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Application of Traditional Cooking Methods in Chestnut Processing: Effects of Roasting and Boiling on Secondary Metabolites and Antioxidant Capacity in *Castanea* spp. Fruits

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Abstract: More information on the effects of traditional cooking methods (roasting or boiling) on the chestnut composition may be important if health-promoting aspects are considered. The main aims of this study were to investigate and describe the phenolic profile and antioxidant capacity of raw, boiled, and roasted chestnuts from several *Castanea* spp. genotypes, evaluating the influence of the application of different traditional cooking methods on the nut phytochemical composition by chromatographic and spectroscopic strategies. The amounts of phenolics were used as selected variables together with total polyphenol content and antioxidant capacity to perform a Principal Component Analysis (PCA). Catechins and tannins were the main molecules in the phenolic phytocomplex, reaching 30–40% of the total, followed by phenolic acids (5–20%) and flavonols (about 5%). Gallic and ellagic acids were the most important phenolic acids in raw and processed chestnuts (about 20–70 mg·100 g^{-1} dried weight-DW and 10–50 mg·100 g^{-1} DW, respectively). Both of the cooking processes significantly influenced the polyphenolic content and the relative antioxidant capacity. This research may support and confirm the potential use of chestnuts for human health, increasing the information on the phenolic pattern of differently processed *Castanea* spp. fruits from different genotypes to (i) assess the potential health-positive effects, (ii) help processing companies to select specific varieties to commercialise in the market, and (iii) increase the use of these fruits with the relative increase in income for the producers.

Keywords: processed chestnuts; phenolics; antioxidants; cooking processes; HPLC fingerprinting

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1. Introduction

Chestnuts (*Castanea* spp.) are highly valuable nuts in some areas of North America, Asia, and Europe, and they present molecules and nutritive substances, potentially positive for human diets. In the few last years, the use and consumption of chestnuts (as fresh or previously transformed products) has increased, also thanks to commercial derived products in the market, such as flour, frozen nuts, and purée [1,2]. In any case, consumers often prefer fresh chestnuts, due to their phytochemical composition and positive influence on human health [3]. Chestnuts are mainly used and consumed in their fresh form from the seasonal harvest after being boiled or roasted [4].

Castanea spp. fruits have recently become popular in human nutrition thanks to their nutritive quality and potential health-promoting traits [5]. Despite this positive influence on human health, chestnuts, as with many vegetables and fruits, can also be potential carriers for toxic compounds (i.e., xenobiotic trace elements dangerous to animals, humans, and

plant systems) [4]. For this reason, it is very important to evaluate the chemical composition of chestnut raw materials and derived products. Moreover, it is necessary to know the specific composition of each product (raw chestnuts or their derived forms) because it may allow innovative opportunities for the producers (i.e., some cultivars are better for fresh utilisation, while others for animal feed, flour, or industrial derived products) [6,7].

Castanea spp. nuts present significant amounts of phenolics, with ellagic and gallic acids predominant compounds, together with phenolic acids, catechins, and hydrolysable tannins [8,9]. Phenolic acids in these fruits occur in different forms (e.g., insoluble-bound, glycosidic, esterified, and free). Free phenolic acids may contribute to the chestnut taste [10]. Flavonoids are a group of polyphenols that may be divided into seven sub-groups: flavanols (flavan-3-ols), flavonoids, flavonols, anthocyanidins, isoflavones, flavones, and flavanones. Flavonoids and phenolic acids may be predominantly conjugated with sugars via ester bonds or O-glycosidic bonds. The interest in flavonoids is significantly increasing because they are important molecules for the human diet [10]. Tannins, another important phenolic class in chestnuts, can be divided, according to their structures, into condensed (flavan-3-ols monomers and polymers) or hydrolysable (ellagitannins and gallotannins). The amounts of polyphenolic compounds in *Castanea* spp. nuts are influenced not only by the 'genotype' factor (i.e., species and cultivars), but also by the orchard location and harvest year, together with processing steps (drying, boiling, and roasting) and storage conditions (duration and temperature) [11].

In recent years, dietary polyphenols have received attention and interest thanks to their many benefits in human health for their positive role and biological effects in the prevention of several diseases in humans, such as neurodegenerative, cardiovascular, and cancers diseases [12]. For this reason, many studies have recently focused on obtaining antioxidant molecules from plants and other natural sources, and replacing synthetic antioxidants responsible for pathological issues (e.g., liver damage and carcinogenesis in animals) [10]. Some studies have been carried out to assess the antioxidant capacity and polyphenolic composition of edible chestnut seeds, but they have been mainly limited to fresh nuts [7,13,14]. Instead, chestnuts are mainly consumed after cooking (roasting or boiling), and some studies have highlighted that cooking processes influence the phytochemical composition of plant material [15]. For this reason, more information on the effects of traditional cooking methods on the chestnut composition may be important if health-promoting aspects are considered. Currently, few data are available regarding the potential changes in the antioxidant capacity and relative phenolic pattern of boiled and roasted chestnuts in relation to fresh fruits [3]. It may be very important to evaluate these changes because the consumption of processed chestnuts could be optimised as a good practice to use antioxidant natural sources [9]. In any case, boiling is known as a cooking method that presents a negative influence on the composition of some constituents with low molecular weight because of the dissolution or loss of these substances into the boiling hot water (leaching) and/or their decomposition (hydrolytic reactions) [15]. Moreover, oven-roasting may cause Maillard reactions (chemical reactions among amino acids and reducing sugars) and acrylamide formation (due to high cooking temperatures); for this reason, high acrylamide levels could be potentially expected, in particular in roasted chestnuts [15].

This preliminary work aimed to study and describe the phenolic profile and antioxidant capacity of raw, boiled, and roasted chestnuts from several *Castanea* spp. genotypes, evaluating the application of different traditional cooking methods on the nut phytochemical composition and quality. In this research, the experimental approach was based on the coupling of chromatographic fingerprint and chemometrics to study the influence of traditional cooking methods on the amounts of specific phenolics, selected for their demonstrated health-promoting and antioxidant properties, to evaluate the potential of processed chestnuts as a natural source of polyphenols in relation to the raw chestnuts. Different phenolic markers were described as the most discriminating variables for chestnuts; these compounds may be utilised for a composition control, distinguishing products derived from specific processes and genotypes. This method, based on the HPLC—chemometrics

coupling, was applied for a full chestnut characterisation; this system detects each considered polyphenolic constituent; important information in comparison to spectrophotometric methods that only consider groups of similar molecules [16].

This research may be important to compare the influence of the most used processing methods on the levels of all the selected phenolics to evaluate the potential of processed chestnuts as a natural source of polyphenolic constituents. Chromatographic and spectroscopic strategies assessed the antioxidant properties and profile of the considered *Castanea* spp. fruits. Several phenolic compounds were used as biomarkers to assess the potential health-promoting capacity of raw and processed chestnuts. Moreover, the influence of these cooking methods on the total polyphenolic content of chestnut seeds was shown and evaluated by the Folin–Ciocalteu method.

2. Materials and Methods

2.1. Plant Material and Preparation of Chestnut Samples

Castanea spp. fruits were collected during the seasonal harvest in the Autumn of 2021 from the germplasm repository of the Chestnut R&D Center—Piemonte (Chiusa di Pesio, Cuneo Province, Italy). For all the twelve selected cultivars, three representative plants were considered, and 1 kg of chestnuts for each tree was randomly collected and stored at 2 °C.

Cultivars were grouped in accordance with their genotype in four groups: (i) Japanese chestnuts (*C. crenata*), (ii) Euro-Japanese hybrids (*C. sativa* × *C. crenata*), (iii) European chestnuts (*C. sativa* 'chestnut type'), and (iv) European chestnuts (*C. sativa* 'marrone type'), as shown in Table 1. The study was performed on fruit pulp. Chestnuts were randomly selected from each 'genotype' group and three replications of mature fruits (n = 3) for each one were considered. All the chestnuts had been previously cut on the top with a knife. Then, for each 'genotype' group, three sub-groups of chestnuts were created according to the used cooking methods; group 1: boiled chestnuts (300 g of chestnuts boiled in 600 mL of water for 15 min at 100 °C); group 2: raw chestnuts (application of any cooking methods); group 3: oven-roasted chestnuts (300 g of chestnuts roasted in an electric oven (WIPA, Stadtlohn, Germany) for 35 min at 180 °C.

Table 1. ID codes of the considered chestnuts (boiled, raw, and oven-roasted). The table shows the cultivars for each selected chestnut group.

		ID Code		
Species/Hybrids	**Cultivars**	**Boiled**	**Raw**	**Oven-Roasted**
C. crenata	Ginyose, Ishizuki, Tsukuba	CB	CC	CF
C. sativa × *C. crenata* hybrids	Bouche de Bétizac, Marigoule, Marissard	IB	IC	IF
C. sativa 'chestnut type'	Gentile, Garrone Rosso, Contessa	SB	SC	SF
C. sativa 'marrone type'	Marrone della Val di Susa, Marrone della Val Pellice, Marrone di Chiusa Pesio	MB	MC	MF

After processing, chestnuts from all the groups were manually peeled to separate the kernel and the shell, the nuts were cut into very small sections and, finally, they were finely powdered by a grinder (Moulinex 505–180 W, Groupe SEB, Écully, France) and sealed in PET plastic bags. Chestnut samples were then stored at room temperature until the phytochemical extraction.

2.2. Extraction Protocols

Chemical reagents and solvents are reported in Supplementary Materials. The phenolic extraction was carried out using a solution of 37% HCl:water:methanol at a 0.5:4.5:95

(*v*/*v*/*v*) ratio. The hydro-methanolic extracts were filtered by a PTFE (polytetrafluoroethylene) membrane micro-filter with pore size of 0.45 µm. Then, they were stored at normal atmosphere (4 °C; 95% R.H.) until phytochemical analysis.

2.3. Spectrophotometric Analysis

The Folin–Ciocalteu colourimetric method [17] was performed to evaluate the total polyphenolic content (TPC), and mg of gallic acid equivalents (GAE) per 100 g of dried weight (DW) were used for the expression of the results.

The FRAP test (Ferric Reducing Antioxidant Power assay) [18] was carried out for the assessment of the antioxidant capacity (AOC), and mmol of Fe^{2+} equivalents per kilogram of DW expressed the results.

Absorbance for TPC (at 760 nm) and AOC (at 595 nm) was assessed by a UV/Vis spectrophotometer (1600-PC, VWR International, Milano, Italy).

2.4. Chromatographic Analysis

A High-Performance Liquid Chromatograph – HPLC (Agilent 1200 series, Agilent Technologies, Santa Clara, CA, USA) was coupled to a UV-Vis diode array detector and used for the chromatographic analysis.

Chromatographic separation of polyphenols was performed by a Kinetex—C18 column (4.6 × 150 mm, 5 µm, Phenomenex, Torrance, CA, USA). Two different methods were applied for the sample analysis following the conditions validated by other studies [19–21], with modifications. The quantitative analyte determination was solved by external standard calibration. All the results were expressed by mg·100 g^{-1} of DW. The chromatographic conditions are reported in Table S1 (Supplementary Materials).

2.5. Data Analysis

Results were assessed as means ± standard deviation. A one-way analysis of variance (ANOVA) of the experimental phytochemical and nutraceutical data was performed to estimate the effects of the genotype, the cooking method, and their interaction. Tukey's HSD post hoc comparison test (n = 3) was utilised to highlight significant statistical differences between means at the 5% level [22]. Pearson's correlation test (r) was performed to define the correlations between nutraceutical variables (i.e., TPC and AOC). Differences at the $p < 0.05$ level were evaluated as statistically significant [2,22]. IBM SPSS Statistics 22.0 (IBM, Armonk, NY, USA) and MS Office Excel 365 (Microsoft Corporation, Redmond, WA, USA) were applied for ANOVA and Pearson's correlation tests.

Principal component analysis (PCA) on the correlation matrix (Varimax rotation) was used to describe the set of phytochemical data and detect the most discriminant variables for assessing the data structure. PCA allowed for observing the relationships among the four different 'genotype' groups depending on the three cooking methods (no treatment, boiling, and roasting) by a correlation matrix which included 36 samples (12 samples with 3 analytical repetitions) and 17 variables (TPC, AOC, and 15 phenolic markers). The PCA biplot, which included chestnut processed samples and single polyphenolic molecules, was assessed using eigenvalues calculated by a correlation matrix among 15 individual phenolics plus TPC and AOC as input. The Bartlett's test of sphericity (BTS) and Kaiser–Meyer–Olkin index (KMO) were used on the same matrix [22,23]. It was column-wise centred and then scaled, and the relative values were turned into Z-scores [24]. The software Minitab 18.1 (State College, PA, USA) was utilised to define the two-dimensional scores and loading plots.

3. Results and Discussion

The phenolic pattern (flavonols, tannins, phenolic acids, and catechins) together with the TPC and AOC evaluation of chestnuts from different genotypes (*C. sativa* 'chestnut' and 'marrone' type, *C. crenata*, and *C. sativa* × *C. crenata*) and differently processed (raw, boiled, and oven-roasted chestnuts) were assessed by screening and fingerprinting analysis [25,26].

3.1. Total Polyphenolic Content and Antioxidant Capacity

The TPC values of chestnuts, expressed as mg of gallic acid equivalents (GAE) on 100 g of dried weight (DW), are reported in Figure 1, while AOC values, expressed as mmol of Fe^{2+} on Kg of dried weight (DW), are reported in Figure 2.

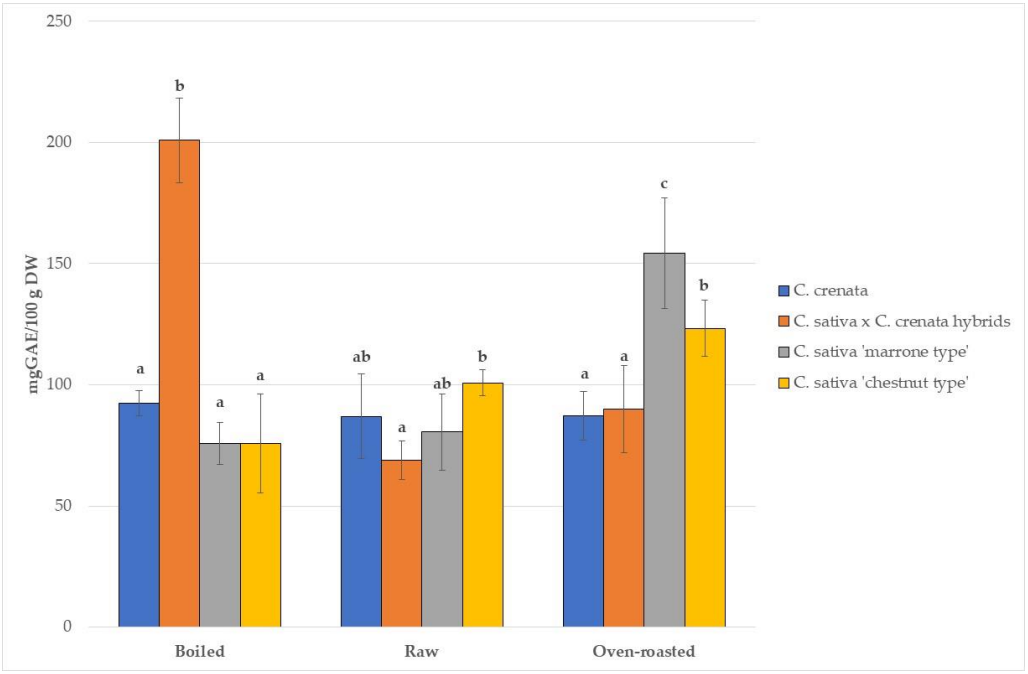

Figure 1. TPCs of all the chestnut groups. Different letters for all the considered groups indicate significant statistical differences ($p < 0.05$) among different species/hybrids in the same "cooking method" groups. Different colours represent the considered genotypes.

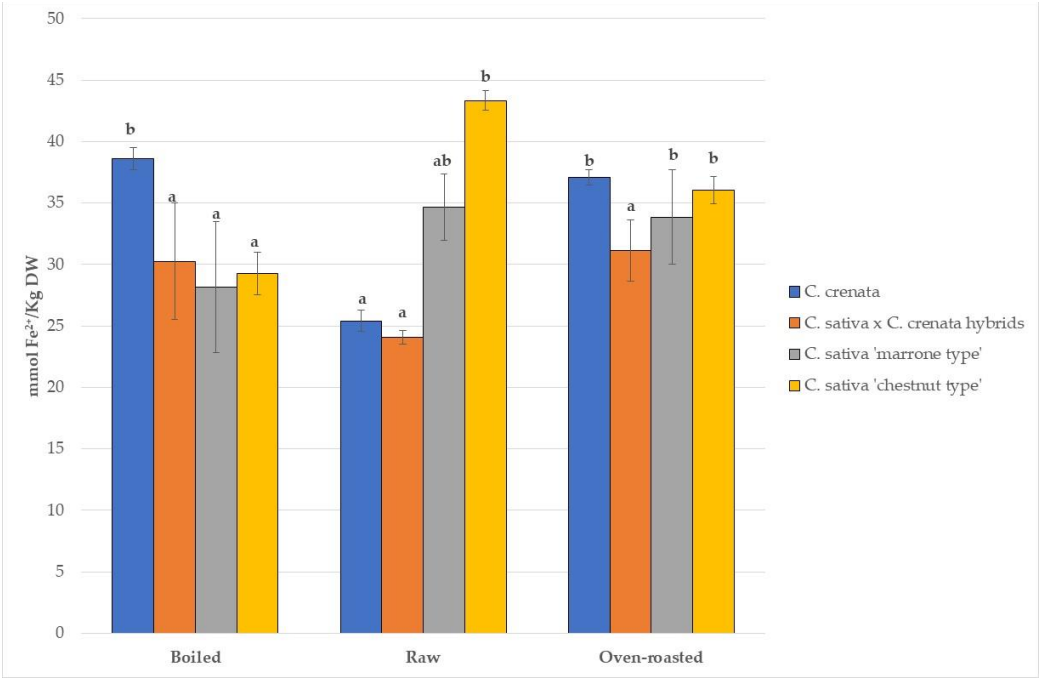

Figure 2. AOCs of the analysed chestnut groups. Different letters for the considered groups indicate significant statistical differences ($p < 0.05$) among different species/hybrids in the same "cooking method" groups. Different colours represent the considered genotypes.

TPC values significantly ranged from 75.78 ± 8.56 mgGAE 100 g^{-1} DW to 200.90 ± 17.50 mgGAE 100 g^{-1} DW for boiled chestnuts, from 87.09 ± 9.98 mgGAE 100 g^{-1} DW to 154.30 ± 22.90 mgGAE 100 g^{-1} DW for oven-roasted chestnuts, and from 68.82 ± 7.89 mgGAE 100 g^{-1} DW to 100.72 ± 5.34 mgGAE 100 g^{-1} DW for raw chestnuts (Figure 1), in agreement with other studies [1,3,15] that presented TPC values varying from 50 to 200 mgGAE 100 g^{-1} DW. The highest total phenolic values were quantified in chestnuts from *C. sativa* × *C. crenata* hybrids and *C. sativa* cultivars (about 100–120 mgGAE 100 g^{-1} DW).

The TPCs of oven-roasted chestnuts are often higher than values of boiled and raw chestnuts; it may be correlated to complex polyphenolics. Indeed, the free-forms of polyphenols are often not detected in plants because they occur as glycosides, bound complexes, and esters. The roasting/boiling of chestnuts may convert bound forms to free ones, as demonstrated in other studies [27]. In this research, the TPC of raw chestnuts for all the considered genotypes was low, while oven-roasted and boiled chestnuts showed very high values. In particular, the oven-roasting process increased the polyphenols mostly in *C. sativa* chestnuts (both the 'chestnut' and 'marrone' types); this increase may be explained by the heating destructive effect on the complex polyphenols and the generation of smaller phenolics more sensitive to the Folin–Ciocalteu reagent test. Raw chestnuts presented many bound-phenolic molecules and the roasting process, but also, the boiling converted many bound polyphenols to free-forms. Moreover, heating processes determine some changes in the structures of specific compounds (e.g., proteins) linked to phenolics, and increasing TPC values in plant-derived foods upon heating may be due to an increase in their ratios [1].

However, these amounts are over-estimated because of interference from non-phenolic compounds (for example, vitamin C and other antioxidants) when compared to the sum of single phenolics detected by high-performance liquid chromatographic (HPLC) methods, as has been shown in other studies [10].

The antioxidant compounds in plant-derived foods like chestnuts can be divided into phytochemical (non-nutrient) and nutrient antioxidant substances. In addition to nutrient antioxidant molecules (e.g., vitamins), there are many non-nutrient compounds with high antioxidant power, such as polyphenols and carotenoids. For example, tannins, phenolic acids, and catechins show high antioxidant properties. Moreover, some previous studies have presented that phenolics possess higher antioxidant capacity than nutrient antioxidants [1]. A high number of screening methods (in vitro biological- and chemical-based tests) have been validated to define the antioxidant capacities of chestnuts, such as the FRAP test used in this research.

Antioxidant properties (AOC) presented statistical differences ($p < 0.05$) among the analysed chestnuts, but the trend was different from the TPC one. AOC values significantly varied from 28.17 ± 5.31 mmol Fe^{+2} kg^{-1} DW to 38.61 ± 0.92 mmol Fe^{+2} kg^{-1} DW for boiled chestnuts, from 31.12 ± 2.47 mmol Fe^{+2} kg^{-1} DW to 37.10 ± 0.61 mmol Fe^{+2} kg^{-1} DW for oven-roasted chestnuts, and from 24.08 ± 0.56 mmol Fe^{+2} kg^{-1} DW to 43.34 ± 0.81 mmol Fe^{+2} kg^{-1} DW for raw chestnuts (Figure 2), as shown in other studies [1,3,27] that presented AOC values varying from 10 to 50 mmol Fe^{+2} kg^{-1} DW. The highest AOC values were observed for the chestnuts from *C. sativa* chestnuts (about 30–45 mmol Fe^{+2} kg^{-1} DW for the 'chestnut' type and about 25–35 mmol Fe^{+2} kg^{-1} DW for the 'marrone' type). Different genotypes, agro-environmental conditions, and analytical protocols may have caused little differences in some previous studies, as already shown in other works [10,28].

Processing methods influenced the AOC of the analysed chestnuts in different ways. The processed chestnuts presented higher AOC values than raw nuts, except for *C. sativa* chestnuts, and these high AOC values may be linked to the increase in TPC levels; in particular, gallic and ellagic acids [3]. In the case of *C. sativa* genotypes, the raw chestnuts showed AOC values slightly higher than processed chestnuts, mainly due to the high content of vitamin C already demonstrated in similar works on the same cultivars [7,11].

The results of this study showed as a high antioxidant capacity was still present in the cooked chestnuts, although low levels of vitamin C are often observed in processed nuts [7]. For this reason, the vitamin C amounts alone cannot define the antioxidant capacity of the cooked chestnuts, but it is also important to consider the TPC, as previously reported [3].

These results showed that the cooking processes did not influence the chestnut antioxidant properties for the different genotypes. Indeed, although statistical differences were quantified in the TPC values of the analysed chestnuts, the AOCs of each differently processed sample were very similar, while differences in the chestnuts from different genotypes were highlighted. The results confirmed that chestnuts may be included in the group of natural sources of polyphenolic constituents, with good antioxidant properties, regardless of the cooking methods, as also shown by several studies [3,29]. However, it is not easy to evaluate the single contribution of phenolics to the AOC because of the combined and synergistic health-promoting effect of the different bioactive substances ("phytocomplex") [30]. This effect may be confirmed by the differences in the AOC levels among the several genotypes [7,21]; for this reason, chestnut groups with the highest TPC contents did not always show the highest AOCs.

Using Pearson's coefficient (R), the AOC values were statistically compared to the TPC ones in each sample. The relationship between AOCs and TPCs is reported in Table 2. AOC and TPC values presented positive Pearson's coefficients for all the samples. The AOCs of raw and processed chestnuts were well correlated to their TPCs (R ranged from 0.43 to 1.00 for *C. crenata*, from 0.79 to 1.00 for Euro-Japanese hybrids, from 0.46 to 0.99 for *C. sativa* 'marrone' type, and from 0.49 to 0.65 for *C. sativa* 'chestnut' type). These results were similar to previous studies that showed a good correlation between the total polyphenolic content and antioxidant capacity in chestnuts [1,7]. Moreover, different levels of other antioxidant compounds, such as vitamin C, in chestnuts may contribute to their antioxidant capacity, even if the main antioxidants are represented by polyphenols. The higher levels of vitamin C and other antioxidants in *C. sativa* chestnuts than in *C. crenata* chestnuts and Euro-Japanese hybrids were confirmed by the lower AOC/TPC R-values in *C. sativa* raw and processed nuts (i.e., 0.56 for *C. sativa* 'chestnut' type, 0.68 for *C. sativa* 'marrone' type, 0.73 for *C. crenata*, and 0.88 for *C. sativa* × *C. crenata* hybrids). Indeed, the levels of other antioxidant molecules highly affected the antioxidant capacity influencing the direct correlation between AOC and TPC values in the analysed samples.

Table 2. Pearson's coefficient values of the analysed chestnuts (boiled, raw, and oven-roasted).

	Pearson's Coefficient
	R
Sample	AOC/TPC
CC	0.43
CB	0.77
CF	1.00
IC	0.79
IB	0.86
IF	1.00
MC	0.58
MB	0.46
MF	0.99
SC	0.65
SB	0.49
SF	0.55

3.2. Phytochemical Composition

The phenolic pattern of the raw and processed chestnuts showed 15 polyphenolic biomarkers, as reported in Table S2 in the Supplementary Materials. Phenolics were grouped into five classes: cinnamic acids, tannins, catechins, flavonols, and benzoic acids.

Catechins and tannins, very important for their antimicrobial, cardioprotective, and chemopreventive capacities [31], were the main molecules in the phenolic phytocomplex, reaching 30–40% of the total, followed by phenolic acids (5–20%), characterised by antimutagenic, probiotic, cardioprotective, and chemopreventive effects [21], and flavonols (about 5%), as shown in Figure 3.

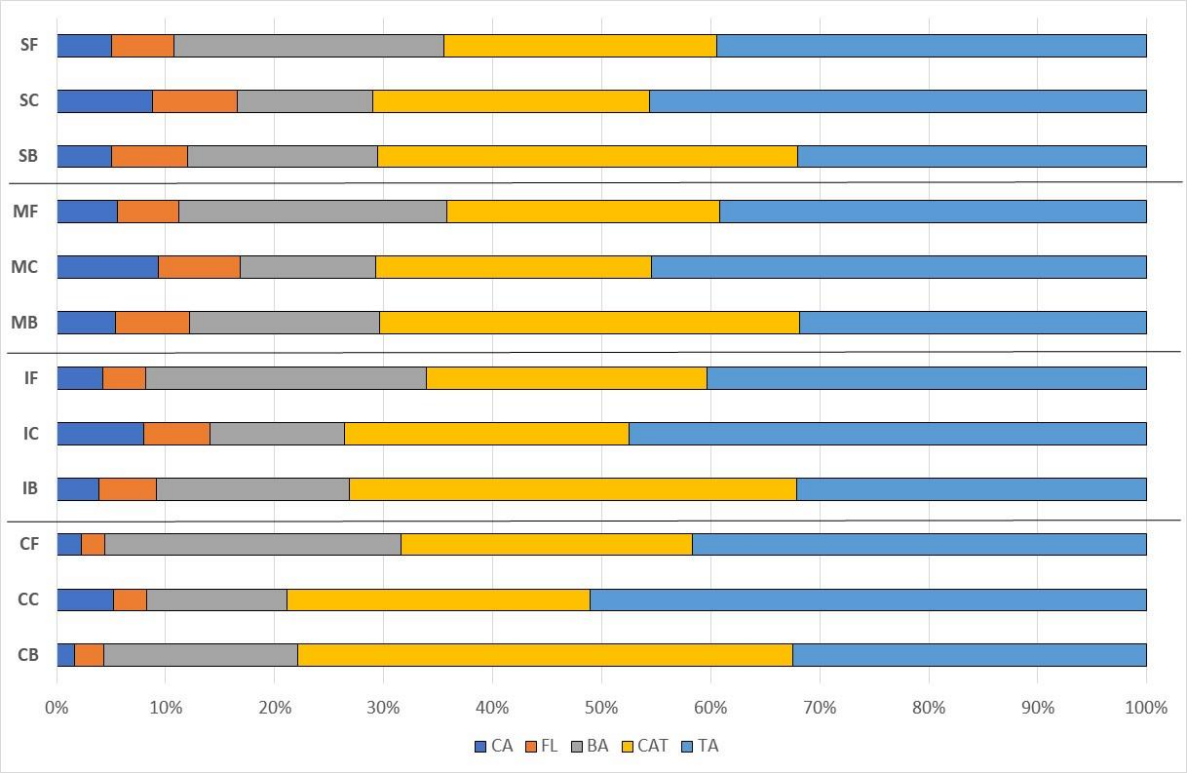

Figure 3. Phenolic phytocomplex of the analysed chestnut groups. Different colours define each chemical class. Blue: cinnamic acids; orange: flavonols; grey: benzoic acids; yellow: catechins; tannins: light blue.

Tannins were a very important class in the analysed chestnut groups, with good variability in the results. The *C. sativa* chestnuts showed higher levels of total tannins (about 130–190 mg·100 g^{-1} DW both for 'chestnut' type and 'marrone' type) than other genotypes (*C. crenata* chestnuts presented tannin amounts of about 95–160 mg·100 g^{-1} DW, while Euro-Japanese hybrids showed values of 110–170 mg·100 g^{-1} DW), similar to previous studies [15,27]. The good tannin levels increase the health-positive capacity of chestnuts because these molecules are free-radical quenchers [32]. Boiled and oven-roasted chestnuts showed high levels of castalagin and vescalagin, despite the effects due to cooking processes (castalagin: 10–30 mg·100 g^{-1} for boiled chestnuts and 30–50 mg·100 g^{-1} for oven-roasted chestnuts; vescalagin: 80–110 mg·100 g^{-1} for boiled chestnuts, and 115–140 mg·100 g^{-1} for oven-roasted chestnuts). In this study, the reduction in the total amount of tannins was influenced by the processing. Natural phenolics may be significantly modified and/or lost in the thermal processes because most of them are relatively thermolabile. The higher loss of tannins in boiled chestnuts than in roasted ones may be mainly due to the direct heat exposure; indeed, in the boiling process, these molecules were directly exposed to the heat and then they were easily leached by hot water. However, the cooking effect may be also influenced by several factors, such as the genotype, the duration and degree of exposure to water and heat, the cooking process, the surface area exposed to oxygen and water, the cooking medium and relative pH, and the extraction solvent [4].

Similar to previous studies [4,15,33], gallic and ellagic acids were the most important phenolic acids in raw and processed chestnuts (about 20–70 mg·100 g^{-1} DW and

10–50 mg·100 g^{-1} DW, respectively). As gallic and ellagic acids are presented to be important anticarcinogenic, antimutagenic, and antioxidant agents in preventing cardiovascular issues, their high levels in chestnuts are especially positive [15]. Cooking methods produce a high number of changes in the chestnut physical–chemical properties [4]. In this study, the results showed that ellagic acid and gallic acid levels increased from raw to processed chestnuts, as already shown in previous works [34,35]. In particular, ellagic acid increased from 10–14 mg·100 g^{-1} DW (raw chestnuts) to 12–17 mg·100 g^{-1} DW (boiled chestnuts) and 40–50 mg·100 g^{-1} DW (oven-roasted chestnuts), while gallic acid increased from 20–28 mg·100 g^{-1} DW (raw chestnuts) to 40–55 mg·100 g^{-1} DW (boiled chestnuts) and 55–70 mg·100 g^{-1} DW (oven-roasted chestnuts), as shown in Figure 4. The increase in the benzoic acid amounts was similar in all the considered genotypes. The increases in the amounts of ellagic acid in the chestnuts may be caused by the thermal hydrolysis in endogenous compounds, such as ellagitannins. The hydrolysis releases HHDP (3,4,5,30,40,50-hexahydroxydiphenic acid), that then rearranges and spontaneously forms the di-lactone ellagic acid, while the increases in the levels of gallic acid may derive from tannin decomposition (galloylesters) during cooking (roasting or boiling) [15,36].

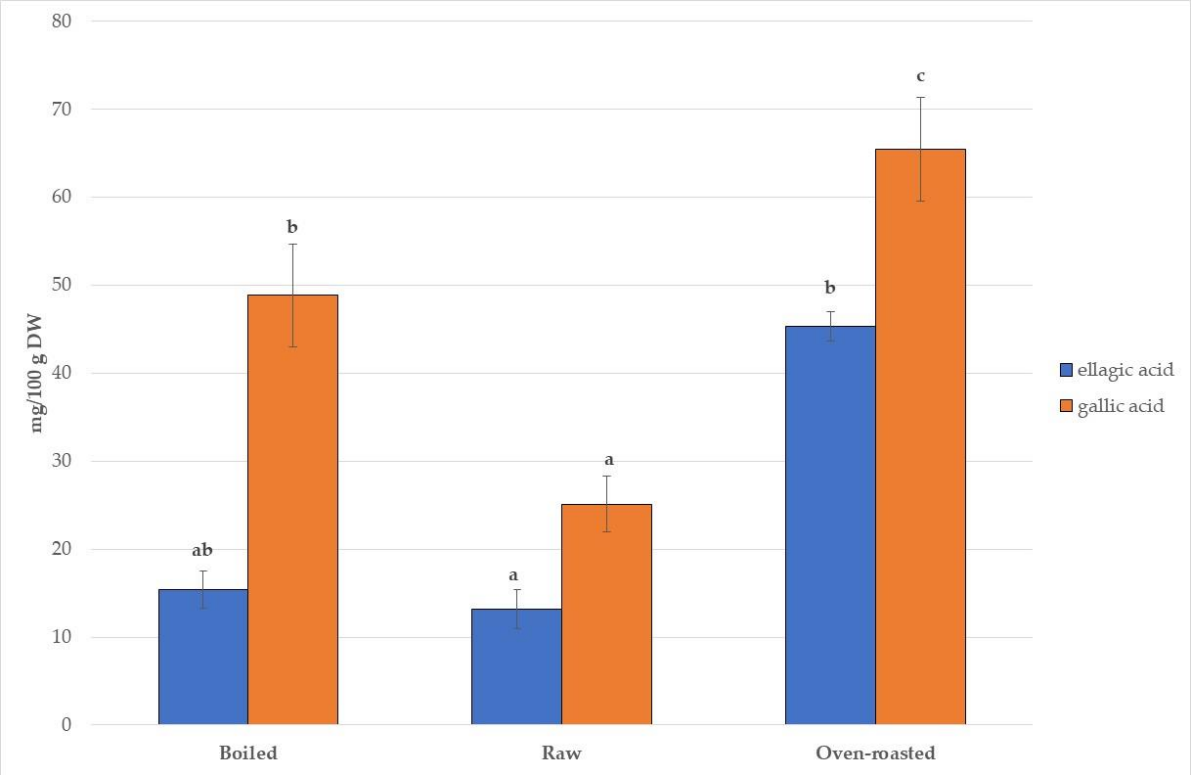

Figure 4. Profile of the benzoic acids in the analysed chestnuts in relation to each heat treatment. Different letters for the considered groups indicate significant statistical differences ($p < 0.05$) among different "cooking method" groups for each compound.

Catechins, another important class in the phenolic phytocomplex, were mainly represented by (+)-catechin (about 70–150 mg·100 g^{-1} DW for all the genotypes) and, secondly, by (−)-epicatechin (about 3–15 mg·100 g^{-1} DW for all the genotypes), as shown in similar studies [8,15]. The levels of (+)-catechin increased due to the heat treatments (processed chestnuts showed nearly double the (+)-catechin amounts in relation to raw nuts). Boiling increased the levels of (−)-epicatechin, while roasting decreased their amounts. The quantification of catechins is an important result to hypothesize the potential use of raw and processed chestnuts as a health-promoting foods thanks to their biological capacities (e.g., inhibition of cyclooxygenase enzymes and lipid peroxidation) [37].

Coumaric and ferulic acids were quantified in each analysed chestnut group in good quantities (about 5–15 mg·100 g^{-1} DW and 3–8 mg·100 g^{-1} DW, respectively) similar to other studies [5]; in particular, *C. sativa* raw chestnuts showed coumaric acid levels of 13.91 ± 0.77 mg·100 g^{-1} DW for 'chestnut' type and 13.62 ± 0.83 mg·100 g^{-1} DW for 'marrone' type, and ferulic acid values of 7.30 ± 0.78 mg·100 g^{-1} DW for 'chestnut' type and 6.98 ± 0.41 mg·100 g^{-1} DW for 'marrone' type. Caffeic acid and chlorogenic acid were also quantified in all the chestnut groups, although at lower levels (<7 mg·100 g^{-1} DW). These simple phenolic acids have been considered very important for their many positive effects on human health (e.g., potential capacity to decrease the risk of cardiovascular diseases, antioxidant properties, anti-inflammatory capacity, and anticancer effects) [35]. Cooking processes greatly decreased the levels of coumaric acid and, to a lesser extent, the amounts of caffeic and ferulic acids. Chlorogenic acid showed no differences between raw and processed chestnuts (Figure 5).

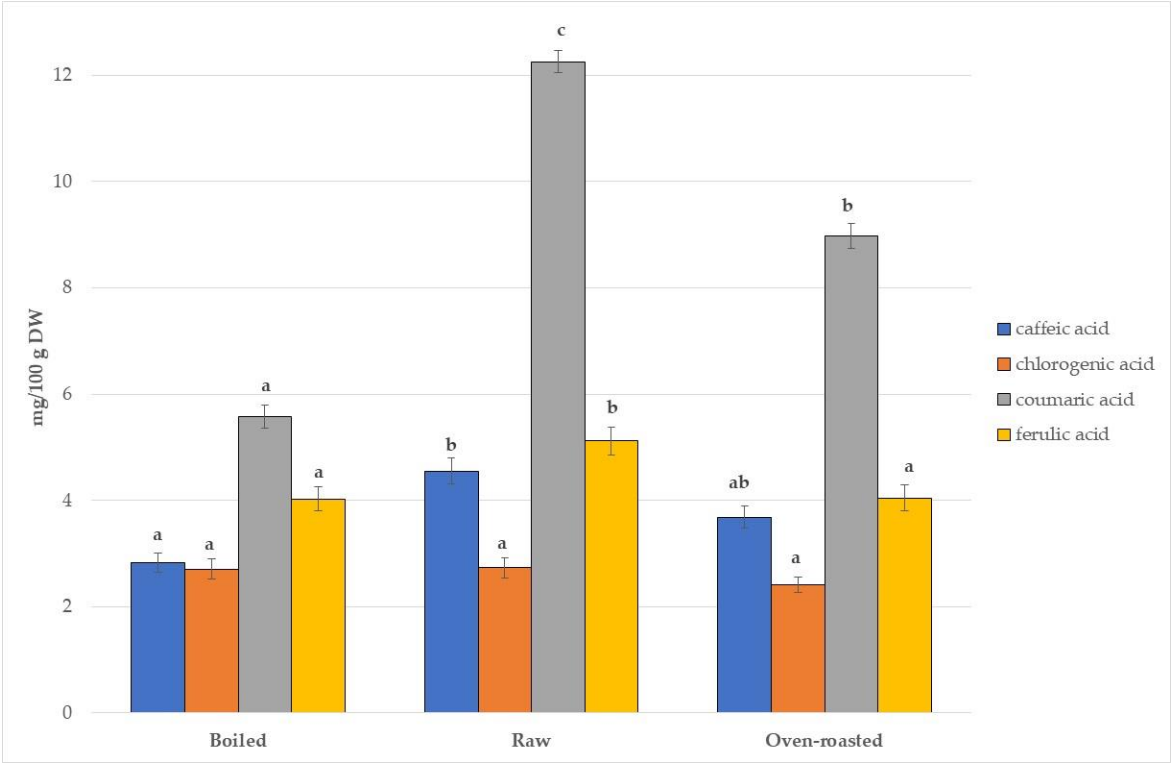

Figure 5. Profile of the cinnamic acids in the analysed chestnuts in relation to each heat treatment. Different letters for the considered groups indicate significant statistical differences ($p < 0.05$) among different "cooking method" groups for each compound.

Flavonols occurred at low levels (<20 mg·100 g^{-1} DW) in all the considered genotypes, except in *C. sativa* chestnuts, which showed values between 20 and 30 mg·100 g^{-1} DW. This class is excellent for (i) quenching active-oxygen substances and (ii) inhibiting in vitro oxidation of low-density lipoproteins [38]. The most important flavonols for all the chestnut groups were hyperoside and isoquercitrin (2–7 mg·100 g^{-1} DW). No differences were highlighted among the considered chestnuts in relation to cooking processes, as already shown in previous studies [36,39].

3.3. Multivariate Analysis

Raw and processed chestnut antioxidant effects derive from the interaction of phenolics rather than the activity of each single molecule [40,41]. For this reason, the quantified amounts of phenolics were used as selected variables together with TPC (total polyphenol content) and AOC (antioxidant capacity) to perform multivariate data analysis (Principal

Component Analysis–PCA). PCA defined the main differences among the considered chestnut groups and evaluated the relative correlations to the different cooking treatments and genotypes based on their single phenolic compounds.

Bartlett's test of sphericity ($p < 0.05$) highlighted collinearity among all the selected 17 variables. The KMO index showed a level of 0.77. The PCA produced two PCs (Principal Components) representing 69.60% of the total system variance; 49.19% explained by PC1, and 20.41% by PC2. The 12 samples (expressed as averages from three replications for each chestnut group) were included in the PCs plan depending on their nutraceutical properties and polyphenolic composition, as indicated in the following score plot (Figure 6).

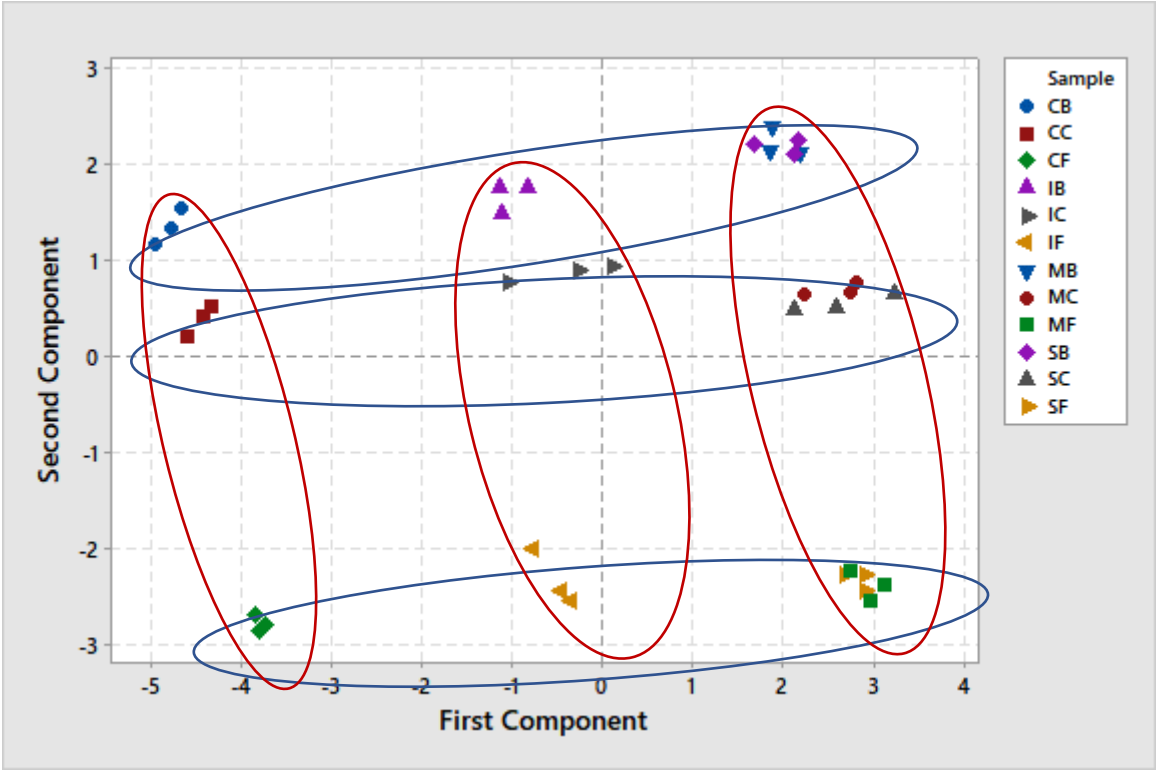

Figure 6. PCA score plot of the considered chestnut groups. Average values ($n = 3$) were included for all the samples. Chestnut group IDs were reported in Table 1.

In the present PC plane, the similarity of the different chestnut groups was correlated to the spatial proximity between the obtained points [42]. Therefore, chestnut groups, derived from similar processes and genotypes, were in nearby spaces in the PCA plane, and a similar phenolic pattern and antioxidant capacity were defined. The genotype influenced the chestnut polyphenolic composition, resulting in three distinct groups (red circles) along the PC1, as shown in Figure 6. The heat processing also showed a substantial effect on the chestnut phenolic composition, resulting in other three different groups (blue circles), as shown in the score plot (Figure 6).

As shown in the loading plot (Figure 7), in PC1 and PC2, the variance of cinnamic acids and flavonols, in particular chlorogenic acid (eigenvector values: 0.331, 0.105), ferulic acid (0.329, 0.081), rutin (0.336, 0.056), and quercitrin (0.333, 0.095), produced the most of the data variability for the 'genotype' effect. Moreover, in the PCs, the variance of tannins, catechins, and benzoic acids, in particular castalagin (values of eigenvectors: 0.208, −0.423), gallic acid (0.094, −0.299), ellagic acid (0.070, −0.489), and (−)-epicatechin (0.151, 0.455), mostly produced the data variability for the 'processing' effect. The PCA loading plot highlighted a strong association between cinnamic acids, flavonols, and PC1 (the lines referring to these phytochemical classes were parallel to the PC1), and a correlation between

tannins, catechins, benzoic acids, and PC2 (the lines referring to these compounds were parallel to the PC2), confirming the results of HPLC phenolic fingerprint (Figure 7).

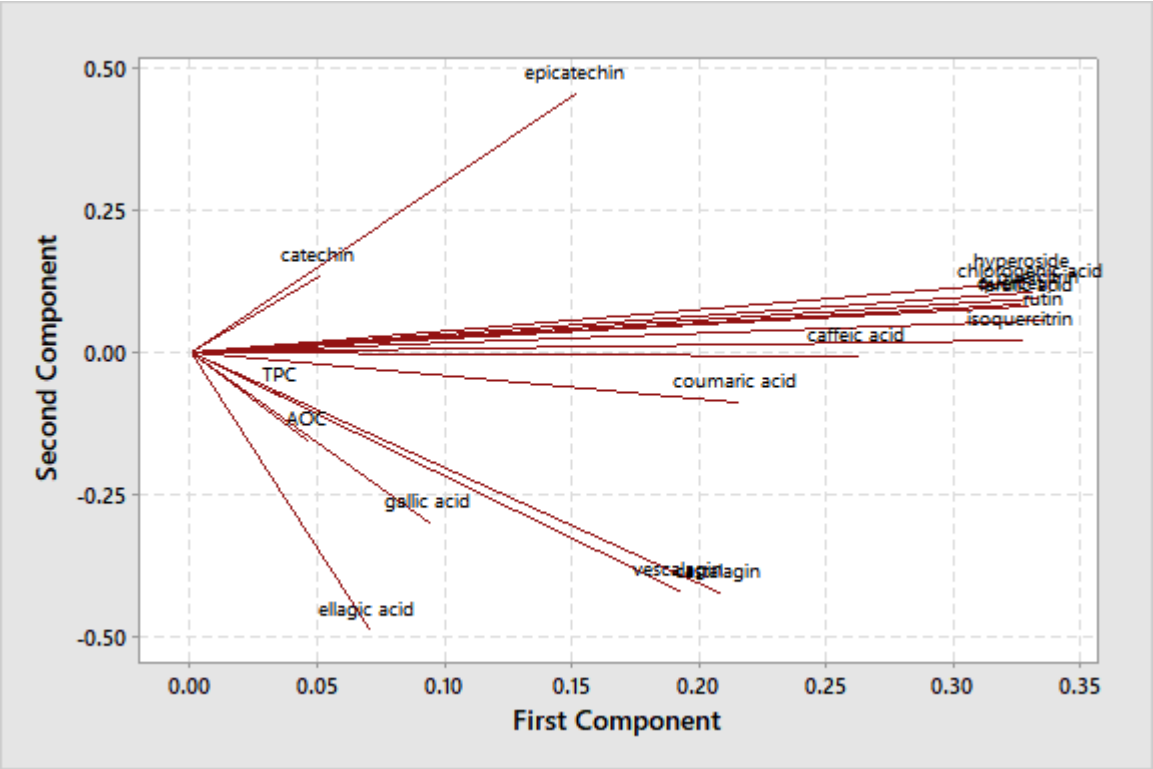

Figure 7. PCA loading plot of the selected variables. If the line referring to a specific variable is parallel to a PC, the relative phytochemical is correlated to the same PC.

Therefore, the data matrix variability (i.e., single phenolics) was shown to be correlated to the processing type (i.e., no cooking, roasting, and boiling) of the samples, but also the chestnut genotype (i.e., *C. sativa*, *C. crenata*, and *C. sativa* × *C. crenata*).

4. Conclusions

The interest in *Castanea* spp. fruits has been increasing thanks to their nutraceutical quality, but the information is almost exclusively focused on raw chestnuts. Not much information is available on processed chestnuts, in particular on the effects of traditional cooking methods on chestnut composition and antioxidant properties in relation to different genotypes. In this study, specific and total phenolic content and antioxidant capacity were monitored to evaluate the phytochemical and antioxidant properties of *Castanea* spp. fruits after two different heat treatments (boiling and oven-roasting) in comparison to raw chestnuts. Different genotypes were considered for a full experimental approach.

Both cooking processes significantly influenced the polyphenolic content and the relative antioxidant capacity in each chestnut group, depending on the genotype and the single compounds. Despite the chemical-structural changes of the considered molecules due to high temperatures (roasting) or the leaching of these compounds due to the use of hot water (boiling), the processed chestnuts maintained excellent levels of polyphenolic compounds and antioxidant capacity. The results of this study showed that roasted and boiled chestnuts may still be good sources of phenolics with excellent health-promoting properties. This research highlighted that a good antioxidant capacity was still present in the processed chestnuts; the results showed that gallic acid, ellagic acid, and (+)-catechin may presumably be the main compounds that significantly contributed to the observed antioxidant properties. In *C. crenata* chestnuts, no differences were observed between the two cooking methods. In *C. sativa* genotypes (both 'chestnut' and 'marrone' types), this

study assessed that roasting was the best method to preserve total polyphenolic content and specific phenolic composition, while in *C. sativa* × *C. crenata* hybrids, an inverse effect of the two traditional cooking methods was observed.

In this study, a statistical multivariate analysis confirmed and better highlighted the fingerprint information obtained by HPLC analysis. The PCA classification showed the selected chestnut groups depending on their polyphenolic composition (single phenolics), adding information on specific phenolic biomarkers that may be mostly influenced by the genotype and heat processes. For this reason, the PCA was applied to evaluate the relationships and potential correlations among different chestnut groups already presented by ANOVA test.

The results of this study could be utilised in future as an important step of the ongoing research on improving and optimizing the chestnut health-positive benefits. This research may support and confirm the potential use of chestnuts for human health, increasing the information on the phenolic pattern of differently processed *Castanea* spp. fruits from different genotypes to (i) assess the potential health-positive effects, (ii) help processing companies to select specific varieties for the market, and (iii) increase the consumption of these fruits with the relative increase in income for the producers. However, this research is only a preliminary study to provide more information on raw and processed chestnuts. In the future, the study should be also improved with a detailed sensory analysis to confirm the potential commercial applications in addition to the phytochemical properties of the processed chestnuts. Finally, biological tests and chromatographic analysis coupled with mass spectrometry are necessary to integrate these preliminary data with information on enzymatic antioxidant capacity and other compounds (e.g., vitamin C, monoterpenes, organic acids, and sugars).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13030530/s1, Table S1: Chromatographic conditions of the used methods. Table S2: Chromatographic fingerprint of the analysed chestnut groups.

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