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PHYSICAL AND COMPOSITIONAL CHARACTERISTICS OF CHESTNUT FRUITS

S. Cosmulescu[1]*, I. Trandafir[2], V. Nour[1] and M. Botu[1]

1Department of Horticulture and Food Science, Faculty of Horticulture, University of Craiova, A.I. Cuza 13, 200585 Craiova, Romania

2 Department of Chemistry, Faculty of Sciences, University of Craiova, Calea Bucureşti Street 107, 200529, Craiova, Romania

*Corresponding author email: sinacosmulescu@hotmail.com

ABSTRACT

The objective of this study was investigationofphysical and compositional fruits characteristics of six chestnut cultivars. The fruits belonging to these cultivars were subjected to physical and chemical composition determinations, and the obtained results were comparatively analysed. It can be noticed that there are significant differences between cultivars for all the analysed traits. A high variability was found between cultivars in terms of nutrients content. The moisture content of chestnuts analysed in this study ranged from 51.63% to 58.42%. The protein content recorded values between 5.40 and 7.18% fresh matter. The polyphenols content in cultivars taken under analysis was between 1.65 and 19.60 mg GAE/g, which is a very large variation range between different cultivars. The obtained values of antioxidant activity of chestnuts are high, ranging from 0.73 to 9.90 mg Trolox/g of fresh substance, revealing also a wide variation range. Among the individual phenolic compounds, elagic and gallic acids were determined in higher amounts. The results of this study have shown that chestnut fruits contain significant concentrations of primary and secondary metabolites that are known for their positive effects on human health.

Keywords*:* antioxidant capacity; chestnut; flavonoid content; nutritional quality parameters; phenolics

INTRODUCTION

For many centuries, sweet chestnuts (*Castaneasativa* Mill.) have been some of the most important food resources in European rural areas, but the emergence of severe pathologies as well as the depopulation of mountain areas have caused a gradual decline in their consumption (Adua, 1998). Recently, the growing demand for traditional food has increased the interest in this resource and the improvement of its capitalization. The assortment includes hundreds of cultivars with specific characteristics and chemical composition. Knowledge of product composition can offer new opportunities for the chestnut market, since some chestnuts types are suitable for fresh consumption while others are more suitable for drying, producing of flour or pastries (Bounouset al., 2005). Numerous studies have been conducted on nutritional composition of European chestnuts (Bassi et al., 1984; Pinnavaia et al., 1993; Künsch et al., 1999; Tarquini et al., 2001; Attanasio et al., 2004; Sacchetti et al., 2005; Bellini, 2005). Data on cultivars cultivated in Romania are still limited

and incomplete because most of these studies do not report all compositional characteristics. An important aspect in the chemical characterization of chestnuts is that the data in the literature shows great variability, and sometimes it is difficult to describe the chemical characteristics of chestnut cultivars. This is due to several reasons, among which are included: i) some data refers to chestnuts and others refers to marrons, which are products with different morphological characteristics and technological characteristics; ii) some cultivars have different ecotypes with different chemical characteristics (Sacchetti et al., 2005) related to ecological environment; iii) different clones of the same cultivar could exhibit a different chemical composition (Pinnavaia et al., 1993); iv) chestnut composition shows a dramatic variation determined by the harvesting year (Pinnavaia et al., 1993; Sacchetti et al., 2005). In addition, the interaction between years and cultivar is also significant (Ferreira-Cardoso et al., 2005). Another source of variation in data about composition is that some literature references relate about the fresh fruit, while others refers to dried fruit, which represent the bulk of commercial products available on the market.

The aim of this study was to investigate the composition of chestnut fruits from six chestnut cultivars that were grown at R.S.F.G. Valcea. Another important objective of this study was to determine the content of bioactive antioxidant compounds (total polyphenols, individual phenolic compounds, flavonoids) in chestnuts, as well as their in vitro antioxidant activity, aswellas to test the potential use of chestnuts as natural source of antioxidant compounds.

MATERIALS AND METHODS

Materials. Six chestnut cultivars were studied: 'Marisol', 'Maraval', 'Casval', 'Bournette', 'PrecoceMigoule' and 'Marissard', all cultivated at Valcea Research Station (45°07' N / 24°22' E). The fruits belonging to these cultivars were subjected to physical determinations and chemical composition, and the results obtained were comparatively analysed.

Regarding the physical properties of fruits, determinations were made on biometric data (fruit diameters and height), individual mass and fruit volume. Laboratory analyses were conducted on moisture content, total dry substance, titratable acidity, total content of phenolic compounds, total flavonoid content as well as antioxidant activity of fruits. 20 fruits for each cultivar have been individually measured and weighed. After harvesting, the samples were stored at -20°C for one month. To determine the moisture content and titratable acidity, the fruits were stored at +4°C and used the next day. **Determination of nutritional quality parameters** (moisture content, total dry matter, titratable acidity). The moisture content was gravimetrically determined by drying the amount of 5 g of fresh vegetable product up to a constant weight in a laboratory oven (Memmert, Germany) that was set at 105°C. Titratable acidity (% citric acid) was measured by titrating an aqueous extract of plant matter with a 0.1 N NaOH solution using phenolphthalein as an indicator.

Determination of total content of phenolic compounds. Total content of phenolic compounds was spectrophotometrically determined by colorimetric method (Singleton et al., 1965) using gallic acid (99% purity, Sigma) as the standard calibration substance. The Folin-Ciocalteu (2N, Merk) reagent and anhydrous sodium carbonate (99% purity, Sigma) were also used. Samples (3 g of plant product) were extracted with 5 mL of methanol in a BandelinSonorex Digital 10P ultrasonic bath for 45 min at ambient temperature. After extraction, the samples were centrifuged for 5 min at 4200 rpm and the supernatants were filtered through polyamide membranes with 0.45 µm holes. To 100 µL of each methanolic extract, 5 mL of distilled water and 500 µL of Folin-Ciocalteu reagent were added. After at least 30 seconds and maximum 8 minutes, 1.5 mL of sodium carbonate solution (20% w / v)

was added. The reaction mixture was diluted with distilled water for a final volume of 10 mL. The same procedure was applied to standard gallic acid solutions. The absorbance at 765 nm of each mixture was measured with a Varian Cary 50 UV spectrophotometer (Varian Co. USA) after incubation for 30 min at 40°C. The results were expressed in mg of gallic acid equivalent (GAE)/100 g.

Determination of total flavonoid content. Determination of flavonoids was performed using the aluminium nitrate colorimetric method described by Mohammadzadeh et al. (2007). Briefly, 0.5 mL of extract was diluted with (1:10) methanol and mixed with 0.1 mL of 10% aluminium nitrate, 0.1 mL of aqueous solution of 1 M potassium acetate and 4.3 mL of methanol. After storing them for 40 minutes at room temperature, the absorbance of the mixture was determined at 415 nm. The mixture of reagents without the sample extract was used as control. Quercetin was used to prepare the standard curve (0-100 mg/l). The samples were analysed in triplicate and the results were expressed in milligrams equivalent of quercetin/100 g (mg QE/100 g).

Determination of antioxidant capacity. Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Oliveira et al. (2008), with some modifications. Extraction of samples was carried out according to the same protocol described in the determination of total polyphenol content. Each methanolic plant extract (50 µL) was mixed with 3 mL of methanolic solution containing 0.004% (v/v) DPPH. The mixture was vigorously stirred and allowed to stand at room temperature and in darkness for 30 min, after which the absorbance decrease was measured at 517 nm using the Varian Cary 50 UV-Vis spectrophotometer. Capture capacity of DPPH free radical was then calculated using the formula: DPPH capture capacity (%) = [1 – Abssample/Abscontrol] × 100, where Abscontrol is the absorbance of control (DPPH solution without sample) and Abssample is the absorbance of the sample to be analysed. The capture capacity of DPPH free radical was then expressed in relation to Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which was used as standard reference. The following substances and reagents were used: methanol (Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Merck). The DPPH radical was freshly prepared and protected from light. A methanol/water mixture served as control and was run in each test. All analyses were performed in triplicate. The results were expressed in mmol of Trolox/100 g.

Determination of individual phenolic compounds by HPLC. High Performance Liquid Chromatography analyses were performed using a Surveyor Thermo Electron HPLC system comprising a Surveyor Plus LCPMPP pump, a Surveyor Plus ASP autosampler, a PDA 5P array diode detector, and the Chrom Quest 4.2 manager system as data processing system, using the method described by Nour et al. (2013). Separation was carried out in reverse phase on a Hypersil Gold C18 column (particle size 5 mm, 250 mm x 4.6 mm) supplied by Thermo Electron Corporation (San Jose, CA). The mobile phase was made up of a solution of 1% acetic acid (A) and methanol (B). Samples were eluted using the following gradient: 90% A from 0 to 27 min, from 90 to 60% A in 28 min, 60% A for 5 min, from 60 to 56% A in 2 min, 56% A for 8 min, from 56 to 90% A in 1 min and 90% A in 4 min to restore the initial conditions, before the next sample is injected. All gradients were linear. The flow rate was 1 mL/min, the injection volume was 5 µL and the column temperature was maintained at 20°C. Chromatograms were recorded at three wavelengths (254, 278 and 300 nm) corresponding to the maximum absorption of the analysed compounds. Each compound was identified by retention time and by standard enrichment under the same conditions. The identity of constituents was also confirmed using the diode array detector by comparison with the UV spectra of standards within the wavelength range 220-450 nm. Quantification was performed by external calibration using a five-point curve obtained by dilutions of

standard solutions. Each standard solution was injected into the HPLC system and the calibration curves were plotted by plotting the peak areas according to respective concentrations for each compound. The content of phenolic compounds investigated in the extracts was expressed in mg/100 g as the mean ± standard deviation. The standards of phenolic acids (gallic, vanilic, chlorogenic, caffeic, siringic, p-coumaric, ferulic, synapic, salicylic, elagic and trans-cinnamic) and flavonoids (catechin, epicatechin, routine, miricetinandquercetin) were purchased from Sigma-Aldrich (Darmstadt, Germany). Methanol and acetic acid were purchased from Merck. The water used in the experiments was treated in a SGWater purification system (Merck KGaA, Darmstadt, Germany).

Statistical analysis. Statistical analysis was performed using Statgraphic Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). The data were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Determinations of physical characteristics of the analysed cultivars reveal, in terms of fruit size, 'Maraval', 'PrecoceMigoule' and 'Marsol' cultivars. All these varieties were also the ones that presented the highest values of fruits mass and volume. 'Bournette' cultivar is worth nothing, because, although having small size fruits, exhibited the highest specific weight among the cultivars analysed. It can be noticed that there are significant differences between cultivars for all the analysed physical traits. The results are shown in Table 1 and Table 2.

Table 1. Diameters, height, shape index and size index determined in analyzed chestnut cultivars

Cultivars	Large diameter (D), mm	Small diameter (d), mm	Height (h), mm	Shape index	Size index
Marsol	35.21±1.69^{cd}	21.23±1.41^{b}	28.9±1.27^{b}	1.01±0.05^{a}	28.28±0.99^{b}
Maraval	39.18±2.24^{e}	23.65±3.68^{c}	33.91±1.32^{d}	1.08±0.07^{bc}	32.25±1.95^{d}
Casval	32.13±1.69^{b}	17.38±1.89^{a}	25.88±1.35^{a}	1.05±0.08^{ab}	25.13±1.26^{a}
Bournette	30.06±2.33^{a}	17.12±2.41^{a}	26.67±1.38^{a}	1.14±0.07^{d}	24.62±1.62^{a}
PrecoceMigoule	36.34±1.64^{d}	20.34±2.09^{b}	31.04±1.16^{c}	1.10±0.05^{cd}	29.24±1.23^{c}
Marissard	34.14±2.32^{c}	18.59±2.62^{a}	31.41±1.51^{c}	1.20±0.10^{e}	28.05±1.74^{b}

*Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$

Table 2. Mass, volume and specific weight determined in chestnut cultivars varieties analyzed

Cultivars	Mass (g)	Volume (cm^3)	Specific weight (g/cm^3)
Marsol	10.13±1.04^{d}	13.80±0.94^{c}	0.73±0.03^{bc}
Maraval	12.76±2.20^{e}	15.64±1.21^{d}	0.81±0.08^{cd}
Casval	5.48±0.77^{a}	9.86±0.65^{b}	0.55±0.04^{a}
Bournette	6.64±0.96^{b}	7.64±0.58^{a}	0.87±0.06^{d}
PrecoceMigoule	10.35±1.38^{d}	13.42±1.02^{c}	0.77±0.04^{bcd}
Marissard	8.72±1.86^{c}	12.62±0.87^{c}	0.69±0.10^{b}

*Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$

The moisture content of chestnuts analysed in this study ranged from 51.63% to 58.42%. In the scientific literature, the values of fresh chestnut humidity were between 41 and 59% (Desmaison et al., 1986; Salvini et al., 1998; De La MontañaMiguelez et al., 2004) with a very high variation coefficient (up to 20%) within the very same cultivar (De La MontañaMiguelezet al., 2004). This fact is due to the chestnut epicarp which is porous and non-lignified, which results in a quick drying of chestnuts compared to other nuts (Bounous

et al., 2005). A series of studies show results of analyses conducted on chestnuts treated in cold water or hot water, therefore some of the variations may be attributed to the influence of these treatments (Sacchetti et al., 2005; Künsch et al., 2001; Jermini et al., 2006).

Nutritional composition of different chestnut cultivars is presented in Table 3. Means and standard deviations are reported for fresh substance. As regards the humidity variation coefficient, it can sometimes be very high (11%), as was reported, for example, by De La MontañaMiguelez et al. (2004) and Sacchettiet al. (2005) in fresh and treated chestnuts, respectively. Regarding the glucidic fraction, it should be noted that sucrose is the main reducing glucid in chestnuts, although small amounts of fructose and glucose traces have also been reported (Desmaison et al., 1986; Sacchetti et al., 2005; Pinnavaia et al., 1993), while maltose was reported only by Attanasio et al. (2004).

Table 3. Chemical composition of fruits in analyzed cultivars

Cultivars	Water (%)	Titrable acidity (g malic acid/100 g)	Lipids (%)	Proteines (%)
Marsol	55.38±1.22^{bc}	0.402±0.03^{b}	0.53±0.03^{c}	5.40±0.33^{a}
Maraval	51.63±1.56^{a}	0.603±0.04^{c}	0.65±0.04^{d}	5.74±0.41^{ab}
Casval	58.42±1.38^{d}	0.670±0.04^{d}	0.35±0.02^{a}	5.90±0.38^{ab}
Bournette	53.81±0.98^{ab}	0.234±0.02^{a}	0.41±0.03^{b}	7.18±0.48^{d}
PrecoceMigoule	57.07±1.33^{cd}	0.268±0.03^{a}	0.64±0.04^{d}	6.34±0.39^{bc}
Marissard	54.77±1.67^{bc}	0.402±0.03^{b}	0.53±0.03^{c}	7.02±0.44^{cd}

*Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$

In the study reported by Neri et al. (2010), sucrose was the representative sugar in all analysed samples, with mean values between 12.95 and 19.84%, values that are consistent with those reported by Senter et al. (1994), Künsch et al. (1999, 2001) and Sacchettiet al. (2005) for ecotypes in Italy, and by De La MontañaMiguelez et al. (2004) for Spanish cultivars. Higher sugars contents (approximately 29-30%) were reported by Attanasio et al. (2004) for chestnuts in Montella, and by Desmaisonet al. (1986) for other Italian ecotypes. Variability of sucrose content may be the result of climatic factors influence and genetic variability. For example, Pinnavaia et al. (1993) reported a high variability of sucrose content (10.45-15.71% d.w.) within the same chestnut ecotype, due both to different years of harvesting and to different clones within the same ecotype. A high variability of sucrose content has also been reported by De La MontañaMiguelez et al. (2004).

Among the different sugars determined by different authors, fructose presented, within the same cultivar, the highest year-to-year variation coefficient (De La MontañaMiguelezet al., 2004; Neri et al., 2010). The protein content recorded values between 5.40 and 7.18% fresh matter. These values are higher than those reported by Pinnavaia et al. (1993) and Sacchettiet al. (2005) for some of the Italian ecotypes. Also, these values are higher than those reported by Kunsch et al. (1999) for some of the Italian and Swiss cultivars, but they are similar to those reported by Ferreira-Cardoso et al. (1993, 2005) in some of the Portuguese cultivars and by De La Montana Miguelez et al. (2004); Pereira-Lorenzo et al. (2006) and Peña-Méndez et al. (2008) in Spanish chestnut cultivars. These authors consider that there is a correlation between chestnut protein content and the type of origin soil; for example, chestnuts in shale areas have a much higher protein content than those grown on granite soils (Gomes et al., 1997). Also, many authors have reported that this protein content showed a very large variation coefficient between different years of analysis (Sacchettiet al., 2005).

Ferreira-Cardoso et al. (2005) also reported that the harvest year and interaction between harvest year and cultivar have significantly influenced chestnut protein content. The highest levels of protein content were determined for 'Bournette' and 'Marissard'

cultivars. The lipid content of chestnuts was found to be between 0.35 and 0.65% f.w., much lower than the data reported by Neri et al. (2010),Bassiet al. (1984),Kunsch et al. (1999),Salvini et al. (1998) and Sacchettiet al. (2005) for Italian chestnuts, but closer to those reported by Senter et al. (1994), De La Montana Miguelez et al. (2004) and Borges et al. (2006) for the Italian, Spanish and Portuguese cultivars, respectively.

The polyphenols content in cultivars taken under analysis (Table 4) was between 1.65 and 19.60 mg GAE/g, which is a very large variation range between different cultivars. Antioxidant activity of chestnuts was determined using a method widely used in the literature and expressed in mg Trolox equivalents per gram. Values obtained are high, ranging from 0.73 to 9.90 mg Trolox/g of fresh substance, revealing also a wide variation range. The highest content of phenolic compounds was determined in 'Marissard' cultivar, followed by 'PrecoceMigoule' cultivar. In direct correlation with the phenolic compounds content, the antioxidant activity has also recorded the highest values in the above-mentioned cultivars.

Table 4. Antioxidant activity, total content of phenolic compounds and total flavonoid content in the fruits of chestnut cultivars

Cultivar	Total content of phenolic compounds (mg GAE/g)	Total content of flavonoids (mg QE/g)	Antioxidant activity (mg Trolox/g)
Marsol	2.79 ± 0.18^{b}	1.80 ± 0.21^{b}	1.78 ± 0.09^{c}
Maraval	1.88 ± 0.14^{a}	2.63 ± 0.17^{c}	1.30 ± 0.11^{b}
Casval	1.65 ± 0.23^{a}	0.84 ± 0.09^{a}	0.73 ± 0.03^{a}
Bournette	2.15 ± 0.22^{ab}	1.79 ± 0.12^{b}	1.55 ± 0.16^{bc}
PrecoceMigoule	12.12 ± 0.68^{c}	8.57 ± 0.56^{d}	6.96 ± 0.36^{d}
Marissard	19.60 ± 0.78^{d}	14.77 ± 0.66^{e}	9.90 ± 0.48^{e}

*Values in the same row followed by different letters as the exponent are significantly different at $p < 0.05$

In general, in case of chestnuts, this high antioxidant activity is only partially based on phenolic compounds, considering that the high content of ascorbic acid in chestnuts has an important contribution to antioxidant activity (Proteggenteet al., 2002). This fact is important due to low thermal stability of ascorbic acid which could cause a dramatic decrease in the antioxidant activity of chestnuts as a result of their roasting.

Values of total phenolic compound content are close to those reported by De Vasconcelos et al.(2007), who found values between 15.8 and 22.7 mg GAE/g in chestnuts of Portuguese cultivars 'Martainha', 'Longal' and 'Judia', while other studies reported a content of 147 mg/g dry matter, which corresponds to about 54 mg/g of fresh substance (Callisteet al., 2005).

Among the individual phenolic compounds (Table 5), elagic and gallic acids were determined in higher amounts.

De Vasconcelos et al. (2007) have also determined large amounts of free gallic acid in Portuguese chestnut cultivars, the highest content recorded being 9.1 mg/g of fresh substance. Also, free elagic acid has been determined in chestnut fruits as well as many elagitinins and procyanidins. De Vasconcelos et al. (2007) reported 9.6, 2.7, and 4.8 mg/g fresh substance in chestnuts of 'Martainha', 'Longal', and 'Judia', respectively. There is strong evidence on beneficial health effects of gallic and elagic acids, with regard to their antioxidant activities, and their positive effects on cardiovascular functions and anticancer activity.

Table 5. Content of phenolic compounds (flavonoids and phenolic acids) (mg/100 g) in chestnuts

Genotypes	Marval	Bournette	PrecoceMigoule	Marsol	Marissard	Casval
Gallic acid	53.48± 0.14	88.5±0.14	193.61± 0.14	58.37±0.23	303.43±0.38	33.62±0.27
Catechinhydrate	33.5±0.27	36.98±0.27	223.52±0.15	24.69±0.27	241.62±0.15	20.72± 0.14
Vanilic acid	7.56± 0.09	27.42±0.22	51.46±0.15	25.04±0.17	8.11±0.08	6.49±0.24
Caffeic acid	nd	nd	7.65±0.08	7.17±0.19	nd	4.52±0.07
Syringic acid	5.46±0.08	9.17±0.15	52.23±0.27	5.32± 0.09	76.24±0.34	17.43±0.25
Epicatechin	10.03± 0.56	0	186.83± 0.56	32.33±0.21	137.606±0.56	10.22±0.22
Coumaric acid	4.32± 0.09	5.11± 0.14	19.13±0.15	9.93±0.08	140.84±0.27	16.57± 0.14
Ferulic Acid	3.28±0.12	3.21±0.08	20.35±0.19	8.68±0.24	124.95±0.35	3.89±0.08
Synapic Acid	5.73± 0.14[a]	5.35± 0.09	38.16±0.17	17.05± 0.14	269.01±0.34	11.67±0.16
Salicilic Acid	11.75±0.15	20.48±0.05	47.49±0.15	19.21±0.07	106.67±0.25	3.71± 0.09
Rutin	14.58±0.23	17.94±0.56	29.36±0.0.655	27.27±0.23	161.41± 0.14	11.78±0.08
Elagic acid	20.07±0.27	28±0.09	251.62± 0.56	36.14±0.27	238.79±0.48	18.76±0.27
Myricetin	2.44±0.08	1.68±0.05	24.24±0.15	3.47±0.15	8.72±0.21	3.29±0.22

CONCLUSIONS

Fruits of six chestnut cultivars of French origin that are grown at Valcea Research Station were characterized with regard to their nutritional and antioxidant composition. A high variability was found between cultivars in terms of nutrients content, a low lipid content but a high protein content.

All cultivars have exhibited a high content of phenolic compounds and high antioxidant activity, these results indicating a potential use of chestnuts as a source of natural antioxidant compounds in a diet.

The results of this study have shown that chestnut fruits contain significant concentrations of primary and secondary metabolites that are known for their positive effects on human health. Chestnuts are an increasingly popular food, equally consumed fresh, frozen or baked, or processed in various ways (marron glacé, pastries).

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