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ORIGINAL PAPER

Nutritional and nutraceutical properties of raw and traditionally obtained flour from chestnut fruit grown in Tuscany

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Abstract

The study of local chestnut and traditional techniques related to their use and consumption are considered of primary importance to promote their nutritional/nutraceutical values. Fruit of four local chestnut cultivars ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona') from Garfagnana (Italy) were analysed under nutritional and antioxidant aspects and compared with their flour obtained through a traditional thermal-drying process. Raw fruit contained significative amounts of P, K and Mg (~ 149, 1960 and 50 mg 100 g^{-1} dry weight, respectively) and they were characterised by a good moisture content (~ 49%) and starch (~ 50 g 100 g^{-1} dw). The traditional thermal-drying processes affected the carbohydrate content of dried chestnut showing a higher sucrose and lower starch content as compared to raw fruits. Traditional thermal-drying processes negatively influenced also total phenol content (TP) and total antioxidant activity: flours from all cultivars contained lower amounts of TP than raw fruit except for 'Morona' in which these compounds remained unchanged. This study provides new useful information about the evaluation of nutritional and nutraceutical characteristics of Tuscany local chestnuts and the effects of a traditional thermal-drying processing method, helping consumers and producers to valorise these "forest products".

Keywords Chemical properties drying process · Flour · Starch · Phenols · Soluble sugars

Introduction

There is a growing interest of consumers toward the consumption of healthy food (fruits, vegetables, nuts, legumes and other) for which a positive correlation between richness in bioactive compounds and human health has been proven [1, 2]. In addition, the beneficial effects of the Mediterranean diet are well known and include reduced risk for chronic diseases such as cancer, cardiovascular diseases, diabetes and neurodegenerative diseases. Nuts are part of the Mediterranean diet and could play an important role to reduce the risks of cardiovascular disease and cancer incidence thanks to their rich content in unsaturated fatty acids, minerals and phytochemicals [3, 4]. On the other hand, among tree nuts, walnuts, pistachios, pecans and chestnuts showed the highest content in antioxidants [5].

Chestnuts (*Castanea sativa* Mill.) are commonly consumed in many European countries, and in addition, there is a growing interest from extra-European markets for chestnuts [6, 7]. Besides their high antioxidant content, compared to other nuts, chestnuts are also a good source of minerals (K, Mg, Mn, and Cu), fatty acids, fibre and vitamins [8–11]. Moreover, chestnuts are also an excellent energy source for human and animal attributable to the high content in carbohydrates [9, 12]. For these reasons, chestnuts might be a valid substitute of potato, corn and wheat or at least be commonly consumed in the human or animal diet [8, 13, 14]. In addition, there is an increasing interest by the industry to create frozen products with the aim to make intact fruits available all year round. The processed chestnuts are

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used in puddings, bakery products or to make gluten-free pasta [15]. However, there are few works available about the effects of processing methods on chestnut and chestnut flour nutritional qualities [16–18]. The comparison between raw fruits and their processed flour, for example, highlighted significant differences in volatile compounds and nutritional qualities [13, 17, 19]. This, raises the need for new researches dealing with the biochemical changes in chestnut end-products (e.g. flours, pasta and bakery products) in order to preserve the nutritional characteristics.

China is the main producer of chestnut in the world, and in Europe, Italy is the first chestnut producer with about 52,300 Mg year^{-1} [20, 21]. The Italian chestnut germplasm includes hundreds of cultivars with different morphochemical characteristics; some of these cultivars are known only at the local level, and few of them are used to make commercial products. This is the case of the chestnut cultivars 'Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona', traditionally grown in Garfagnana (Tuscany) and used for the production of an Italian Protected Designation of Origin (PDO) product [22], namely: "Farina di Neccio della Garfagnana'. Although these local cultivars are commonly used for the aforementioned purpose, scarce information are available about their nutritional and nutraceutical proprieties as well as the effect of processing for flour preparation [23]. For these reasons, this work analysed raw fruits of chestnut cultivars 'Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona' for their morphological, nutritional and nutraceutical features. Moreover, the comparison in terms of carbohydrate, mineral content and nutraceutical properties between raw fruit and the corresponding flour obtained with the traditional drying method "metato" was conducted to analyse the effects of this drying process on those characteristics.

Materials and methods

Plant material

Four local chestnut cultivars ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona') were harvested in a field localised at 650 mt above the sea level in Isola di Castiglione di Garfagnana (44°10′22″ N, 10°25′40″ E), Lucca, Italy. The chestnut field is situated on sandy acid soils characterised by arenaceous flysch lithology [24]. The climatic conditions of the growing area during all the experiment (September/October 2017) were characterised by maximum and minimum temperatures (21.5 and 7.1 °C, respectively) and total rainfall (128 mm). The field is characterised by a planting distance of 10 mt between trees, with similar fruit production rate among cultivated cultivars (about 0.4 Mg ha^{-1}). In the 2017 chestnut ripening season, three representative trees from each cultivar were selected, and fruit samples (about 1 kg per sample) from each cultivar were randomly collected. Morphological indexes such as fresh weight (g), height (mm), width (mm) and thickness (mm) were estimated on 30 randomly chosen fruits of each cultivar. About 0.5 kg of raw fruits of each sample were peeled and milled in liquid nitrogen for biochemical analyses. The remained 0.5 kg of each sample was used to produce flour through the traditional thermal-drying processes in the "metato" structure. "Metato" is a little building constituted by two floors, in the ground floor heating is produced by combustion of chestnut wood and scraps. Chestnut fruits were placed on the second floor at a temperature of 35–40 °C and were left for 30 days. After the drying process and peeling, chestnuts were milled at low temperatures using a beater mill with filter ø 0.7 mm (SK 100 Cross Beater Mill, Retsch, Germany) and the flour was immediately frozen in liquid nitrogen, and stored at − 80 °C for biochemical analyses.

Mineral analysis

The digestion of dried chestnut samples (0.5 g dry matter) were carried out in concentrated sulfuric acid (96%, v/v) and H_2O_2 (30%, w/v) as follow. Dry samples were added to 5 mL sulfuric acid (96%, v/v) and incubated overnight at room temperature. Then, 4 mL of H_2O_2 (30%, w/v) were added and samples were mineralized at 370 °C for 30 min. Samples were then transferred in a final volume of 50 mL, reached by addition of double distilled water. After mineralization, K, Mg, Ca, Cu, Zn, Mn and Na content was determined using an atomic absorption spectrometer (Thermo Scientific ICE 3000 Series). P content was determined spectrophotometrically by an Ultrospec 2100 Pro spectrophotometer (GE Healthcare Ltd., Little Chalfont, England) following the molybdenum blue method according to Murphy and ruley [25] modified by Benini et al. [26].

Proximate analysis

Fruits were peeled and cut in small pieces and moisture content of chestnut raw samples was determined gravimetrically by oven drying at 65 °C until constant weight [27]. Three replicates ($n = 3$) were run per each sample. Moisture was expressed in percentage (%).

Ash content was determined according to the ISS method accessible in the Official Journal of the EU by the L 54/50/2009 [28]. Three replicates of chestnut dried samples (weight 5 g) were ashed at 550 ± 10 °C to constant weight. Ashes were expressed as g 100 g^{-1} dry weight (dw).

Crude fat (ethyl ether extract) was estimated gravimetrically by filter bag XT4 technique after petroleum ether extraction of the dried sample in an extraction system Ankom XT10 (Ankom Technology inc., Macedon, NY) [29]. The difference of starting weight and final weight

determined the presence of crude fats, dissolved in the petroleum ether. Crude fat concentration was expressed as g 100 g^{-1} dw.

To determine the content of raw fiber, the Weende technique adapted to the filter bag XT4 technique was applied. This method determines the organic residue of dried chestnut samples remaining after digestion with 2 L acid solution of H_2SO_4 0.255 N for 30 min at 100 °C and with 2 L basic solution of NaOH 0.313 N, using an Ankom 200 fibre analyser (Ankom Technology Inc.) [30]. Finally, the bags were ashed and the raw fiber concentration were expressed as g per 100 g^{-1} dw. Neutral detergent fibre (NDF) was determined according to Van Soest et al. [31], using dried chustnut samples in an extraction system Ankom XT10 using with an Ankom 200 fibre analyser added to heat stable at 100 °C α-amylase (4 mL) and sodium sulphite (20 g).

Acid detergent fibre (ADF) was determined using dried chestnut samples digested in filter bags using an Ankom 200 fibre analyser added to a detergent acid solution (20 g cetyltrimethylammonium bromide in 1 L H_2SO_4 1 N). The ADF content was determined by the difference between pre-treatment and post-treatment weight according to Van Soest et al. [31], and it was expressed as g 100 g^{-1} dw. Acid detergent lignin (ADL) was determined using the treatment of ADF with 72% sulfuric acid (v/v). Filter bags used for the ADF determination added with solution of sulfuric acid were incubated in DAISY incubator (Ankom Technology Inc.) for 3 h [32]. The filter bags were ashed at 550 °C and weighted. ADL concentration was expressed as g per 100 g^{-1} dw.

Crude protein content was calculated by multiplying the total nitrogen content, obtained by the Kjeldahl method by a conversion factor of 5.3 [33]. In the Kjeldahl procedure, after the digestion of dried chestnut samples in concentrated sulfuric acid (96%, v/v) and H_2O_2 (30%, w/v), the total organic nitrogen is converted to ammonium sulphate. Ammonia is formed and distilled into boric solution 4% (w/v) under alkaline conditions. The formed borate anions formed were titrated with standardized HCl solution (0.1 N), by which is calculated the content of nitrogen representing the amount of crude protein in the sample. Crude protein concentration was expressed as g per 100 g^{-1} dw.

Soluble sugar analysis was conducted on chestnut fruit and flour according to Yusof et al. [34] and Sotelo et al. [35] with some modifications. For soluble sugar extraction, 100 mg of samples were finely ground in a mortar, suspended in 10 mL of 80% aqueous ethanol (v/v), and placed in an ultrasonic water bath at 60 °C for 30 min. The solution was centrifuged at 10,000*g* for 10 min at 10 °C, and the supernatant was filtered using a HPLC filter (pore size: 0.45 µm). Sucrose, glucose and fructose were quantified using K-SUFRG commercial kits (Megazyme, Wicklow, Ireland), following the manufacturer's protocol. The residual pellet of the centrifuged solution was used for starch quantification using commercial kit K-TSTA (Megazyme) according to the manufacturer's protocol. Soluble sugar and starch concentration were expressed as g per 100 g^{-1} dw.

Phenol extraction and analysis

About 1 g of chestnut fruit and flour ($n = 3$) was homogenized with 10 mL of 80% (v:v) methanol solution by sonication for 30 min, keeping the temperature within the range 0–4 °C. After centrifuging samples at 6000*g* for 10 min at 4 °C, supernatants were collected and passed through PTFE filters (0.20 µm pore size; Sarstedt, Verona, Italy). Extracts were stored at − 80 °C before analysis.

Phenols content was evaluated according to the method reported by Dewanto et al. [36] based on Folin–Ciocalteau reagent. Briefly, 50 µL of phenolic extract was added to 75 µL of ultrapure H_2O and 125 µL of Folin–Ciocalteau reagent. The obtained solution was vigorous shaken and was incubated for 6 min at room temperature. After the incubation, 1.25 mL of 7% $NaHCO_3$ was added and then the solution was incubated for further 90 min at room temperature. The solution absorbance at 760 nm against a prepared blank was recorded. Values were expressed as mg gallic acid equivalent (GAE) g^{-1} dw.

Total antioxidant activity analysis

Total antioxidant activity (TAA) was measured using the method reported by Brand-Williams et al. [37]. Briefly 10 µL of phenolic extract were added to 990 µL of a solution containing 3.12×10^{-5} M 2,2-difenil-1-picrylhydrazyl (DPPH) in methanol. The decrease in absorbance at 515 nm was measured against a blank solution (without extract) after a reaction time of 30 min at room temperature using a spectrophotometer. Results were expressed as percentage reduction of the initial DPPH absorption in the extracts and expressed as mg Trolox equivalent (TE) g^{-1} dw.

Statistical analysis

Data obtained from morphological, mineral and proximate analysis (except for carbohydrates) were subjected to a one-way ANOVA with cultivar as the source of variation and then the means were separated with Fisher's least significant difference (LSD) post hoc test ($P = 0.05$). Data are expressed as mean ± standard deviation. The starch, soluble sugars and phenol content as well as total antioxidant activity values were analysed by two-way ANOVA using cultivar and food processing as variability factors. Before performing ANOVAs, the assumption of homogeneity of variances was tested using Bartlett's test. Pearson's correlation coefficient and

other statistical analyses were performed using GraphPad (GraphPad, La Jolla, CA, USA).

Results and discussion

Analyses on raw fruit

The morphological parameters of fruit from the four chestnut cultivars 'Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona' are summarised in Table 1. Fruit morphological characteristics evidenced different fruit shapes in the four chestnut cultivars. Fresh weight varied from 9.60 to 8.30 g with 'Morona' showing the highest weight. Moreover, among cultivars, 'Morona' also showed the highest size (height and width, 29.71 and 30.25 cm, respectively). The selection of chestnut cultivars is made to increase the nut size and the early harvesting [38, 39]. Chestnuts over 15 g are considered as large-size fruits, and are very appreciated by agri-food industries; those between 10 and 15 g are considered medium-sized and sold in the fresh market whilst smaller than 10 g are used for industrial uses (flour, starch extraction) [38–41]. All the cultivars shown a size below than 10 g and so they are not suitable for the candy preparation (e.g. Marron glacè), but only for fresh utilisation in the local market or for production of traditional products as flour.

The mineral element content in the raw fruit of the four chestnut cultivars was significantly different (Table 2). P and K were the most abundant fruit mineral elements in all the cultivars. The P content ranged from 186.56 mg 100 g^{-1} dw in 'Carpinese' to 124.04 mg 100 g^{-1} dw in 'Morona', while K content was higher in 'Carpinese' and 'Pontecosi' with 2094.33 and 2050.38 mg 100 g^{-1} dw, respectively, as compared to the values recorded in the other fruits. Mg content was highest in fruits of 'Capannaccia with about 58.77 mg 100 g^{-1} dw, whereas the lowest value ws found in 'Carpinese' with 41.74 mg 100 g^{-1} dw. Ca ranged from 16.86 mg 100 g^{-1} dw in 'Pontecosi' to 12.51 mg 100 g^{-1} dw in 'Capannaccia'. In 'Morona' the highest content in Cu, Zn and Mn (0.59, 1.22 and 3.71 mg 100 g^{-1} dw, respectively) were recorded, while Na content ranged from 2.35 mg 100 g^{-1} dw in 'Pontecosi' to 1.07 mg 100 g^{-1} dw in 'Capannaccia'. The values of element content found in fruit of chestnut cultivars are in accordance with those found in literature [9, 12, 42]. However, the levels of K detected in all the cultivars were higher when compared with the mean values obtained by Borges et al. [9] and De Vasconcelos et al. [10] in Portuguese cultivars and other authors in Spanish cultivars [12]. Mineral elements have a key role in the

Table 1 Morphological characteristics of four local chestnut cultivars fruit ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona', respectively) from Garfagnana (Italy)[a]

Parameters	Units	Chestnut cultivar			
		'Carpinese'	'Pontecosi'	'Capannaccia'	'Morona'
Weight	g	8.30 ± 1.16^{b}	8.30 ± 1.15^{b}	8.79 ± 1.06^{b}	9.60 ± 1.37^{a}
Height	mm	28.58 ± 1.41^{b}	28.11 ± 1.12^{bc}	27.68 ± 1.25^{c}	29.71 ± 0.99^{a}
Width	mm	29.98 ± 1.87^{b}	28.32 ± 1.61^{c}	31.60 ± 1.34^{a}	30.25 ± 1.74^{b}
Thickness	mm	18.24 ± 2.60	17.79 ± 2.15	18.66 ± 1.82	18.51 ± 1.39

[a]Means ± SD ($n = 30$) were subjected to one-way ANOVA with cultivar as source of variation. Means flanked by the same letter are not statistically different for $P = 0.05$ after Fisher's least significant difference post hoc test

Table 2 Mineral content (mg 100 g^{-1} dw) of four local chestnut cultivars fruit ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona', respectively) from Garfagnana (Italy)[a]

Elements	Chestnut cultivar			
	'Carpinese'	'Pontecosi'	'Capannaccia'	'Morona'
P	186.56 ± 47.36^{a}	163.66 ± 1.50^{ab}	127.51 ± 2.33^{b}	124.04 ± 2.04^{b}
K	2094.33 ± 79.61^{a}	2050.38 ± 2.68^{a}	1897.52 ± 26.73^{b}	1816.74 ± 33.11^{b}
Mg	41.74 ± 0.59^{d}	47.77 ± 1.00^{c}	58.77 ± 1.08^{a}	51.76 ± 2.88^{b}
Ca	9.81 ± 2.91^{b}	16.87 ± 2.56^{a}	12.51 ± 1.05^{b}	12.59 ± 0.67^{b}
Cu	0.54 ± 0.02^{b}	0.56 ± 0.03^{ab}	0.53 ± 0.00^{b}	0.59 ± 0.01^{a}
Zn	0.97 ± 0.01^{b}	1.13 ± 0.07^{ab}	1.04 ± 0.10^{b}	1.22 ± 1.22^{a}
Mn	3.21 ± 2.00^{b}	3.45 ± 0.00^{ab}	2.62 ± 0.04^{c}	3.71 ± 0.33^{a}
Na	1.57 ± 0.56^{b}	2.35 ± 0.31^{a}	1.07 ± 0.36^{b}	1.51 ± 017^{b}

[a]Means ± SD ($n = 3$) were subjected to one-way ANOVA with cultivar as source of variation

Means flanked by the same superscript letter are not statistically different for $P = 0.05$ after Fisher's least significant difference post hoc test

human nutrition as they are involved in many human cellular metabolic activities [43, 44], and fruit of the analysed cultivars were rich in macroelements K, P and Mg (values compared to recommended daily intake) [45]. K is needed to ensure the proper functions in nerve cells, blood pressure, carbohydrate metabolism and protein synthesis. P is associated to: (1) to cell growth, maintenance and reparation; (2) ATP and energy production; (3) mineralisation of bones and teeth. Finally, Mg has an important role in immune system health and nerve transmission [44].

The results of proximate analysis on fruit evidenced differences among cultivars in terms of moisture, ash, raw fibre and crude protein concentration (Table 3). Analysed fruit showed good moisture content ranging from 53.67 in 'Carpinese' to 47.79% in 'Morona'. Overall moisture averaged 50%, value which is closed to the mean value (51%) measured by Borges et al. [9] in several Portuguese chestnuts cultivars, and lower than those found in several Turkish cultivars (53%) [46]. Fruit moisture is a parameter potentially influenced by the environmental conditions typical of the territory (e.g. soil type, temperatures, rainfall) [12]. The moisture content is an important parameter for the storage of chestnuts because a high moisture content increases proliferation of moulds (mainly due to *Fusarium*, *Clamidosporium*, *Alternaria* and *Pennicillium* fungal genera) causing consequently the depreciation and loss of the product [47, 48]. Ash values detected in analysed fruit were similar to those obtained from Turkish (about 3.01 g 100 g^{-1} dw) [49], but higher than some Portuguese cultivars (about 1.54 g 100 g^{-1} dw) [50]. 'Morona' showed the lowest ash content (2.25 g 100 g^{-1} dw), whereas the other cultivars showed similar concentration values (on an average of 2.54 g 100 g^{-1} dw).

The lowest raw fibre content (2.25 g 100 g^{-1} dw) was obtained in 'Morona', while the highest was found in 'Capannaccia' (3.48 g 100 g^{-1} dw). However, the range of values found for raw fibre content was similar to those obtained by Borges et al. [9] and Pereira-Lorenzo et al. [12] for Spanish and Portuguese chestnut cultivars, respectively. The crude protein content showed significative differences among cultivars with the highest value in 'Capannaccia' (5.16 g 100 g^{-1} dw) and the lowest in 'Pontecosi' (3.80 g 100 g^{-1} dw). Results are in line with data obtained with other European cultivars that have similar protein content values [9, 12, 51]. No significant differences among cultivars in crude fat, neutral detergent fibre, acid detergent fibre and acid detergent lignin were detected in fruits (Table 3). Mean value of crude fat content was 2.59 g 100 g^{-1} dw according to Portuguese chestnut fruit cultivars (2.47 g 100 g^{-1} dw) and cultivars from different origins as well [9, 12, 49, 52]. However, crude fat content in chestnut fruit is lower if compared to the values detected in other nuts such as almond, pistachios and walnuts (~48 and 61%, respectively) [52]. Neutral and acid detergent fibre and acid detergent lignin are important parameters to evaluate the nutritional characteristics of food consumed by animals, their average values (22.58, 4.78 and 0.62 g 100 g^{-1} dw, respectively) were slightly higher to those obtained by other researchers in several chestnut cultivars [9, 12, 53].

Differences on carbohydrate, phenol content and total antioxidant activity between raw fruit and flour

Nutritional properties of chestnuts can be influenced by thermal-drying processes which are responsible for changes to the quality of product [16, 53, 54]. Indeed, the carbohydrate content was strongly influenced by thermal-drying process (Fig. 1). Starch content in raw chestnut fruit was not significantly different among cultivars with values around 50 g 100 g^{-1} dw. The traditional process used to obtain the flour reduced significantly the starch content that decreased by about 40% in 'Carpinese', 'Pontecosi' and 'Capannaccia' cultivars, reaching a decline close to 50% in 'Morona' as compared to the content detected in raw fruit (Fig. 1a).

Table 3 Proximate analysis of four local chestnut cultivars fruit ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona', respectively) from Garfagnana (Italy)[a]

Parameter	Units	Chestnut cultivar			
		'Carpinese'	'Pontecosi'	'Capannaccia'	'Morona'
Moisture	%	53.67 ± 0.58^{a}	49.71 ± 0.96^{b}	47.86 ± 0.89^{bc}	47.79 ± 1.50^{c}
Ash	g 100 g^{-1} dw	2.59 ± 0.09^{a}	2.52 ± 0.10^{a}	2.52 ± 0.03^{a}	2.25 ± 0.00^{b}
Crude fat	g 100 g^{-1} dw	2.38 ± 0.44	2.86 ± 0.66	2.93 ± 0.38	2.20 ± 0.08
Raw fibre	g 100 g^{-1} dw	3.19 ± 0.26^{ab}	2.86 ± 0.26^{b}	3.48 ± 0.24^{a}	2.25 ± 0.32^{c}
Neutral detergent fibre	g 100 g^{-1} dw	21.77 ± 3.85	19.75 ± 3.76	21.94 ± 0.78	26.88 ± 0.12
Acid detergent fibre	g 100 g^{-1} dw	5.73 ± 0.78	4.53 ± 0.47	4.93 ± 1.25	3.94 ± 0.08
Acid detergent lignin	g 100 g^{-1} dw	0.82 ± 0.24	0.62 ± 0.18	0.64 ± 0.36	0.41 ± 0.06
Crude protein	g 100 g^{-1} dw	4.83 ± 0.58^{b}	3.80 ± 0.05^{c}	5.16 ± 0.39^{a}	4.69 ± 0.32^{b}

[a]Means ± SD ($n = 3$) were subjected to one-way ANOVA with cultivar as source of variation.

Means flanked by the same superscript letter are not statistically different for $P = 0.05$ after Fisher's least significant difference post hoc test

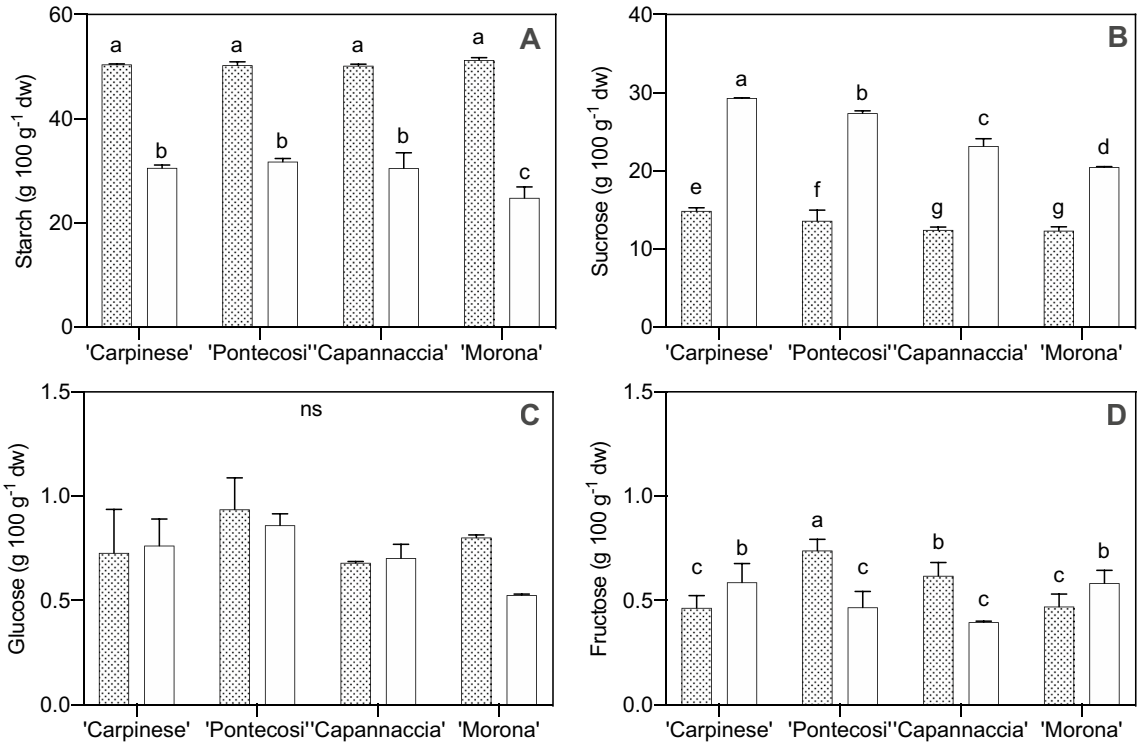

Fig. 1 Total starch (**a**), sucrose (**b**), glucose (**c**) and fructose (**d**) contents in raw chestnut fruits (closed bars) and thermal-drying processed fruits (open bars) of four local chestnut cultivars ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona', respectively). Means ± SD ($n = 3$) were subjected to two-way ANOVA with cultivar and treatment as sources of variations. Means flanked by the same letter are not statistically different for $P = 0.05$ after Fisher's least significant difference post hoc test

On the contrary, sucrose content was positively affected by "metato", with higher values in flours (about 50% higher) than their relative raw fruit. Among cultivars, 'Carpinese' had the highest sucrose content in raw fruit and flour (14.88 and 29.32 g 100 g^{-1} dw, respectively), whereas 'Morona' the lowest (12.34 and 20.48 g 100 g^{-1} dw, respectively) (Fig. 1c). No significative differences in glucose content among different fruit and relative flours were detected (Fig. 1c). The change in fructose content in raw fruits and flour and among cultivars was different. In some cultivars ('Carpinese' and 'Morona'), the thermal-drying process increased the fructose content (about 20%), whereas in the other cultivars ('Capannaccia' and 'Pontecosi') decreased by about 40% (Fig. 1d). The highest fructose content in raw fruit (0.73 g 100 g^{-1} dw) was detected in 'Pontecosi', whereas the lowest in 'Carpinese' and "Morona' (0.46 and 0.47 g 100 g^{-1} dw, respectively) (Fig. 1d). The carbohydrate values reported in the present work were similar or higher than those obtained by other authors for several Portuguese [55] and Spanish cultivars [12, 56]. The results showed that the traditional drying method "metato" (35–40 °C of temperature) prolonged for 30 days induced an increase in sucrose content, whereas the starch content resulted decreased by the dry treatment. It has been already reported that carbohydrate profile in chestnut was influenced by thermal processes [54, 57]. Indeed, starch content in chestnut fruit is affected by high temperatures that in turn can influence the enzymatic reactions leading to a partial starch hydrolysis [54, 57]. However, the dynamic interconversion of starch to soluble sugars in chestnut is not clear and it is hard to establish the factors (e.g. the mechanism that precedes germination or to increase the osmotic cellular potential to contrast the water loss during the drying process) underpinning these enzymatic processes [16, 54, 58–61]. Therefore, many variables such as the available substrate, the temperature inside the kernel and the activity of enzymes involved in the sucrose synthesis/degradation, make it necessary further studies to elucidate the carbohydrate interconversion process in chestnuts.

Nutraceutical properties can also be affected by thermal processes [17, 42, 62]. Total phenol content was significatively influenced by cultivar and thermal-drying processes (Fig. 2). The TP content in raw chestnut fruit ranged between 10.30 in 'Capannaccia' and 6.95 mg GAE g^{-1} dw in 'Morona'. Data of TP content are in agreement with previous data for raw chestnut [53, 63, 64]. It is well known as polyphenol content in fruit is strictly influenced by environmental conditions [65, 66]. However, in this work, plants

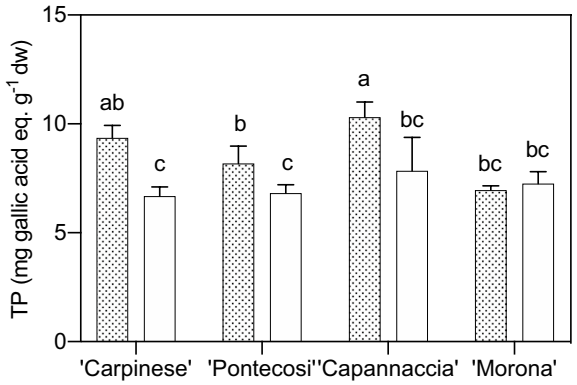

Fig. 2 Total phenol content (TP) in raw chestnut fruits (closed bars) and thermal-drying processed fruits (open bars) of four local chestnut cultivars ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona', respectively). Means ± SD ($n = 3$) were subjected to two-way ANOVA with cultivar and treatment as sources of variations. Means flanked by the same letter are not statistically different for $P = 0.05$ after Fisher's least significant difference post hoc test

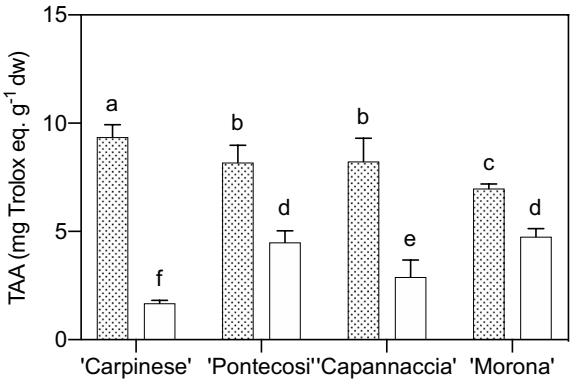

Fig. 3 Total antioxidant activity (TAA) in raw chestnut fruits (closed bars) and thermal-drying processed fruits (open bars) of four local chestnut cultivars ('Carpinese', 'Pontecosi', 'Capannaccia' and 'Morona', respectively). Means ± SD ($n = 3$) were subjected to two-way ANOVA with cultivar and treatment as sources of variations. Means flanked by the same letter are not statistically different for $P = 0.05$ after Fisher's least significant difference post hoc test

were grown in the same microclimate conditions (soil, temperature, humidity, and light) and then the difference in TP content is attributable to the cultivar genetic background. No statistical significative differences in TP content were detected among traditionally processed chestnuts, their content was about 7.14 GAE g^{-1} dw, values were higher than those found in chestnut commercial flour [67]. "Metato" process decreased the TP content significantly in 'Carpinese', 'Pontecosi' and 'Capannaccia' (about 29, 16 and 24%, respectively), whereas in 'Morona' it remained substantially unchanged. This indicates a different behaviour among cultivars during the thermal-drying process, in which it is conceivable that part of phenols was oxidised by polyphenol oxidase, that reach is optimum temperature in chestnuts around 30–40 °C [68].

Total antioxidant activity (TAA) varied significantly between raw fruit and related flour (Fig. 3). The total antioxidant activity was positively correlated to the total phenol content (Pearson's correlation coefficient: 0.68, $P < 0.001$). The chemical structure of phenols, thanks to their − OH groups (mainly 3′,4′-dihydroxy catechol group) and other substitutions, plays a pivotal role in the antioxidant activity [69]. Raw fruits of 'Carpinese' showed the highest TAA (9.35 mg TE g^{-1} dw), whereas 'Morona' the lowest (6.98 mg TE g^{-1} dw). Analysed chestnut fruit belonging to different cultivars showed a good TAA in accordance with other authors [70, 71]. This represents an important aspect considering that a high TAA is the essential requisite for the definition of a functional food [72]. The decrease in TAA, as observed in all obtained flours (Fig. 3), was related partially to the decrease in TP content. The highest decrease in TAA was detected in 'Carpinese' (82%) whereas the lowest in 'Morona' (31%).

Conclusions

In conclusion, among four chestnut cultivars, 'Carpinese' showed the best compromise between nutritional and nutraceutical properties, characterised by a high content in minerals macronutrients (especially P, K and Mg) and good nutraceutical characteristics (high TP and TAA). The differences in nutritional and nutraceutical properties detected among chestnut cultivars from Garfagnana (Italy) was related to the genotype, i.e. the cultivar, that influences the peculiar fruit nutritional composition. The traditional thermal-drying process ("metato") affected the carbohydrate content, total phenol content and total antioxidant activity of flours when compared to the values of raw fruits. These results can increase the knowledge about the nutritional and nutraceutical properties of these old chestnut cultivars and could help producers to valorise these "forest products" which is already an essential part of the Garfagnana area traditions. However, we are aware that further analyses (e.g. activity of enzymes related to starch hydrolysis and sucrose synthesis and metabolome analysis) are needed to fully elucidate the physiological processes that affect chestnuts during this traditional thermal-drying process. Indeed, an in-depth knowledge of the physiological processes that take place during the industrial processing of chestnut is necessary to preserve and enhance the nutritional characteristics of the final products.

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Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contains any studies with human participants or animals performed by any of the authors.

Consent to participate All the co-authors are fully aware and agree.

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