumaric acid and quercetin were not found in any of the chestnuts. The phenolic compounds that were found in chestnut samples are given in Table 2 along with the statistical analyses. The results of analyses were expressed as ppm.

Although some phenolic compounds were found in roasted and boiled chestnuts, they were not detected in raw

samples; these compounds include ellagic acid, p-coumaric acid and naringin. The likely reason is that the phenolics are bound. Some phenolic compounds convert to free forms with temperature. Thus, they can be detected in roasted and boiled chestnuts.

The HPLC chromatograms of raw, roasted and boiled Aydin chestnuts are given in Figure 2–4.

### Conclusions

Roasting and boiling processes were applied to chestnut samples from three provinces (Aydin, Bursa and Zonguldak) and the total phenolic contents, total antioxidant capacities and phenolic compounds were determined. According to the total phenolic content analyses, there were statistically significant differences between processes, with roasted chestnuts having the highest values. However, the total antioxidant capacities of all chestnut samples were similar. From the 15 phenolic compound standards; myricetin, kaempferol, fumaric acid and quercetin were not been found. Some phenolic compounds were determined in roasted and boiled chestnuts, but they were not detected in raw samples. It is possible that some phenolic compounds converted to free forms as a result of the temperatures used in processing.

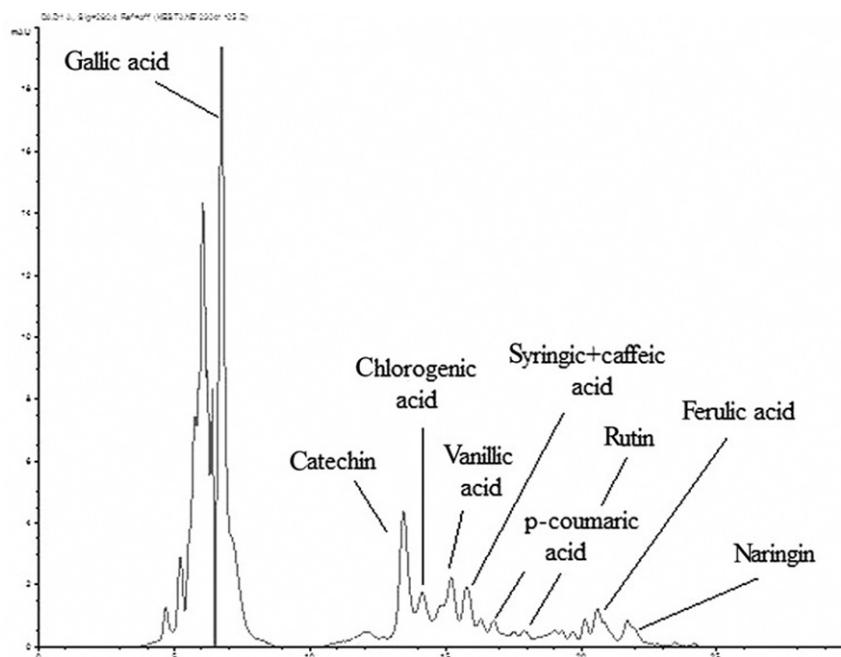


Figure 3 The HPLC chromatogram of roasted Aydin chestnut (280 nm).

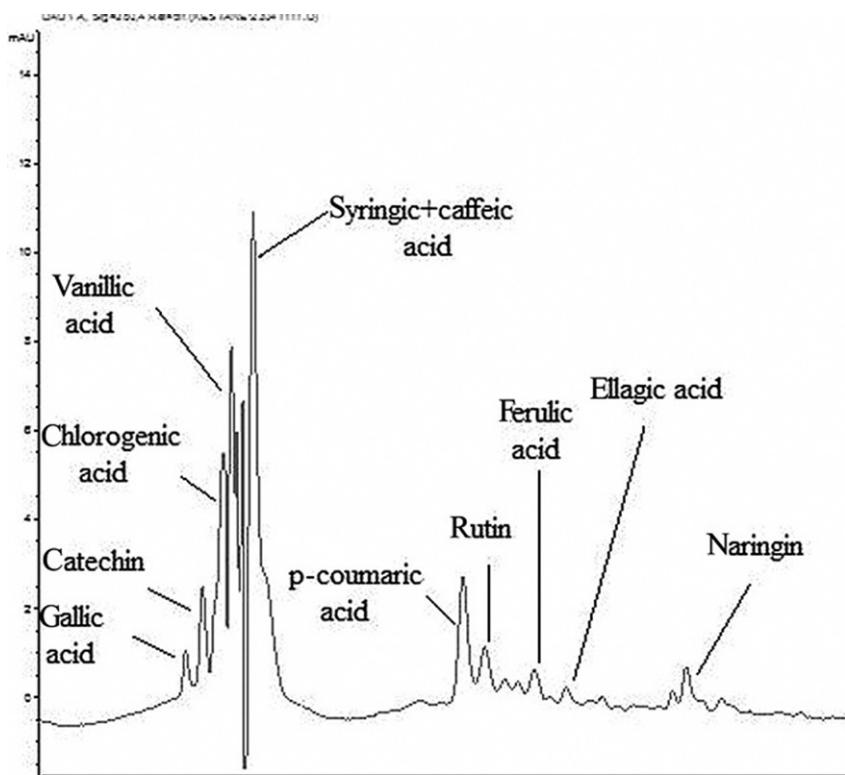


Figure 4 HPLC chromatogram of boiled Aydin chestnut (280 nm).

**Table 2** The amounts of phenolic compounds of all chestnut samples\* (ppm)

	Sample	Gallic acid	Syringic + caffeic acid	Vanillic acid	Rutin	Ellagic acid	Catechin	Chlorogenic acid	p-coumaric acid	Ferulic acid	Naringin
Aydin	Raw	126.3 <sup>b</sup>	16.2 <sup>a</sup>	486.4 <sup>b</sup>	30.5 <sup>a</sup>	0.0 <sup>b</sup>	49.0 <sup>b</sup>	15.0 <sup>a</sup>	0.0 <sup>b</sup>	12.4 <sup>a</sup>	0.0 <sup>c</sup>
	Roasted	219.5 <sup>a</sup>	16.0 <sup>a</sup>	1229.4 <sup>a</sup>	16.5 <sup>b</sup>	0.0 <sup>b</sup>	167.4 <sup>a</sup>	33.0 <sup>a</sup>	17.7 <sup>a</sup>	15.7 <sup>a</sup>	44.4 <sup>a</sup>
	Boiled	204.6 <sup>a</sup>	15.6 <sup>a</sup>	278.0 <sup>b</sup>	13.5 <sup>b</sup>	24.0 <sup>a</sup>	49.0 <sup>b</sup>	15.0 <sup>a</sup>	0.0 <sup>b</sup>	12.4 <sup>a</sup>	0.0 <sup>c</sup>
Bursa	Raw	263.3 <sup>a</sup>	23.5 <sup>a</sup>	417.4 <sup>b</sup>	18.7 <sup>ab</sup>	28.5 <sup>a</sup>	168.5 <sup>a</sup>	57.5 <sup>a</sup>	20.5 <sup>a</sup>	8.3 <sup>a</sup>	36.3 <sup>a</sup>
	Roasted	205.0 <sup>b</sup>	7.6 <sup>a</sup>	580.9 <sup>a</sup>	23.8 <sup>a</sup>	26.9 <sup>b</sup>	142.8 <sup>ab</sup>	32.8 <sup>b</sup>	19.7 <sup>a</sup>	5.1 <sup>a</sup>	29.0 <sup>ab</sup>
	Boiled	191.1 <sup>b</sup>	11.2 <sup>a</sup>	144.2 <sup>c</sup>	12.4 <sup>b</sup>	0.0 <sup>c</sup>	108.8 <sup>b</sup>	0.0 <sup>c</sup>	14.7 <sup>b</sup>	20.3 <sup>a</sup>	24.1 <sup>b</sup>
Zonguldak	Raw	179.8 <sup>a</sup>	13.8 <sup>a</sup>	1241.7 <sup>a</sup>	35.5 <sup>a</sup>	14.2 <sup>a</sup>	230.0 <sup>a</sup>	39.2 <sup>a</sup>	11.9 <sup>a</sup>	10.5 <sup>a</sup>	35.3 <sup>a</sup>
	Roasted	183.0 <sup>a</sup>	0.0 <sup>b</sup>	475.9 <sup>a</sup>	16.9 <sup>b</sup>	24.2 <sup>a</sup>	197.8 <sup>b</sup>	0.0 <sup>b</sup>	12.3 <sup>a</sup>	11.1 <sup>a</sup>	0.0 <sup>b</sup>
	Boiled	169.5 <sup>a</sup>	0.0 <sup>b</sup>	623.8 <sup>a</sup>	28.5 <sup>a</sup>	0.0 <sup>a</sup>	128.8 <sup>c</sup>	0.4 <sup>b</sup>	2.9 <sup>a</sup>	13.0 <sup>a</sup>	38.6 <sup>a</sup>

\*Statistical analyses were applied on each provinces and phenolic compounds separately. <sup>a,b,c</sup>Means in the same column with unlike superscripts differ significantly ( $P < 0.01$ ).

According to the results of earlier studies (De Vasconcelos *et al.*, 2009a,b; De Vasconcelos *et al.*, 2010b), the industrial processing of chestnut fruits has both positive and negative effects on composition. An extension of the shelf life of the fruits and an increase in crude energy, fibre, amino acid, tocopherols and phenolics are positive effects. Reductions in the levels of total starch, fat and vitamin C are negative effects. This study showed that processing can affect phenolics positively. It is especially notable for roasting processing. It may be attributed to bound phenolics and the effect of processing temperature.

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