

Chemical Composition of Seed Propagated Chestnut Genotypes from Northeastern Turkey

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Abstract

Turkey ranked third place in the world for chestnut production after China and Bolivia and the country has unique chestnut populations including valuable and diverse seed propagated chestnut genotypes. In this study, chestnuts (*Castanea sativa*) were collected during the 2016 harvest season from Northeastern part of Turkey from promising 12 different seedling origin genotypes. Tree growth habit, nut weight, kernel ratio, kernel color, moisture, crude protein, crude fat, dietary fiber, total polyphenols, antioxidant activity and fatty acid content of fruits belongs to 12 genotypes were determined. The results showed that, the majority of genotypes had semi upright tree growth habit. The nut weight and kernel ratio were between 5.05 g (K-10) and 10.10 g (K-5) and 71.10% (K-1) and 82.44% (K-3) among genotypes. The total crude fat content ranged from 0.87% (K-7) to 2.61% (K-1) while the crude protein ranged from 4.80% (K-7) to 7.65% (K-1). The dietary fiber content made up no more than 3.61% of the remaining portion of the kernel. It was found that total polyphenols was between 1.66 and 2.70 g GAE/kg and antioxidant activity was between 5.80 and 9.07 μ mol Trolox equivalent/g dry weight basis. Oleic and linoleic acid were the major fatty acids in all chestnut fruits and followed by palmitic and linolenic acid. The results showed that there was enough variations among seed propagated chestnut genotypes for most of the searched parameters and this highlights the importance of conserving the genotypes, as their high levels of heterogeneity allow them to respond to abiotic and biotic stresses and adapt to low-input farming systems.

Keywords: bioactive content, *Castanea sativa*, diversity, fatty acids, pomology

Introduction

Chestnut (*Castanea sativa*) is the oldest cultivated fruit in the world. The chestnut fruits have a high nutritional value and high-quality wood. In addition, chestnuts have significant economical value and medicinal importance for human health as sources of antioxidants, and as sources of other useful bioactive substances (Vasconcelos *et al.*, 2010). Chestnut fruits are highly regarded and widely consumed throughout Europe, America and Asia. Various commercial forms are available, e.g. fresh and industrially processed. People consume it in large quantities; therefore, it has a very important role in public nutritional habits. Chestnut has a high calorie level, rich nutrient composition. Chestnuts contain high amount of carbohydrates, protein and dietary fiber. In addition, chestnuts differ from the other nuts for

their low fat and salt content, which make them ideally suited for human nutrition and health (Mujic *et al.*, 2010).

Chestnut is geographically distributed in three major areas: Europe with *Castanea sativa* Mill., Asia with *Castanea creanata* Sieb. and Zucc. (Japan) and *Castanea mollissima* Bl. (China and Korea), and North America with *Castanea dentata* Borkh. All *Castanea* species and their hybrids are edible and some are used in commercial nut production around the world (Bounous, 2005; Lang *et al.*, 2006).

Major chestnut-producing countries in the world are China (1,685,000 tons), Bolivia (78,000 tons) Turkey (64,000 tons), South Korea (56,000 tons), and Italy (52,000 tons) (FAO, 2014). Turkey has a remarkable chestnut population of seed propagated chestnut genotypes when compared to other parts of the world (Erturk *et al.*, 2006; Ertan, 2007; Serdar *et al.*, 2009; Yildiz *et al.*, 2009; Ormeci *et al.*, 2016).

Chestnut growing areas in Turkey spread from East of the Black Sea Region, through Marmara and Aegean Regions and reaches to in the Mediterranean Region in Anatolia (Ertan, 2007). In Turkey, chestnut production is mainly based on local heterogeneous genotypes, which represent examples of farmer selection carried out on-farm, and are named based on the geographical area of cultivation, without genetic or origin identification. Thus chestnut populations show considerable variations aspect of nut quality, tree characteristics, productivity, health status, and climatic adaptability. These genetic resources provide a good opportunity for genetic improvement. It seems that there were a lot of chestnut genotypes that free of chestnut blight (*Cryphonectria parasitica*) and ink disease (*Phytophthora cambivora*) damage in Turkey's forests (Ormeçi *et al.*, 2016).

The most important chestnut varieties in Turkey are 'Akkozak', 'Alimolla', 'Demirci', 'Dursun', 'Firdola', 'Hacıbıç', 'Hacıömer', 'Halilibrahim', 'İnegölkestanesi', 'İzmitli', 'Karamehmet', 'Korucu', 'Osmanoğlu', 'Öküçgözü', 'Sarışlama', 'Sarkestan', and 'Tepeköysarı' and all of them are selected among seed propagated chestnut populations (Mert and Erturk, 2017).

Recently, an increasing concern on chestnut and chestnut cultivation has been showing again in Turkey. The nuts are consumed directly in roasted or boiled form or evaluated as value added products such as chestnut desserts and candied chestnut (Mert and Erturk, 2017).

Previously, various composition and health studies clearly indicated that chestnut fruits, and potentially other extracts from chestnut trees, have considerable potential as functional foods or as food ingredients, e.g. chestnut polyphenolic extracts as a natural source of antioxidants and other beneficial compounds (Attanasio *et al.*, 2004; Bernardez *et al.*, 2004; De La Montana Miguelez *et al.*, 2004; Pereira-Lorenzo *et al.*, 2006). It has been previously shown that many ellagitannins, including castalagin and vescalagin, have potent antitumor, antioxidant, antimicrobial and antimalarial properties (Cerda *et al.*, 2004; Seeram *et al.*, 2005; Reddy *et al.*, 2007).

In our study, a compositional comparison between different seed propagated chestnut genotypes was undertaken aiming at exploiting the nutrient profiles of natural growing chestnut fruits and promoting the further development of the rich chestnut resources. The present work was to investigate the twelve chestnut genotypes from Northeastern Turkey in terms of pomological (tree growth habit, nut weight, kernel ratio, kernel color) and proximate nutritive compounds (including moisture, crude protein, crude fat, crude fat, dietary fiber, total carbohydrates, total polyphenols, antioxidant activity and fatty acids).

Materials and Methods

Plant material

This research was conducted at Rize province of Turkey in 2016. Among population, a total 12 seed propagated promising chestnut genotypes were marked in terms of better yield, nut size, earliness and resistance to chestnut blight and ink disease characteristics. The nut samples were collected from 12 promising genotypes. The K letter (K-1 to K-12) was assigned to the 12 genotypes.

Pomological traits

Pomological characteristics and chemical content were conducted with four replications on a total 40 nuts per genotype. Nut weight was measured by using a digital balance with a sensitivity of 0.001 g. Kernel ratio (%) was counted considering nut and kernel weight (Ertan *et al.* 2007).

Proximate analysis

Kernel parts of nuts samples were used to assess moisture, crude protein, crude fat, dietary fiber, total carbohydrates, total polyphenols, and antioxidant activity. The moisture content of the chestnuts was determined by the gravimetric method using a drying oven at 105 ± 2 °C. The total nitrogen was analysed using the Kjeldahl method, and crude protein content was calculated using a nitrogen conversion factor of 5.30, which is specific for chestnut fruits (AOAC, 2000). Total fat was determined after extraction with ether for 16 h in a Soxhlet device (AOAC, 2000). The dinitrophenol method was utilized in the analysis of total carbohydrates (Ross, 1959) using spectrophotometer.

Total polyphenol and antioxidant activity

For total polyphenol analysis, the sample extraction was carried out combining 3 g of sample with 6 mL of 70% (v/v) ethanol and homogenizing with an Ultra-Turrax homogenizer. The extract was shaken at 210 rpm under refrigerated conditions for 15 min and then centrifuged for 15 min at $2346 \times g$ (5 °C). Before analysis, phenolic compounds were extracted by solid-phase extraction because substances such as reducing sugars, alcohol and tartaric acid, as well as antioxidant compounds (ascorbic acid) could interfere in the determination of polyphenols with the Folin-Ciocalteu reagent (Naczki and Shahidi, 2004). Commercially available octadecyl C18 cartridges (1 g, 6 mL) were used for the extraction of the phenolic fraction according to the following protocol: 2 mL of sample was loaded onto the column previously conditioned with 5 mL of methanol and 10 mL of water. The column was eluted with 4 mL of 0.02 N sulphuric acid to eliminate all the water-soluble compounds. The compounds retained by the column were recovered by eluting with 4 mL of 60% (v/v) methanol solution. Total polyphenol content was determined by the colorimetric reaction with the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Gallic acid was used as an external standard for the calibration curve and results were expressed as g of gallic acid equivalents (GAE) per kg of dry weight.

For antioxidant activity determination, the sample extraction was carried out combining 3 g of sample with 6 mL of 70% (v/v) ethanol and homogenizing with an Ultra Turrax homogenizer. The extract was shaken at 180 rpm under refrigerated condition for 15 min then centrifuged for 15 min at $2346 \times g$ (5 °C). The radical-scavenging activity was determined soon after extraction by the ABTS⁺ radical cation decolorization assay, as described by Re *et al.* (1999). The bleaching rate of ABTS⁺ in the presence of the sample was monitored at 734 nm by a spectrometer. A volume of 2.97 mL of ABTS⁺ solution was used. The reaction was started by the addition of 30 mL of the ethanolic extract diluted up to 1:10. ABTS⁺ bleaching was monitored at 25 °C for at least 30 min and the percentage of decoloration after 7 min was used as the measure of antioxidant activity. In this dilution range, the ABTS⁺ bleaching was proportional to the concentration of the

sample added to the medium, and a linear model fit to the dose-response curve. Antioxidant activity was calculated by the ratio of the regression coefficient of the dose-response curve of the sample and the regression coefficient of the dose-response curve of Trolox (hydrophilic homologue of α -tocopherol), and was expressed as μmol of Trolox equivalents per g of sample (dry weight basis).

Fatty acid analysis

Fatty Acids were prepared by hydrolysis with a 2 M methanolic potassium hydroxide solution, and extraction with n-heptane, in accordance with ISO 5509 method (ISO, 2000) and following a procedure described in a previous work (Barreira *et al.*, 2009). The fatty acids profile was evaluated with a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, a flame ionization detector (FID), and a Chrompack CP-9050 auto-sampler. Separation was achieved on a 50 m \times 0.25 mm i.d. fused silica capillary column coated with a 0.19 μm film of CP-Sil 88 (Chrompack). Helium was used as carrier gas at an internal pressure of 120 kPa. The results are expressed in relative percent-age of each fatty acid, calculated by internal normalization of the chromatographic peak area, and assuming that the detector response was the same for all compounds.

Statistical analysis

The experiment was a completely randomized design with four replications. The replications were done from the measuring solutions. Data were subjected to analysis of variance (ANOVA) and means were separated by LSD test at $p < 0.05$ significant levels.

Results and Discussion

Pomological traits

In present study, tree growth habits and kernel color of 12 genotypes are mostly semi upright and light cream (Table 1). Serdar *et al.* (2011) reported that the chestnut cultivars 'Serdar' and 'Marigoule' have semi upright tree growth habit and light cream and cream kernel color.

The nut weight varied from 5.03 g (K-10) to 10.10 g (K-5) (Table 1). Serdar (2002) selected 13 new chestnut genotypes in

Camili vicinity of Northeastern part of Turkey and average nut weight varied between 4.79 to 7.45 g respectively. Nut weight of 12 chestnut genotypes were comparable to previously selected genotypes of chestnuts in China (Ding, 1993), in India (Pandit *et al.* 2011), in Slovenia (Solar *et al.* 2005) and in Bosnia & Herzegovina (Mujic *et al.*, 2010).

Kernel ratio of the 12 chestnut genotypes varied from 71.10% (K-1) to 82.44% (K-3), respectively (Table 1). In our study, kernel ratio of 12 genotypes was similar to other studies (Serdar, 2002; Ertan *et al.* 2007). Mujic *et al.* (2010) reported the percentage of kernel was ranged from 78.5 to 87.3% in non-grafted chestnuts in Bosnia & Herzegovina. Ertan (2007) reported percentage of kernel among European chestnuts from ten different areas in Turkey between 75.9 to 86.1%.

Proximate composition

The moisture, total crude protein, crude fat, total carbohydrates and dietary fiber content are shown in Table 2. Moisture, protein, fat, carbohydrate and fiber content varied from 45.11% (K-1) to 54.27% (K-7); 4.80% (K-7) to 7.65% (K-1); 0.87% (K-7) to 2.61% (K-1); 52.33% (K-7) to 62.10% (K-1) and 2.06% (K-4) to 3.61% (K-1), respectively (Table 2). Mert and Erturk (2017) reported kernel composition of 19 local and foreign chestnut cultivars were 5.58-7.35 g/100 g protein; 58.18-66.21 g/100 g total carbohydrates (on the dry matter basis). Bernardez *et al.* (2004) reported moisture contents of chestnuts in Spain between 48.37-59.35%. Pereira-Lorenzo *et al.*, (2006) and De La Montana Miguelez *et al.* (2004) determined the same parameter as 54% and 49%, respectively. The total protein content was reported between 4.50 and 10.87 g/100 g by different researchers in *C. sativa* Mill. (De La Montana Miguelez *et al.*, 2004; Ertan and Kiliç, 2005; Ertürk *et al.*, 2006; Pereira-Lorenzo *et al.*, 2006; Mert and Erturk (2017). Our results are similar to the previous results. Mert and Erturk (2017) reported total carbohydrate quantities of chestnuts fruits between 58.18 and 66.21% depending on cultivar (Table 2). Erturk *et al.* (2006) reported that this component in *Castanea sativa* Mill. cultivars between 75.32 and 86.31%. Results of this research were comparable with above results.

Table 1. Some pomological characteristics of genotypes

Genotyp/ Cultivars	Tree growth habits	Average nut weight (g)	Average kernel ratio (%)	Kernel color
K-1	Semi upright	6.27	71.10	Cream
K-2	Spreading	5.83	74.43	Light cream
K-3	Semi upright	9.32	82.44	Light cream
K-4	Semi upright	7.16	76.38	Cream
K-5	Spreading	10.10	80.76	Cream
K-6	Semi upright	8.74	75.40	Dark cream
K-7	Semi upright	7.49	77.62	Cream
K-8	Spreading	5.56	81.59	Light cream
K-9	Semi upright	9.38	80.37	Light cream
K-10	Semi upright	5.03	75.29	Light cream
K-11	Semi upright	6.27	78.44	Cream
K-12	Semi upright	7.81	80.15	Light cream
LSD _{0.05}		2.18	4.57	

Table 2. Proximate composition of chestnut fruits of 12 genotypes (dry weight basis per 100 g)

Genotypes/ Cultivars	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Carbo- hydrate (%)	Dietary Fiber (%)
K-1	45.11	7.65	2.40	62.10	3.61
K-2	47.98	6.98	1.97	58.64	2.14
K-3	46.16	7.45	2.49	60.18	2.73
K-4	47.43	6.94	1.44	57.52	2.06
K-5	50.30	6.05	1.65	55.44	2.55
K-6	49.15	6.37	1.16	59.51	2.80
K-7	54.27	4.80	0.93	52.23	2.27
K-8	48.35	5.67	1.12	53.17	2.44
K-9	47.66	7.10	1.35	57.41	3.15
K-10	50.10	5.98	1.51	56.49	2.38
K-11	52.11	5.57	0.97	54.49	3.03
K-12	49.55	6.59	1.96	60.87	2.95
LSD _{0.05}	4.22	1.98	0.36	3.89	0.34

Comparing to the other nuts such as walnut, hazelnut etc., chestnuts differ from other nuts and have lower fat (2.0-5.0%) content. The crude indicate significant classes among the cultivars ($p < 0.05$). Mert and Erturk (2017) reported the crude fat amount chestnut cultivars ranged from 0.87 to 2.61%. Our results are in accordance with those obtained by Erturk *et al.* (2006) for *C. sativa* and hybrid cultivars, for Italian cultivars (Sacchetti and Pinnavaia, 2005) and for Spanish cultivars (De La Montana Miguez *et al.*, 2004 and Pereira-Lorenzo *et al.*, 2006)

Previous studies indicated cultivar/genotype differences on chemical content of fruit species, including stone fruits, nuts, berries and subtropical fruits (Celik *et al.*, 2007; Ercisli, 2009; Ercisli *et al.*, 2010; Erturk *et al.*, 2010; Saridas *et al.*, 2016; Yazici and Sahin, 2016; Zorenc *et al.*, 2016).

Total polyphenols and antioxidant activity

Total polyphenols was between 1.66 and 2.70 g GAE/kg on dry weight basis and antioxidant activity was between 5.80 and 9.07 μmol Trolox equivalent/g dry weight (Table 3). There were statistical differences among genotypes on these parameters ($p < 0.05$). These results make it possible to classify chestnut among fruits with low polyphenol content (Vinson *et al.*, 2001). Our polyphenol data were slightly higher than those results (1.27-2.35 g/kg d.w.) reported by Vekiari *et al.* (2006), which found the highest values in Spanish chestnuts followed by Greek nuts. In this study, the antioxidant activity of chestnuts was measured with a method extensively used in literature, expressed in terms of μmol of Trolox equivalents on g dry fresh and the values were compared with those obtained on other fruits and vegetables by the same method of analysis. The antioxidant activity of the 12 chestnut genotypes represents a median value between the TEAC values of other fruits as reported by Pellegrini *et al.* (2003).

Fatty acids

Table 4 shows the fatty acids profiles data reported as mean value of each genotype. The results show that chestnuts genotypes were a significant source of variation for the majority of fatty acids. Oleic acid is the most abundant, varying from 34.3% (K-6) to 41.7% (K-2) among genotypes. Polyunsaturated fatty acid (PUFA) seemed to be favored for most of the genotypes and linoleic acid was evidently the major PUFA, with contents ranging from 33.2% (K-4) and 39.4% (K-1) (Table 4). The high amount of this PUFA represents a well-known advantage, since it is classified as an essential fatty acids (Emken *et al.*, 1994).

Results demonstrated that chestnut lipidic fraction is mainly constituted by three fatty acids: linoleic, oleic and palmitic acids accounting for more than 90% of the total fatty acid content, a value slightly higher or similar when compared with the results obtained by other research groups (Borges *et al.*, 2007; Barreira *et al.*, 2012), most likely due to the different origin of chestnut samples. Currently, consumers are showing an increased interest in chestnuts because of their nutritional qualities and potentially beneficial health effects, including the well-known advantages of omega-3 and omega-6 polyunsaturated fatty acids (PUFA), whose intake is insufficient in Western diets (Simopoulos, 1991). Particularly linoleic acid plays an important role in preventing cardiovascular diseases in adults, promoting the brain and

retina development in infants (Simopoulos, 1991; Senter *et al.*, 1994; Künsch *et al.*, 1999) or preventing DNA damage (Kok *et al.*, 2003).

Table 3. Means of the polyphenols concentration and antioxidant activity of chestnuts (dry weight basis)

Genotypes/ Cultivars	Total polyphenols (g GAE/kg)	Antioxidant activity (μmol Trolox equal/g)
K-1	2.56	8.85
K-2	1.94	7.11
K-3	1.82	6.94
K-4	2.42	8.34
K-5	2.10	7.68
K-6	2.70	9.07
K-7	2.20	8.02
K-8	1.78	5.80
K-9	1.82	6.38
K-10	1.88	6.11
K-11	2.14	7.96
K-12	1.66	5.97
LSD _{0.05}	0.14	2.12

NS: not significant.

Table 4. Means of the fatty acids of genotypes (dry weight basis per g fruit)

Genotypes/ Cultivars	16:0 (%)	18:1 (%)	18:2 (%)	18:3 (%)
K-1	13.2	38.7	39.4	3.8
K-2	16.3	41.7	35.1	3.9
K-3	15.5	40.3	35.7	3.9
K-4	12.1	36.2	33.2	3.6
K-5	17.2	40.9	38.6	4.0
K-6	15.1	34.3	37.8	3.8
K-7	18.1	36.5	35.3	4.7
K-8	17.8	35.9	38.6	3.7
K-9	14.7	34.9	33.9	4.3
K-10	13.8	37.6	36.8	4.1
K-11	15.9	37.0	37.5	4.3
K-12	17.0	37.9	35.0	4.0
LSD _{0.05}	1.7	2.8	2.6	0.5

Conclusions

The present study highlighted the pomological values and bioactive compositions varied among wild grown chestnuts. This may have important for future breeding activities to select better genotypes to use in cross breeding studies. Present study also showed that chestnut fruits are one of the most promising natural antioxidant sources. Therefore, utilizing wild grown chestnuts as sources of phytochemicals could offer enormous opportunities for the functional food industry. These efforts highlight the need for the assessment of this traditional germplasm under different profiles, fundamental for its protection and conservation. The value of plant genetic resources lies in producing new cultivars, and in responding to new challenges based on systems of sustainable production and improved nutritional quality.

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