Effect of cooking on free amino acid and mineral profiles of sweet chestnut (*Castanea sativa* Mill.)

Berta GONÇALVES[1]*, Olga BORGES[2], Eduardo ROSA[1], João COUTINHO[3], Ana Paula SILVA[1]

[1] CITAB - Cent. Res. Technol. Agro-Environ. Biol. Sci., Univ. Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal, bertag@utad.pt

[2] Deleg. Reg. Agric. Nordeste Transmont., Ave. General Humberto Delgado, 5300-167 Bragança, Portugal

[3] UTAD-CQ-Cent. Chem., Univ. Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

* Correspondence and reprints

Received 17 August 2011
Accepted 15 November 2011

Fruits, 2012, vol. 67, p. 201–214

DOI: 10.1051/fruits/2012013
www.fruits-journal.org

RESUMEN ESPAÑOL, *p. 214*

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Abstract — Introduction. Chestnut represents one of the most important crops for the Portuguese economy. The most common consumption mode of these fruits is roasted or boiled. In this context, the major aim of our study was to evaluate the amino acid contents and the mineral composition of raw and cooked chestnuts. **Materials and methods**. Amino acids were determined by HPLC and minerals were determined by molecular absorption spectrophotometry, by atomic absorption spectrophotometry or by flame emission photometry. The most important Portuguese cultivars were evaluated: Aveleira, Boaventura, Côta, Judia, Lada, Lamela, Longal Padrela, Longal Soutos da Lapa, Negra and Martaínha. **Results and discussion**. The cooking method significantly affected the total amino acid composition with contents in roasted samples 13% and 12 % higher than in boiled and raw chestnuts, respectively. Roasted chestnuts presented higher alanine, arginine, isoleucine, leucine, phenylalanine, threonine, tyrosine and valine contents than either raw or boiled chestnuts. Moreover, serine presented the highest content in raw or roasted chestnut kernels. Regarding the mineral composition, potassium (K) was the predominant macronutrient in the chestnuts, whereas phosphorus (P), calcium (Ca) and magnesium (Mg) were found in low contents. Cooking significantly affected the mineral composition, except for iron (Fe), copper (Cu), and manganese (Mn) contents. In general, calcium decreased upon cooking. K, Ca, Mg and B decreased with boiling. The present data confirm that cooked chestnuts are a good source of amino acids and minerals, both affected by boiling and roasting, which have been associated with positive health benefits.

Portugal / *Castanea sativa* / fruits / cooking / variety trials / proximate composition / mineral content / protein content / free amino acids

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Effet de la cuisson sur les profils des acides aminés libres et des éléments minéraux de la châtaigne (Castanea sativa Mill.).

Résumé — Introduction. Le châtaigner est l'une des cultures les plus importantes de l'économie portugaise. Ses fruits sont le plus fréquemment consommés grillés ou bouillis. Dans ce contexte, l'objectif principal de notre étude a été d'évaluer les teneurs en acides aminés et la composition minérale des marrons crus et cuits. **Matériel et méthodes**. Les acides aminés ont été déterminés par HPLC et les minéraux, par spectrophotométrie d'absorption moléculaire, spectrophotométrie d'absorption atomique ou photométrie d'émission de flamme. Les cultivars portugais les plus importants ont été évalués : Aveleira, Boaventura, Côta, Judia, Lada, Lamela, Longal Padrela, Longal Soutos da Lapa, Negra and Martaínha. **Résultats et discussion**. La méthode de cuisson a significativement affecté la composition totale en acides aminés avec, dans les échantillons torréfiés, des teneurs accrues de 13 % et 12 % par rapport à la composition respective des châtaignes bouillies ou crues. Les marrons torréfiés ont présenté des teneurs en alanine, arginine, isoleucine, leucine, phénylalanine, thréonine, tyrosine et valine supérieures à celles des marrons crus ou bouillis. En outre, la sérine a présenté une plus haute teneur dans les marrons crus ou grillés. En ce qui concerne la composition minérale, le potassium (K) a été le macronutriment prédominant dans les châtaignes, tandis que phosphore (P), calcium (Ca) et magnésium (Mg) ont été trouvés avec des teneurs faibles. La cuisson a sensiblement affecté la composition minérale, sauf pour les teneurs en fer (Fe), cuivre (Cu), et manganèse (Mn). En général, la teneur en calcium a diminué lors de la cuisson. Celle en K, Ca, Mg, B a diminué dans les fruits bouillis. Les données actuelles confirment que les châtaignes cuites sont une bonne source d'acides aminés et de minéraux ; ces deux éléments sont affectés par l'ébullition et la torréfaction qui ont été associées à des avantages bénéfiques pour la santé.

Portugal / *Castanea sativa* / fruits / cuisson / essai de variété / composition globale / teneur en éléments minéraux / teneur en protéines / acide aminé libre

1. Introduction

Free amino acids are implicated in secondary plant metabolism and in the production of compounds which directly or indirectly play an important role in the plant-environment interaction and in human health [1]. Additionally, amino acids directly contribute to the flavour and colour of food induced during thermal reactions in the stages of production, processing and storage of food [2]. They are precursors of heme, glutathione, various hormones, nucleotides, nucleotide coenzymes, neurotransmitters [3, 4] and alkaloids [5]. In mammals, the extra dietary amino acids are converted to metabolic intermediates, such as pyruvate, oxaloacetate, acetyl-CoA and α-ketoglutarate and, therefore, they are also precursors of glucose, fatty acids and ketone bodies [3].

There have been some published studies on the free amino acid content of chestnuts, such as Desmaisson *et al.* [6], De Vasconcelos *et al.* [7], Borges *et al.* [8] and De Vasconcelos *et al.* [9]. De Vasconcelos *et al.* [7] identified fifteen amino acids in the Portuguese cultivars (cvs.) Martaínha, Judia and Longal: alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine. However, some of them (glycine, threonine, tryptophan and tyrosine) were only present in trace amounts. In another study dealing with eight Portuguese chestnut cultivars, the amino acid profiles were dominated by aspartic acid, followed by alanine, arginine, glutamic acid and leucine [8].

Regarding the mineral composition, a recent study from De Vasconcelos *et al.* reported that chestnuts are rich in K and P, but poor in Ca, Mg, I, Zn and Mn [10]. However, Borges *et al.* reported that nuts are a good source of K, P, Mg, Fe, Mn and Cu [8].

Chestnut production is concentrated in the northern region of Portugal, representing one of the most important crops for the economy of the country. For the most part, chestnuts are consumed roasted and boiled, and less fried, using fresh, seasonally harvested fruits. During the last decade there was an increase in the use of frozen chestnuts to cover the needs throughout the year. Nevertheless, earlier studies have mainly focused on the composition of raw fruits, and the information about the changes in primary and secondary metabolite chestnut composition after roasting and boiling is quite limited. From other studies it is known that boiling affects the content of amino acids and minerals. For instance, boiling led to a significant decrease in both essential and non-essential amino acids in kidney beans [11]. The major reduction of essential amino acids affected methionine, tyrosine and threonine. In chickpeas, the only essential amino acid that was affected by cooking was lysine. In lentils, a decrease in the content of isoleucine, leucine and valine was observed, but data showed a significant increase in lysine, phenylalanine and tyrosine. Additionally, K and Mg are strongly leached from chestnuts by cooking [12]. In contrast, the Ca content increased during cooking with tap water. Therefore, the aims of this study were to describe the variation in free amino acids and minerals from ten Portuguese chestnut cultivars when submitted to the most common cooking methods, boiling and roasting; to establish the relationships between the different variables and to detect the most important causes of variability. From this information we inferred the potential beneficial human health effects of the cooked fruits.

2. Materials and methods

2.1. Sample collection and cooking procedures

The most important Portuguese cultivars were selected in the northern region of Portugal: Aveleira, Boaventura, Côta, Judia, Lada, Lamela, Longal Padrela, Longal Soutos da Lapa, Martaínha and Negra [13]. Three representative trees from each cultivar were selected and three replications of mature chestnuts (1 kg of fruits per sample) of each selected tree were randomly collected and kept at ± 2 °C until analysis for a maximum of 3 days.

Raw chestnuts (500 g) from each cultivar, previously cross-cut, were boiled for 20 min in 2 L of tap water (low hardness), with 5 g of NaCl added. Another 500 g of raw nuts from each cultivar were slit with a knife, spread with 7 g of NaCl, and then roasted in a preheated electric oven with air circulation (Nabertherm Mod. – L9R, Lilienthal, Germany), at 200 °C for 40 min. These conditions were selected after previous tests. After processing, the samples were peeled and the nuts were rapidly cut into small pieces and milled in a Model SK 100 Cross Beater (Retsch, Haan, Germany) to a uniformly fine powder. Samples were stored in a freezer at –80 °C until analyses were performed. Both the boiling and roasting processes were done in triplicate.

2.2. Chemicals

All chemicals and reagents were of analytical grade and were obtained from various commercial sources (Sigma/Aldrich, St. Louis, USA; Merck, New Jersey, United States, and Pronalab, Lisbon, Portugal). All solvents were of high-performance liquid chromatography (HPLC) grade, and all water was ultrapure. All amino acid standards were obtained from Sigma-Aldrich. For all standards, HPLC calibration curves were constructed by injection of 20 µL of different stock contents of the standards.

2.3. Extraction and HPLC analysis of free amino acids

The freeze-dried samples of raw, boiled and roasted chestnut kernels were weighed in triplicate (0.2 g) into 15-mL glass centrifuge tubes. Extraction was performed by sequential extraction with first 70% and then 90% v/v methanol in combination with a heating plate and a homogeniser [1, 7]. After each extraction, the samples were centrifuged. The purification of the extracts was done using a previously described method [14]. Basically, samples were loaded into plastic columns (Chromabond®, Macherey-Nagel, Germany) containing 1 mL of cationic ion-change resin (Dowex (H^+) 50WX8-400, Sigma) connected to a solid-phase extraction unit (Vac Elut SPS 24, Varian, Harbor City, California, USA). The final eluates obtained were evaporated on a heating plate at 35 °C with an air flow vacuum pump, and the evaporated fractions were resuspended with 300 µL of ultrapure water and then filtered (Whatman general purpose 0.2-µm filter, Banbury, Oxon, UK) and stored in vials in the refrigerator until HPLC analysis. The analysis was performed using a 150 mm × 4.6 mm, 3-µm C18 Spherisorb S3 ODS2 reverse-phase column (Waters, Foster City, California, United States) in combination with a Gilson HPLC system (Gilson, Middleton, Wisconsin, USA) consisting of a model 118 mixing chamber, model 402 high-pressure pump, model 231 XL detector, and a Jones chromatography (Grace Vydac/Jones Chromatography, Ontario, Canada) thermostatically controlled oven for the column set at 30 °C. Data were processed using the uniPoint software (uniPoint Corporate**,** Winnipeg, Manitoba, Canada). The solvents were (I): 350 $mol \cdot L^{-1}$ disodium hydrogen phosphate with 250 $mol \cdot L^{-1}$ propionic acid (1:1), acetonitrile, and ultrapure water (40:8:52), and (II): acetonitrile, methanol and ultrapure water (30:30:40). Both solvents were filtered (0.2 µm) and degassed before use. The gradient and flow rates were as follows: 0 min, 100% solvent (I); 9.5 min, 89% solvent (I); 11.0 min, 88% solvent (I); 13.6 min, 80% solvent (I); 20.4 min, 55% solvent (I); 23.4 min, 50% solvent (I) at 1.3 $mL \cdot min^{-1}$; then 25.4 min, 40% solvent (I); 32.0 min, 100% solvent (II) at 0.8 $mL \cdot min^{-1}$; followed by 34.0–37.0 min, 100% solvent (I) at 1.3 $mL \cdot min^{-1}$. A mixture of amino acid standards was freshly prepared and run with each set of samples.

2.4. Quantification of minerals

Quantification of P and B was determined by molecular absorption spectrophotometry, after wet digestion with H_2SO_4 and H_2O_2 for P and dry combustion for B [15]. Chestnut content of Ca, Mg, Fe, Cu, Zn and Mn was determined by atomic absorption spectrophotometry or by flame emission photometry (K), after wet digestion with

HNO_3 and $HClO_4$ [15]. Element content was calculated on a dry mass basis.

2.5. Scanning electron micrographs

For scanning electron microscopy (SEM) observations, transverse and tangential sections of the raw, boiled and roasted chestnuts were conducted at 12.5 kV using a Philips/FEI SEM/ESEM Quanta 400 (FEI, Hillsboro, Oregon, USA). The samples were held with double-sided carbon adhesive tape on aluminium sample holders.

2.6. Statistical analysis

Data were tested using a two-way ANOVA test to determine the main effects of the cooking process and cultivar, followed by Duncan's new multiple range test with a 0.05 significance level. Additionally, a Fisher correlation analysis and a principal component analysis (PCA) including several of the studied parameters were also performed using the StatView 4.0 package (Abacus Corporation, Berkeley, California, USA).

3. Results and discussion

3.1. Amino acid contents

From the data presented in a previous study by our team, the means of crude protein of raw, boiled and roasted chestnuts were estimated to be (6.5, 6.3 and 6.7) $g \cdot 100\ g^{-1}$, respectively [13]. This represents approximately 14% of the reference daily intake (RDI) for females and 12% of the RDI for males [16]. A total of 13 amino acids were detected within the sampled chestnut cultivars: arginine, isoleucine, leucine, phenylalanine, threonine and valine as the essential amino acids; and alanine, asparagine, aspartic acid, glutamic acid, glycine, serine and tyrosine as the non-essential amino acids (*table I*). These data show a variation in free amino acid content between raw and cooked (boiled and roasted) chestnuts, as well as among cultivars. The methods of cooking can be classified according to the method of heat transfer to the food. Heat conduction is used when cooking in liquid medium (boiling); the radiant heat is used in roasting.

The cooking method significantly affected ($P < 0.001$) the total amino acid composition, with contents 13% and 12% higher in roasted samples than in boiled and raw samples, respectively (*table I*). Regarding the essential amino acids, it was observed that, regardless of the treatment (raw, boiled or roasted), threonine and valine dominated, with 39% and 24% of the essential amino acids, and 7% and 4% of the total amino acids; the major non-essential amino acids were asparagine, glutamic acid and aspartic acid, which represent 62%, 23% and 11% of the non-essential amino acids and 37%, 14% and 7% of the total amino acids. These data are in agreement with those of De Vasconcelos *et al.* [7].

Roasted chestnuts presented higher alanine, arginine, isoleucine, leucine, phenylalanine, threonine, tyrosine and valine than both raw and boiled chestnuts (*table I*). Moreover, there was a higher content of serine in raw and roasted kernels. There were no significant differences in asparagine, aspartic acid or glutamic acid contents among raw, boiled or roasted kernels. In contrast, a previous study from Candela *et al.* reported that boiling led to a significant decrease in all amino acids in kidney beans, especially methionine, threonine and tyrosine, with reduction percentages of 65%, 51% and 49%, respectively [11]. Also, a decrease in the content of isoleucine, leucine and valine and a significant increase in lysine, phenylalanine and tyrosine were observed in lentils.

In our study, the content of total amino acids was highest ($P < 0.001$) in roasted chestnuts (419 $mg \cdot 100\ g^{-1}$ dry weight); 12% and 13% higher compared with boiled and raw nuts, respectively (*table I*).

According to Klein and Mondey, quantitative variations in the composition of amino acids can depend on their structural composition, solubility in water, vulnerability to heat treatment and also on the type of tissue under investigation [17]. In addition, Lisiewska *et al.* stated that a food product is

Table I.
Content of amino acids in kernels of raw, boiled and roasted chestnuts (Portuguese cultivars).

Kernel of chestnuts	Essential amino acids					
	Arginine	Isoleucine	Leucine	Phenylalanine	Threonine	Valine
	(mg·100 g^{-1} dry weight)					
Raw	2.36 ± 2.19 a	6.80 ± 4.53 a	3.23 ± 2.47 a	6.86 ± 4.27 a	23.81 ± 18.75 a	16.14 ± 7.45 a
Boiled	2.09 ± 2.00 a	6.34 ± 3.19 a	3.17 ± 2.11 a	6.50 ± 3.61 a	27.76 ± 23.93 b	14.69 ± 5.03 a
Roasted	3.21 ± 2.30 b	9.13 ± 4.71 b	5.76 ± 3.13 b	9.23 ± 4.30 b	29.25 ± 22.16 b	19.39 ± 8.57 b

Kernel of chestnuts	Non-essential amino acids						
	Alanine	Asparagine	Aspartic acid	Glutamic acid	Glycine	Serine	Tyrosine
	(mg·100 g^{-1} dry weight)						
Raw	24.76 ± 7.94 b	143.83 ± 73.71 a	27.08 ± 22.48 a	49.80 ± 34.70 a	3.97 ± 2.34 b	6.69 ± 3.58 b	0.034 ± 0.197 a
Boiled	19.34 ± 6.21 a	145.22 ± 87.16 a	27.15 ± 20.20 a	51.54 ± 38.53 a	1.89 ± 1.37 a	5.05 ± 2.71 a	0.007 ± 0.040 a
Roasted	33.27 ± 7.91 c	144.21 ± 66.89 a	24.35 ± 16.64 a	57.34 ± 45.64 a	1.59 ± 1.39 a	6.29 ± 2.66 b	0.264 ± 0.469 b

Kernel of chestnuts	Total of essential + non-essential amino acids (mg·100 g^{-1} dry weight)
Raw	374.55 ± 165.21 a
Boiled	371.31 ± 190.63 a
Roasted	419.25 ± 175.79 b

Means (n = 33) ± standard deviations followed by the same letter within a line are not significantly different at $P < 0.05$, according to the Duncan Multiple Range test.

a complex matrix, with peptides, polypeptides and free amino acids [18]; therefore, it is difficult to determine the reason for changes in individual amino acids. However, the decomposition of certain protein fractions rich in amino acids can bring about increases in the content of other amino acids [19].

Among non-essential amino acids, asparagine was the predominant one, followed by glutamic acid and aspartic acid (*table I*). Asparagine is the amide of the dicarboxylic amino acid aspartic acid that is either deaminated during food processing or converted into aspartate by the mucosal cells [4]. In the human body, low levels of asparagine may indicate low synthesis of aspartic acid, which can result in the inability to properly synthesise and excrete urea, which is the major waste product of excess dietary protein. The incapacity to excrete urea can result in buildup of nitrogen-containing toxic metabolites that can lead to confusion, headaches, depression, irritability, or, in extreme cases, psychosis. On the other hand, glutamic acid is essential for cerebral functions and increases the amount of gamma-aminobutyric acid (GABA) required for brain functioning and mental activity [3]. According to Belitz *et al.* [2], this amino acid is abundant in most proteins, but is particularly high in milk proteins (22%), wheat (31%), corn (18%), and soya (19%).

Among essential amino acids, threonine and valine perform various important functions in the human body. In fact, threonine was discovered by Rose in 1935; it helps to maintain the proper protein balance in the body and supports cardiovascular, liver, and central nervous and immune system function [4]; threonine is present at 4.5–5% in meat, milk and eggs and 2.7–4.7% in cereals [2]. In the human body, valine is needed for muscle metabolism and repair and tissue growth and for the maintenance of the nitrogen balance in the body. It can be utilised as an energy source in the muscles, preserving the use of glucose [2].

Regarding the [cultivar × treatment] interaction (*table II*), the threonine content ranged between (2 and 82) mg·100 g^{-1} dry weight in raw cv. Martaínha chestnuts and in boiled cv. Lada, respectively; valine changed from (3 to 33) mg·100 g^{-1} dry weight in raw cv. Martaínha chestnuts and roasted cv. Negra, respectively; phenylalanine varied between (1 and 16) mg·100 g^{-1} dry weight in raw cv. Boaventura and roasted cv. Negra, respectively; and isoleucine varied between (0.14 and 16) mg·100 g^{-1} dry weight in raw cv. Martaínha chestnuts and roasted cv. Longal SL, respectively. Chestnuts also presented arginine and leucine at low contents.

In our study, asparagine was the non-essential amino acid with the highest content in chestnut kernels; it ranged from (56 to 336) mg·100 g^{-1} dry weight in boiled chestnuts of cv. Côta and cv. Aveleira, respectively (*table III*). Glutamic acid appeared in second place and it varied between (8 and 166) mg·100 g^{-1} dry weight in roasted chestnuts of cv. Martaínha and cv. Longal SL, respectively. Moreover, it was found that aspartic acid ranged from (4 to 55) mg·100 g^{-1} dry weight in raw chestnuts of cv. Boaventura and boiled chestnuts of cv. Lada, respectively; and alanine varied between (13 and 51) mg·100 g^{-1} dry weight in boiled chestnuts of cv. Côta and roasted chestnuts of cv. Lada, respectively. Chestnuts also presented serine, glycine and tyrosine, but at low contents.

The amino acids that constitute the proteins are of high importance in achieving a healthy and nutritionally balanced diet [20]. Several studies reported that nut consumption has been linked to a lowered risk of cardiovascular heart disease [21, 22], which may be partly explained by the cholesterol-lowering effect due to a favourable fatty acid composition. In addition, several amino acids exert a synergistic effect with vitamin C on the antioxidant activity of vitamin E and their effectiveness is partially related to their lipophilicity [23]. The additive and synergistic actions of phytochemicals in nuts are responsible for the potent antioxidant and anticancer activities [24, 25]. Although roasting induced the highest levels of amino acids, it is known that it might also lead to Maillard reactions with the associated formation of acrylamide (a probable human carcinogen), but, according to Karasek *et al.*, it is unlikely that this kind of food has a significant impact on the total acrylamide intake of humans via food [26].

3.2. Mineral contents

In our study, nine minerals were determined in the ten chestnut cultivars: the main elements P, K, Ca and Mg; the trace elements Fe, Cu, Zn and Mn; and the ultra-trace element B (*tables IV, V)*.

Potassium (K) was the predominant main element in the kernels, and low contents of P, Ca and Mg were observed (*table IV*). Among the trace elements, manganese (Mn) was present at a high level, followed by Fe, and low contents of Zn, Cu and the ultra-trace mineral B were also present in raw, boiled and roasted kernels. Similar results were found for chestnuts by Souci *et al.* [27]. However, Borges *et al.* [8] reported that nuts are rich in K, P, Mg, Fe, Mn and Cu.

It is well known that minerals have an important role in the human organism. Several epidemiological, observational and clinical trials [28–30] have demonstrated a significant reduction in blood pressure, and in the incidence of cardiovascular and cerebrovascular accidents with increased dietary K^+ intake. Second only after K, magnesium (Mg) is one of the major body minerals, primarily found in muscle and bone. It is a constituent and activator of many enzymes, particularly those associated with the conversion of energy-rich phosphate compounds, and a stabiliser of plasma membranes, intracellular membranes and

Table II.
Content of essential amino acids in kernels of raw, boiled and roasted chestnuts from ten Portuguese cultivars (means ± standard deviations; $n = 3$).

Cultivar (C)	Treatment (T)	Arginine	Isoleucine	Leucine	Phenylalanine	Threonine	Valine
		(mg·100 g^{-1} dry weight)					
Aveleira	raw	2.43 ± 1.75	4.23 ± 1.20	2.21 ± 1.40	5.85 ± 1.47	8.81 ± 3.40	10.23 ± 2.01
	boiled	7.15 ± 0.16	11.91 ± 0.44	6.87 ± 0.61	12.25 ± 0.56	41.98 ± 1.90	22.67 ± 1.29
	roasted	3.20 ± 0.96	6.95 ± 1.13	5.67 ± 1.21	8.25 ± 1.29	10.19 ± 3.14	13.81 ± 2.19
Boaventura	raw	0.88 ± 0.55	1.61 ± 0.40	0.55 ± 0.47	1.37 ± 0.30	32.49 ± 5.98	9.14 ± 1.20
	boiled	1.73 ± 1.00	2.83 ± 0.82	1.01 ± 0.48	2.75 ± 1.45	50.79 ± 10.42	10.93 ± 2.87
	roasted	0.22 ± 0.18	3.55 ± 0.95	1.74 ± 1.30	3.56 ± 1.62	55.51 ± 4.69	11.70 ± 1.70
Côta	raw	3.11 ± 0.91	6.17 ± 0.40	2.56 ± 0.95	11.87 ± 3.10	10.98 ± 8.33	17.26 ± 1.29
	boiled	1.49 ± 1.29	3.76 ± 1.91	1.18 ± 1.21	9.45 ± 1.66	7.82 ± 3.55	12.60 ± 5.04
	roasted	1.71 ± 1.31	4.47 ± 2.04	2.44 ± 1.22	9.91 ± 5.06	9.57 ± 7.71	13.53 ± 5.13
Judia	raw	0.76 ± 0.44	6.55 ± 1.04	2.36 ± 0.33	3.59 ± 1.86	26.42 ± 10.77	14.31 ± 1.88
	boiled	0.60 ± 0.52	6.79 ± 0.87	2.91 ± 0.84	3.49 ± 0.70	14.95 ± 4.56	13.51 ± 2.30
	roasted	1.97 ± 0.52	9.38 ± 1.91	4.55 ± 1.49	7.82 ± 2.07	22.75 ± 3.97	19.18 ± 2.93
Lada	raw	2.99 ± 2.68	14.84 ± 2.57	6.50 ± 1.65	12.05 ± 1.95	67. 49 ± 10.01	25.45 ± 4.14
	boiled	2.43 ± 0.24	9.48 ± 0.81	3.87 ± 0.76	8.03 ± 1.72	82.10 ± 19.78	17.58 ± 0.93
	roasted	5.23 ± 2.01	14.42 ± 2.49	8.27 ± 1.48	12.45 ± 2.27	73.15 ± 0.71	25.51 ± 4.22
Lamela	raw	3.85 ± 1.92	5.01 ± 2.43	3.07 ± 0.95	3.46 ± 2.02	31.22 ± 11.80	13.79 ± 4.61
	boiled	1.41 ± 1.03	3.51 ± 1.32	2.14 ± 1.04	2.62 ± 1.34	36.93 ± 6.43	10.11 ± 2.32
	roasted	2.46 ± 1.36	5.21 ± 0.97	3.23 ± 0.50	4.33 ± 1.26	41.23 ± 11.93	10.38 ± 2.25
Longal P	raw	2.77 ± 1.89	5.47 ± 2.32	2.34 ± 1.37	7.31 ± 2.47	17.48 ± 10.07	15.35 ± 4.22
	boiled	2.37 ± 0.97	5.71 ± 2.15	2.21 ± 1.21	8.63 ± 1.64	15.51 ± 4.91	15.56 ± 3.22
	roasted	3.75 ± 1.11	9.50 ± 1.75	5.10 ± 1.17	13.48 ± 2.65	15.18 ± 4.64	22.40 ± 3.66
Longal SL	raw	1.12 ± 0.65	7.46 ± 2.81	2.86 ± 1.58	5.43 ± 2.77	11.60 ± 6.84	18.09 ± 4.73
	boiled	0.29 ± 0.26	5.49 ± 0.29	2.11 ± 0.33	4.48 ± 1.16	9.04 ± 1.36	12.55 ± 0.41
	roasted	5.47 ± 0.92	15.77 ± 2.73	9.98 ± 1.99	10.02 ± 1.98	42.63 ± 3.20	30.08 ± 4.79
Martaínha	raw	Not detected	0.14 ± 0.13	Not detected	2.63 ± 1.89	1.78 ± 1.03	3.31 ± 0.60
	boiled	0.88 ± 0.63	2.69 ± 1.30	1.88 ± 0.82	2.55 ± 0.58	3.51 ± 0.88	7.46 ± 2.99
	roasted	1.60 ± 0.94	4.34 ± 0.77	4.66 ± 1.54	4.55 ± 0.25	2.65 ± 2.42	9.05 ± 1.05
Negra	raw	3.00 ± 2.63	11.47 ± 1.05	7.01 ± 1.12	10.16 ± 0.79	33.77 ± 9.33	24.10 ± 2.03
	boiled	3.50 ± 1.09	10.16 ± 1.09	6.97 ± 0.71	10.59 ± 2.85	30.99 ± 4.32	21.21 ± 2.25
	roasted	6.84 ± 1.30	14.97 ± 3.06	10.50 ± 3.02	15.56 ± 2.53	37.55 ± 4.76	33.08 ± 5.70
Trigueira	raw	5.83 ± 2.05	11.81 ± 2.40	6.17 ± 1.72	11.79 ± 3.16	20.59 ± 5.98	26.54 ± 3.57
	boiled	1.92 ± 0.63	7.42 ± 0.76	3.75 ± 0.84	6.70 ± 0.75	11.73 ± 2.38	17.46 ± 1.57
	roasted	3.29 ± 3.09	11.86 ± 1.56	7.23 ± 1.53	11.59 ± 1.86	11.38 ± 3.12	24.54 ± 3.03
P (C)		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P (T)		0.0053	0.0001	0.0001	0.0001	0.0088	0.0001
P (C × T)		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table III.
Content of non-essential amino acids in kernels of raw, boiled and roasted chestnuts from ten Portuguese cultivars (means ± standard deviations; $n = 3$).

Cultivar (C) and PDO	Treat-ment (T)	Alanine	Asparagine	Aspartic acid	Glutamic acid	Glycine	Serine	Tyrosine	Total amino acids
		(mg·100 g^{-1} dry weight)							
Aveleira	raw	31.42 ± 6.49	136.72 ± 38.40	24.37 ± 22.29	64.50 ± 58.82	5.39 ± 1.51	8.34 ± 4.80	Not detected	338.25 ± 135.99
	boiled	27.82 ± 1.74	335.86 ± 22.92	53.32 ± 17.92	110.55 ± 36.64	3.31 ± 0.13	11.15 ± 0.73	Not detected	747.69 ± 74.28
	roasted	31.38 ± 4.28	118.39 ± 17.43	24.13 ± 16.05	52.83 ± 35.61	1.87 ± 0.22	4.50 ± 1.29	Not detected	329.26 ± 90.53
Boaventura	raw	19.59 ± 2.45	139.62 ± 32.77	4.44 ± 1.52	14.95 ± 4.73	0.45 ± 0.48	4.85 ± 1.17	Not detected	275.80 ± 52.84
	boiled	24.01 ± 2.09	174.49 ± 29.56	16.01 ± 18.08	73.91 ± 13.91	0.82 ± 0.73	7.38 ± 2.41	Not detected	435.23 ± 151.23
	roasted	37.84 ± 3.21	182.35 ± 57.84	22.14 ± 15.91	55.29 ± 13.74	0.18 ± 0.31	6.76 ± 0.62	Not detected	457.04 ± 94.61
Côta	raw	19.28 ± 2.46	72.36 ± 16.25	13.59 ± 8.90	33.11 ± 25.14	2.40 ± 1.01	5.35 ± 1.87	Not detected	249.96 ± 73.90
	boiled	12.53 ± 2.73	56.23 ± 10.15	5.23 ± 1.53	20.01 ± 1.13	0.31 ± 0.36	3.89 ± 1.50	Not detected	170.79 ± 42.90
	roasted	19.55 ± 5.46	64.03 ± 29.54	7.54 ± 6.48	19.25 ± 5.60	0.70 ± 0.29	4.14 ± 2.10	0.22 ± 0.13	197.81 ± 104.89
Judia	raw	20.88 ± 2.09	269.97 ± 35.23	34.96 ± 10.21	80.16 ± 32.06	6.49 ± 0.26	5.11 ± 0.61	Not detected	524.89 ± 65.01
	boiled	20.04 ± 1.72	156.89 ± 36.75	22.18 ± 13.10	62.98 ± 36.28	1.45 ± 0.29	3.34 ± 0.74	Not detected	351.38 ± 96.97
	roasted	33.81 ± 2.59	198.26 ± 13.61	30.47 ± 11.45	97.97 ± 31.38	1.58 ± 0.70	5.81 ± 1.23	Not detected	499.211 ± 80.05
Lada	raw	40.02 ± 5.32	245.13 ± 39.86	49.32 ± 26.70	79.11 ± 46.13	4.37 ± 1.26	7.43 ± 1.69	Not detected	683.99 ± 130.43
	boiled	29.58 ± 1.48	253.16 ± 14.38	55.42 ± 12.20	61.09 ± 15.86	3.13 ± 0.50	5.35 ± 0.63	Not detected	654.69 ± 78.12
	roasted	50.74 ± 3.52	239.50 ± 24.10	35.19 ± 22.83	44.43 ± 32.03	3.42 ± 2.65	8.03 ± 1.76	0.40 ± 0.23	659.58 ± 88.78
Lamela	raw	21.93 ± 3.03	215.64 ± 63.83	19.44 ± 12.43	36.41 ± 25.40	2.84 ±1.45	5.50 ± 1.87	Not detected	422.56 ± 147.32
	boiled	19.85 ± 2.31	185.69 ± 45.69	14.51 ± 5.39	39.17 ± 17.79	1.34 ± 0.59	3.36 ± 1.07	Not detected	377.35 ± 85.58
	roasted	29.86 ± 2.07	157.41 ± 37.49	18.51 ± 15.12	36.16 ± 12.67	0.76 ± 0.70	4.09 ± 1.13	Not detected	380.47 ± 85.87
Longal P	raw	29.44 ± 5.25	122.95 ± 39.13	17.37 ± 13.01	40.89 ± 33.69	5.03 ± 1.92	9.03 ± 2.39	Not detected	326.14 ± 130.72
	boiled	16.45 ± 2.09	113.23± 22.60	48.02 ± 17.21	40.57 ±12.87	3.25 ± 2.11	6.98 ± 1.41	0.13 ± 0.08	328.77 ± 84.97
	roasted	30.38 ± 3.22	103.31 ± 22.94	38.18 ± 22.77	54.63 ± 25.48	1.31 ± 0.59	5.50 ± 0.76	0.59 ± 0.56	372.71 ± 103.25
Longal SL	raw	29.44 ± 5.25	76.51 ± 29.00	11.85 ± 7.43	28.14 ± 15.62	2.24 ± 1.13	4.71 ± 2.37	Not detected	235.90 ± 93.45
	boiled	16.45 ± 2.09	67.40 ± 15.15	14.21 ± 10.86	32.16 ± 25.11	1.40 ± 0.60	2.32 ± 0.32	Not detected	194.87 ± 36.90
	roasted	30.38 ± 3.22	245.34 ± 25.15	34.60 ± 3.52	165.53 ± 17.56	3.73 ± 1.26	11.37 ± 0.94	0.45 ± 0.26	719.67 ± 45.81
Martaínha	raw	14.19 ± 1.29	70.23 ± 10.91	21.42 ± 5.17	43.55 ± 8.53	1.67 ± 0.40	2.35 ± 0.68	Not detected	167.58 ± 21.28
	boiled	14.99 ± 2.95	86.41 ± 13.19	13.80 ± 9.64	44.89 ± 34.47	0.45 ± 0.57	2.26 ± 0.92	Not detected	200.73 ± 71.40
	roasted	32.84 ± 3.50	74.05 ± 9.24	6.28 ± 2.03	8.08 ± 3.00	0.66 ± 0.37	2.54 ± 0.17	Not detected	178.14 ± 17.69
Negra	raw	29.18 ± 4.62	119.40 ± 41.42	54.98 ± 42.54	66.19 ± 48.99	7.20 ± 2.08	6.80 ± 1.99	Not detected	462.78 ± 163.82
	boiled	20.34 ± 0.82	90.06 ± 10.84	22.24 ± 0.37	15.23 ± 4.98	3.61 ± 0.77	4.81 ± 0.54	Not detected	323.12 ± 30.19
	roasted	38.14 ± 3.74	122.90 ± 21.15	9.62 ± 3.52	49.45 ± 19.74	0.90 ±0.31	8.29 ± 0.16	1.20 ± 0.77	467.49 ± 76.15
Trigueira	raw	26.13 ± 5.54	113.57 ± 19.81	46.19 ± 15.56	60.77 ± 22.95	5.55 ± 0.52	14.11 ± 3.31	0.65 ± 0.38	432.15 ± 81.20
	boiled	17.59 ± 3.67	77.77 ± 19.68	33.77 ± 18.32	66.33 ± 44.54	1.69 ± 0.79	4.68 ± 0.73	Not detected	299.79 ± 97.69
	roasted	30.44 ± 3.06	80.79 ± 13.07	41.21 ± 6.78	47.14 ± 10.99	2.35 ± 0.63	8.19 ± 1.61	0.49 ± 0.31	350.38 ± 54.52
P (C)		0.0001	0.0001	0.0001	0.0013	0.0001	0.0001	0.0026	0.0001
P (T)		0.0001	0.9812	0.6992	0.5819	0.0001	0.0005	0.0001	0.0674
P (C × T)		0.0001	0.0001	0.0192	0.0003	0.0001	0.0001	0.0024	0.0001

Table IV.
Minerals in kernels of raw, boiled and roasted chestnuts (Portuguese cultivars).

Kernel of chestnuts	P	K	Ca	Mg	B	Fe	Cu	Zn	Mn
	($g \cdot kg^{-1}$ dry weight)				($mg \cdot kg^{-1}$ dry weight)				
Raw	1.13 ± 0.20 ab	9.36 ± 1.40 b	0.548 ± 0.143 b	0.539 ± 0.144 b	7.07 ± 2.97 b	25.91 ± 8.58 a	8.22 ± 2.33 a	8.91 ± 1.89 a	36.61 ± 17.85 a
Boiled	1.12 ± 0.25 a	8.09 ± 2.33 a	0.482 ± 0.109 a	0.472 ± 0.150 a	6.22 ± 1.86 a	25.78 ± 9.91 a	8.14 ± 2.64 a	9.81 ± 2.52 b	35.97 ± 18.52 a
Roasted	1.18 ± 0.25 b	9.05 ± 1.65 b	0.505 ± 0.12 a	0.521 ± 0.186 b	6.74 ± 2.55 b	25.72 ± 9.46 a	8.89 ± 2.18 a	9.06 ± 1.88 a	36.31 ± 21.10 a

Means (n = 33) ± standard deviations followed by the same letter within a line are not significantly different at $P < 0.05$, according to the Duncan Multiple Range test.

nucleic acids [2]. Additionally, magnesium may be helpful in the treatment of diseases such as hypertension, acute myocardial infarction and atherosclerosis [31]. Phosphorus (P) has an important role in mineralisation of bones and teeth, energy metabolism, and absorption and transport of nutrients [32]. Higher dietary calcium (Ca) is associated with lower blood pressure and a decreased risk of developing hypertension [33]. Belitz *et al.* indicates that milk and milk products are the most important source of Ca, followed at a considerable distance by fruit and vegetables [2]. Moreover, iron (Fe) is necessary for heme synthesis, serves as a catalyst for oxidation-reduction reactions by readily accepting/donating electrons, and is also present in a number of enzymes, such as peroxidase, catalase, hydroxylase and flavine enzymes [2]. Also, zinc (Zn) is an essential trace element that is present in over 200 metalloenzymes that work at several biochemical levels including protein, fat and carbohydrate metabolism; nucleotide polymerase activity; and elongation and desaturation of fatty acids, among others. Copper (Cu) and manganese (Mn) are the component or activator of many important body enzymes [2].

Cooking significantly affected ($P < 0.001$) the mineral composition of the chestnuts, except for P, Fe, Cu and Mn contents (*tables IV, V*). In general, calcium significantly decreased upon cooking. In addition, boiling led to severe leaching of K, Ca, Mg and B, mainly in cvs. Judía, Negra and Trigueira. In a previous study dealing with the same species, Künsch *et al.* found that cooking led to decreases in K and Mg, but not in Ca contents [12].

In relation to the [cultivar × treatment] interaction and among the main elements, potassium (K) ranged between (5 and 13) $g \cdot kg^{-1}$ dry weight in boiled cv. Negra chestnuts and boiled cv. Martaínha, respectively; calcium (Ca) ranged from (0.31 to 0.77) $g \cdot kg^{-1}$ dry weight in raw cv. Longal SL chestnuts and raw cv. Côta, respectively; and magnesium (Mg) varied between (0.33 and 0.92) $g \cdot kg^{-1}$ dry weight in boiled cvs. Judia and Lada chestnuts and roasted cv. Lamela, respectively (*table V*).

3.3. Principal component analysis and correlations between amino acids and minerals

In order to establish the relationships between the different variables and to detect the most important causes of variability, a principal component analysis (PCA) was applied to the total amino acids and the individual amino acids which present greater relevance (asparagine, aspartic acid, glutamic acid, threonine and valine), and to the minerals (P, K, Ca, Mg, B, Fe, Cu, Zn and Mn) of raw, boiled and roasted chestnuts (*figure 1*).

For raw chestnuts (*figure 1A*), two principal components (PC) were obtained, accounting for 60% of the total variance. Component 1 explained 36.9% of the total variance and the parameters that correlated best with this principal component were total amino acids (0.981), aspartic acid (0.820), glutamic acid (0.792), threonine (0.779), asparagine (0.760), valine (0.671) and phosphorus (P) (0.530). Similarly, for boiled chestnuts (*figure 1B*), two principal

Table V.
Mineral contents in kernels of raw, boiled and roasted chestnuts from ten Portuguese cultivars (means ± standard deviations; *n* = 3).

Cultivar (C) and PDO	Treatment (T)	P	K	Ca	Mg	B	Fe	Cu	Zn	Mn
		($g \cdot kg^{-1}$ dry weight)				($mg \cdot kg^{-1}$ dry weight)				
Aveleira	raw	1.36 ± 0.14	10.62 ± 0.54	0.57 ± 0.03	0.69 ± 0.15	3.85 ± 0.83	21.67 ± 6.66	5.53 ± 0.97	10.33 ± 1.53	36.33 ± 14.43
	boiled	1.60 ± 0.10	9.33 ± 1.46	0.56 ± 0.08	0.47 ± 0.03	6.73 ± 1.40	18.33 ± 6.66	7.50 ± 2.04	13.00 ± 2.00	49.67 ± 13.28
	roasted	1.55 ± 0.28	10.97 ± 2.14	0.51 ± 0.07	0.45 ± 0.06	5.07 ± 1.08	19.33 ± 3.06	7.30 ± 1.60	9.00 ± 0.01	34.67 ± 9.29
Boaventura	raw	0.76 ± 0.04	8.75 ± 0.35	0.52 ± 0.04	0.46 ± 0.03	4.46 ± 0.32	30.00 ± 8.19	9.13± 0.38	11.33 ± 0.58	47.00 ± 7.00
	boiled	0.71 ± 0.04	7.47 ± 0.54	0.55 ± 0.10	0.44 ± 0.05	6.02 ± 1.20	24.00 ± 5.57	9.10 ± 0.60	11.33 ± 0.58	42.33 ± 5.86
	roasted	0.76 ± 0.03	8.40 ± 0.35	0.47 ± 0.03	0.41 ± 0.03	6.49 ± 1.75	27.33 ± 4.16	9.73 ± 1.31	11.00 ± 1.73	50.00 ± 9.17
Côta	raw	1.12 ± 0.07	11.03 ± 1.23	0.77 ± 0.08	0.46 ± 0.06	5.66 ± 0.10	23.67 ± 7.23	7.90 ± 0.66	7.33 ± 0.58	39.33 ± 8.08
	boiled	1.01 ± 0.14	11.00 ± 1.12	0.65 ± 0.07	0.42 ± 0.05	4.10 ± 0.97	21.33 ± 1.16	8.33 ± 0.61	9.00 ± 1.00	31.33 ± 7.02
	roasted	1.17 ± 0.01	11.35 ± 2.19	0.75 ± 0.10	0.40 ± 0.03	4.65 ± 0.79	34.50 ± 2.12	7.05 ± 0.07	8.50 ± 0.71	30.00 ± 7.07
Judia	raw	1.29 ± 0.03	10.50 ± 1.40	0.45 ± 0.10	0.42 ± 0.07	13.42 ± 1.26	20.33 ± 4.04	7.13 ± 3.07	9.00 ± 1.00	17.67 ± 9.07
	boiled	1.23 ± 0.10	6.83 ± 0.18	0.37 ± 0.07	0.33 ± 0.03	9.09 ± 0.77	19.33 ± 6.11	8.43 ± 1.99	11.00 ± 1.73	14.67 ± 6.03
	roasted	1.40 ± 0.06	9.10 ± 1.40	0.40 ± 0.05	0.34 ± 0.05	12.02 ± 1.82	44.33 ± 7.51	10.93 ± 3.05	9.33 ± 0.58	15.33 ± 6.66
Lada	raw	1.40 ± 0.07	9.57 ± 1.07	0.62 ± 0.05	0.43 ± 0.03	8.54 ± 0.67	35.00 ± 6.25	6.90 ± 0.50	10.67 ± 1.53	23.33 ± 4.04
	boiled	1.30 ± 0.09	6.59 ± 0.62	0.37 ± 0.01	0.33 ± 0.06	8.17 ± 0.49	35.67 ± 8.51	6.60 ± 2.04	10.33 ± 0.58	21.67 ± 7.51
	roasted	1.51 ± 0.01	9.45 ± 0.70	0.45 ± 0.07	0.46 ± 0.04	9.15 ± 0.29	23.00 ± 9.64	6.80 ± 1.85	11.67 ± 0.58	24.33 ± 6.11
Lamela	raw	1.08 ± 0.30	9.22 ± 0.54	0.58 ± 0.13	0.78 ± 0.06	4.64 ± 0.84	40.00 ± 6.08	8.10 ± 0.70	11.67 ± 0.58	82.00 ± 11.00
	boiled	0.75 ± 0.03	6.77 ± 0.40	0.54 ± 0.07	0.66 ± 0.12	4.40 ± 1.01	40.00 ± 7.55	7.77 ± 2.47	12.67 ± 0.58	80.00 ± 3.00
	roasted	0.91 ± 0.03	8.28 ± 1.13	0.53 ± 0.13	0.92 ± 0.08	3.97 ± 0.83	25.33 ± 5.03	9.63 ± 0.31	11.67 ± 0.58	88.33 ± 10.60
Longal P	raw	1.07 ± 0.04	8.75 ± 0.35	0.56 ± 0.06	0.40 ± 0.02	6.25 ± 0.67	18.67 ± 1.53	7.60 ± 0.92	8.00 ± 1.00	30.67 ± 4.04
	boiled	1.09 ± 0.06	9.10 ± 0.99	0.55 ± 0.03	0.40 ± 0.01	6.54 ± 0.28	24.50 ± 10.61	7.40 ± 2.26	12.50 ± 2.12	22.50 ± 7.78
	roasted	1.12 ± 0.05	9.98 ± 0.18	0.58 ± 0.05	0.44 ± 0.01	6.25 ± 0.10	26.00 ± 9.17	8.30 ± 2.03	8.33 ± 0.58	26.33 ± 6.81
Longal SL	raw	1.06 ± 0.10	7.12 ± 0.20	0.31 ± 0.06	0.47 ± 0.02	10.46 ± 0.88	18.00 ± 3.46	5.93 ± 1.35	7.00 ± 1.00	30.67 ± 1.53
	boiled	1.14 ± 0.01	6.07 ± 0.54	0.38 ± 0.03	0.41 ± 0.02	8.04 ± 0.95	26.33 ± 12.50	5.73 ± 1.10	6.33 ± 0.58	39.33 ± 6.81
	roasted	1.22 ± 0.02	7.12 ± 0.20	0.43 ± 0.02	0.45 ± 0.01	8.16 ± 0.86	18.33 ± 2.08	7.17 ± 0.45	6.33 ± 0.50	31.00 ± 4.58
Martaínha	raw	1.02 ± 0.07	9.10± 0.35	0.46 ± 0.08	0.51 ± 0.01	8.01 ± 1.01	18.33 ± 3.22	9.53 ± 1.88	7.00 ± 0.01	32.67 ± 2.52
	boiled	1.20 ± 0.11	12.62 ± 2.13	0.44 ± 0.10	0.64 ± 0.31	5.99 ± 1.49	16.33 ± 2.08	5.50 ± 1.10	7.33 ± 0.58	36.00 ± 7.00
	roasted	1.18 ± 0.11	8.40 ± 0.70	0.42 ± 0.04	0.49 ± 0.02	6.48 ± 1.39	18.33 ± 4.51	9.07 ± 2.51	7.00 ± 0.01	34.67 ± 4.73
Negra	raw	1.07 ± 0.09	7.70 ± 1.21	0.47 ± 0.07	0.52 ± 0.02	8.07 ± 0.77	32.33 ± 5.51	13.17 ± 0.81	7.33 ± 0.58	23.00 ± 4.36
	boiled	1.11 ± 0.06	5.25 ± 0.35	0.40 ± 0.04	0.43 ± 0.06	5.39 ± 0.96	37.00 ± 6.08	13.67 ± 3.00	8.67 ± 0.58	24.33 ± 3.51
	roasted	1.04 ± 0.02	6.88 ± 0.54	0.41 ± 0.09	0.48 ± 0.01	7.42 ± 1.29	32.33 ± 7.02	11.20 ± 0.56	7.33 ± 0.58	15.00 ± 6.08
Trigueira	raw	1.23 ± 0.14	10.62 ± 0.54	0.73 ± 0.12	0.77 ± 0.06	4.46 ± 1.47	27.00 ± 4.00	9.53 ± 1.10	8.33 ± 0.58	40.00 ± 4.58
	boiled	1.21 ± 0.03	8.28 ± 0.40	0.52 ± 0.05	0.64 ± 0.12	4.11 ± 0.22	20.33 ± 5.13	9.30 ± 1.02	6.67 ± 0.58	29.33 ± 8.74
	roasted	1.13 ± 0.13	10.38 ± 0.73	0.69 ± 0.09	0.85 ± 0.10	3.82 ± 0.28	17.00 ± 3.00	10.03 ± 2.97	9.33 ± 0.58	47.67 ± 7.57
P (C)		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P (T)		0.0697	0.0001	0.0034	0.0028	0.0051	0.9873	0.1946	0.0001	0.8518
P (CxT)		0.0092	0.0001	0.0467	0.0040	0.0001	0.0001	0.2249	0.0001	0.1069

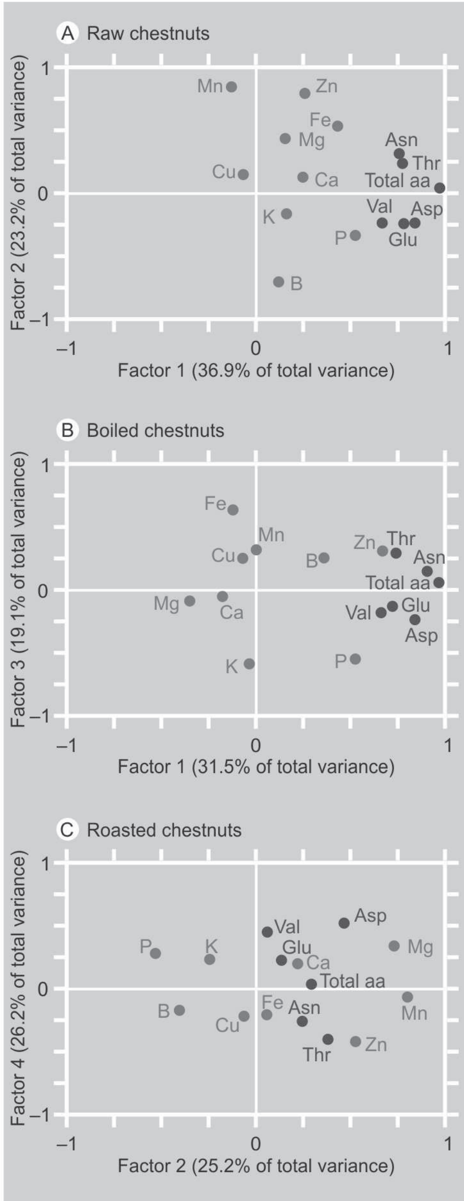

Figure 1.
Principal component analysis plot for nutritional parameters of raw, boiled and roasted chestnuts (Portuguese cultivars).

components were obtained, accounting for 51% of the total variance. Component 1 explained 31.5% of the total variance and the parameters that correlated best with this principal component were total amino acids (0.976), asparagine (0.900), aspartic acid (0.845), threonine (0.728), glutamic acid (0.723), Zn (0.669), valine (0.653) and phosphorus (P) (0.529). For roasted chestnuts (*figure 1C*), two principal components were obtained, accounting for 51% of the total variance. Component 4 explained 26.2% of the total variance and the parameters that correlated best with this component were aspartic acid (0.534), valine (0.453) and magnesium (Mg) (0.338).

It is interesting to note that the amino acids valine, aspartic acid, glutamic acid and total amino acids from raw chestnuts, and threonine, asparagine, aspartic acid and total amino acids from boiled chestnuts were positively correlated with phosphorus (P) (data not shown). However, there were no positive correlations between amino acids and P in roasted chestnuts. Also, some positive correlations between threonine, asparagine and total amino acids and iron (Fe) were found for raw chestnuts, but not in either boiled or roasted chestnuts (data not shown). Threonine and asparagine from raw chestnuts, and threonine, asparagine, aspartic acid, glutamic acid and total amino acids from boiled chestnuts correlated positively with zinc (Zn), but not in roasted chestnuts. In roasted chestnuts, asparagine, glutamic acid and total amino acids correlated positively with boron (B) and correlated negatively with calcium (Ca) (data not shown). Jiang *et al.* reported that eight mineral element contents had correlations with different amino acid contents [34]. Magnesium, calcium and zinc contents were significantly correlated with most of the 17 amino acid contents, but sodium (Na) content did not correlate with amino acid contents except with aspartic acid of rice. Moreover, Zhou *et al.* indicated positive correlations between amino acid content and copper or zinc content [35].

In summary, since more than 90% of the consumption is made up of cooked chestnuts, apart from a good level of amino acids and minerals, the cooking process also has a positive effect regarding the digestibility of macro-molecules such as proteins and polysaccharides (fibre) in terms of denaturation and increasing bioavailability, as can be observed in the scanning electron micrographs (*figure 2*): the cells of chestnut starchy endosperm showed cell walls and large and small starch grains (*figure 2A*); after cooking, both the boiling (*figure 2B*) and roasting (*figure 2C*) processes led to

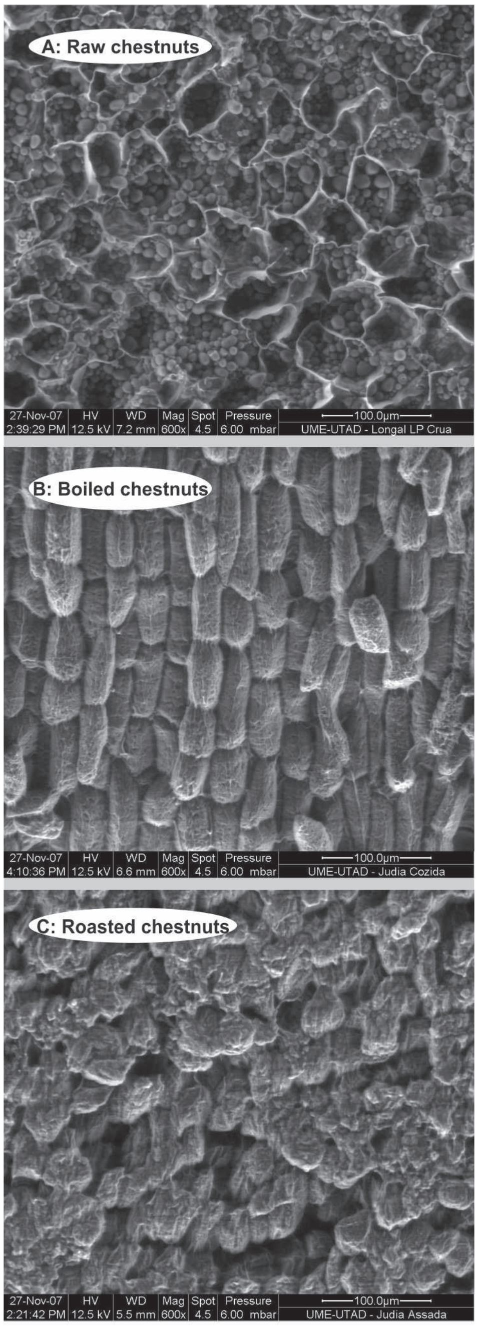

Figure 2.
Scanning electron microscopy (SEM) micrographs of raw, boiled and roasted sections of chestnuts (Portuguese cultivars).

cell wall destruction and protein matrix disruption.

In relation to amino acid content, the best cultivars were cvs. Aveleira, Boaventura and Martainha for boiling; and cvs. Longal P, Longal SL and Negra for roasting. In addition, cvs. Aveleira, Boaventura, Longal P and Martainha were the most stable cultivars upon cooking regarding the mineral content. Since they showed a larger decrease in total amino acid content after cooking, the other cultivars Côta, Judia, Lamela and Trigueira might be better used for flour, for a gluten-free diet in cases of celiac disease. However, based on the present data, cooked chestnuts are a good source of amino acids and minerals, as well as phenolics and organic acids and low in fat [13], thus meeting the requirements of health products.

Acknowledgements

This work was sponsored by the PO AGRO – Acção 8.1 – Desenvolvimento Experimental e Demonstração, Proj. Nº 939/2006.09.004006.1. We are grateful to Rosa Carvalho and Rui Pires for their technical assistance in the laboratory.

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Efecto de la asación en los perfiles de aminoácidos libres y de elementos minerales de la castaña (Castanea sativa Mill.).

Resumen — Introducción. El castaño es uno de los cultivos más importantes de la economía portuguesa. La mayoría de las veces, sus frutos se consumen asados o hervidos. En este contexto, el objetivo principal de nuestro estudio fue evaluar los índices de aminoácidos y la composición mineral de las castañas crudas y asadas. **Material y métodos**. Los aminoácidos se determinaron por HPLC (cromatografía líquida de alto rendimiento) y los minerales por espectrofotometría de absorción molecular, espectrofotometría de absorción atómica o fotometría de emisión de llama. Se evaluaron los cultivares portugueses más importantes: Aveleira, Boaventura, Côta, Judia, Lada, Lamela, Longal Padrela, Longal Soutos da Lapa, Negra y Martaínha. **Resultados y discusión**. El método de asación alteró significativamente la composición total de aminoácidos, presentando, en las muestras tostadas, unos índices incrementados de un 13% y un 12%, en relación con la composición respectiva de las castañas hervidas o crudas. Las castañas tostadas presentaron unos índices de alanina, arginina, isoleucina, leucina, fenilalanina, treonina, tirosina y valina superiores a los de las castañas crudas o hervidas. Además, la serina presentó un índice más elevado en las castañas crudas o asadas. En cuanto a la composición mineral, el potasio (K) fue el macronutriente predominante en las castañas, mientras que los índices encontrados de fósforo (P), calcio (Ca) y magnesio (Mg) fueron más flojos. La asación alteró sensiblemente la composición mineral, salvo para los índices de hierro (Fe), cobre (Cu), y magnesio (Mn). Por lo general, con la asación, el índice de calcio disminuyó. El índice de K, Ca, Mg, B disminuyó en los frutos hervidos. Los datos actuales confirman que las castañas asadas son una buena fuente de aminoácidos y de minerales; ambos elementos están alterados por la ebullición y el tueste, los cuales, a su vez, están asociados a ventajas saludables.

Portugal / *Castanea sativa* / frutas / cocción / ensayos de variedades / composición aproximada / contenido mineral / contenido proteico / aminoácidos libres