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Castanea sativa shells and fruits: Compositional analysis by proton induced X-ray emission

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ABSTRACT

Chestnut fruits, from *Castanea sativa* Miller species, have been previously characterized mainly to study the nutritional and biochemical parameters, with mineral contents receiving less attention from the researchers, although these minerals are essential for several mechanisms in human body. In this study, a detailed elemental compositional analysis was performed using Proton Induced X-ray Emission (PIXE) to characterize two varieties of *Castanea sativa* Miller chestnuts (Longal and Judia). This study comprises the composition of the outer shells (pericarp), inner shells (episperm) and the edible fruits. From our knowledge, this is the first time that the presence of up to 20 elements on the shells and the fruits are reported. Furthermore, by PIXE it was possible to quantify them, allowing significant improvements in the assessment of chestnuts composition. Essential and non-essential elements were quantified on a food item that is used mainly for human consumption but it is also incorporated in animal feeding.

1. Introduction

Chestnut trees are grown in several regions of the planet, with their fruits and sub-products being widely consumed and not limited to the production areas. The global production is rising through the last decades, reaching almost 2.3 millions of tons in 2016, being China the main responsible for this production, almost 83% [1]. Considering the EU countries, chestnut fruits market represents a quantity of about 100 thousand tons per year and an amount of about 200 million Euros. Portugal is the third largest producer with an average production of 30 thousand tons per year in 2017 [2], mainly produced in the Northern region of Trás-os-Montes. The majority of the chestnuts production is exported [3], contributing the incomes to this area development and industrialization.

These edible fruits are being consumed since ancient times by the local populations and nowadays is globally consumed due to their multiple uses as subproducts, being for example a real alternative source of starch due to the high amounts present in the fruits and functional properties. Currently, chestnuts fruits are consumed fresh, cooked, roasted or incorporated as flour, pastry or candies ("*marron glacé*") being also suitable for celiac population. Furthermore, they are available all the year, as peeled-dried or frozen, to be used by gourmet restaurants sector as an exquisite food.

Although the chestnut is classified as a nut, it has lower lipid content, approximately only 3%. They are source of carbohydrates, fibre, fatty acids, proteins, vitamins and minerals, among others [4]. A better knowledge of its nutritional content could valorise even more the product, answering also to the demand of better informed consumers. Regarding the minerals contents, different authors [4–8] reported on the major minerals like potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) and on the minor elements (manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu)). Their concentration depends on their species (American, Chinese or European) and variety or cultivar [9], region of production and external factors as climatic conditions and fertilizers, although differences even between cultivars from the same place have been reported [7].

The elemental composition techniques most used by the previously referenced research works are the atomic absorption and ultraviolet–visible (UV–VIS) spectrophotometry techniques, depending on the elements to be quantified [7], focusing their analysis only to the fruit. As far as we could gather, none of them have analysed the shells (pericarp and episperm) minerals content. Although human consumption is limited to the fruit, due to the non-digestibility or bitterness of shells, relevant bioactive substances have been identified on them [10].

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A better knowledge of the contents of the shells minerals could valorise what are, until now, sub-products of chestnut food industry.

The major and minor elements are present in small quantities when compared with the organic part content of the chestnuts, however they have relevant metabolic functions in several human body mechanisms. Potassium is an essential mineral in many physiological processes, commanding nerve impulse transmission and muscle contraction [11]. Phosphorus is associated with several mechanisms, such as mineralisation of bones and teeth, as well as being a component of cell structure [12]. Calcium is essential for human bones and teeth formation, among other functions [13,14]. Minor elements such as iron or zinc also have a fundamental role in several biological mechanisms [15], iron is present in human haemoglobin and its deficiency is related with several disorders since it is an essential element of enzymes involved in the synthesis of collagen and some neurotransmitters [16]. Other trace elements such as copper and nickel are essential nutrients to sustain the health and function of the human body although an imbalance ratio can be a concern due to their toxicity. The same applies with more relevance to other contaminants and heavy metals, which could be detected using more sensible techniques, as is the case of lead (Pb) or arsenic (As) [17], although their presence is more related with unintentional contamination than with regular food ingest.

In this study, the Proton Induced X-ray Emission (PIXE) technique was applied to characterize the elemental composition of the fruit and shells (pericarp and episperm) of the two most cultivated varieties of European chestnut (*Castanea sativa* Miller) in Portugal, being protected designation of origin (PDO): Longal and Judia. While the Longal variety is the preferred one for industrial processing because it is easy to peel, the Judia is preferred for being marketed as fresh because of their higher size and deep colour of the pericarp [18].

PIXE is a very sensitive technique and it is able to identify the presence of elements with atomic number above 12 (Mg), with detection limits in the order of µg/g [19]. Besides its application in the field of materials science, PIXE is referred as particularly useful in the detection of trace elements in biological samples and it has already been applied in the study of different food products such as canned tuna fish [20], honey [21], flour and bread [22], millet [23], human milk [24] or coffee [25]. It was also used to characterize soy beans cultivated with regular and genetically modified seeds [26], among other food items.

The aim of this work is to use PIXE technique to perform the elemental analysis of the two most frequent varieties of chestnuts cultivated in Portugal, Longal and Judia, from a broad perspective. That includes not only the analysis of the edible fruit, but also de shells (pericarp and episperm), extending the number of elements that until now has been studied by other researchers. This information can be helpful to food processing industries to promote the added-value of chestnuts by-products.

2. Materials and methods

Two varieties of *Castanea sativa* Miller (Longal and Judia) from Bragança (Northeast of Portugal) were chosen for this study. Chestnuts (a bag of about 35 kg) were collected randomly in autumn and stored in refrigerated conditions (at 4 °C).

For preliminary PIXE analysis, 1 kg of chestnut fruits were arbitrary selected, hand peeled (the out and the inner shells were separated for analysis too, see Fig. 1), ground, freeze-dried and kept refrigerated (4 °C) until two days before the measurements. Previous to PIXE analysis, the fruits and shells were powdered using a refrigerated (liquid nitrogen container) vibratory mill (Mixer Mill MM 200, Retsch, Germany) which produces very fine and homogenised powder. The powders were freeze-dried for 24 h and part of the powder material compressed into pellets (hydraulic press reading 8 ton) with 15 mm diameter and 1 mm thick. For each variety, 4 pellets for the fruits and 2 pellets for each, the inner and outer shells, were analysed.

PIXE measurements were performed at CTN/IST (Portugal), at the PIXE line of the 2.5 MV Van de Graaff accelerator using a broad proton beam (5 mm in diameter) to induce the X-ray emission from the samples. The sample irradiation was performed under vacuum conditions and a 8 µm Be windowed Si(Li) detector with 145 eV resolution placed in air at 110° relative to the proton beam direction was used to detect the X-ray emission. Notice a 6 µm thick Mylar vacuum window and 5 mm air path between the 22.5° tilted sampled and the Si(Li) detector.

Fig. 1. Fruit (A), inner (B) and outer (C) shells of the analysed chestnuts.

It is known that biological samples when analyzed by PIXE in vacuum conditions suffer a loss of low atomic number elements as C, O, N from the matrix, thus affecting the intensity of the X-ray emission peaks in the spectrum which will be used to quantify the elemental concentration. The most affected emission peaks correspond to the P, K, Ca and Fe elements. To overcome this issue, Reis et al. proposed a methodology to perform the PIXE measurements and to quantify the elements concentration [27]. This methodology was followed in this work, which basically consist of obtaining X-ray spectra from the same point using different beam energies (1.220 and 2.220 MeV) and currents. Briefly, first a spectrum is taken to measure major and minor elements with a low beam current density ($\leq$0.25nA/mm^2) at 1.220 MeV and no absorber in front of the Si(Li) detector, afterwards another spectrum with high current density (~2.5 nA/mm^2) and 2.220 MeV energy is obtained (using a 250 µm Mylar filter in front of the detector) from which the trace elements can be determined; finally, an additional spectrum using a low beam current density and 1.220 MeV energy is obtained to calculate change in concentration of high Z elements due to target irradiation damage (loss of low Z matrix elements). More details can be found in [27].

PIXE spectra recorded for each pellet (obtained with different proton energies and beam currents) were fitted with the AXIL code [28] to extract the peak areas used as input for the DATTPIXE code [27] which converts elemental peak area into sample elemental concentration values in an iterative procedure, initially considering matrix composition based on cellulose and the experimental conditions used. Accuracy of the determined elemental concentrations can be affected by X-ray peaks overlap as it is the case of Pb-Lα (10.551 keV) and As-Kα (10.543 keV). This can be unraveled taking into account the other elemental characteristic emissions and their relative intensities: Pb-Lβ (12.614 keV) for Pb and As-Kβ (11.726 keV) for As, during the AXIL spectra deconvolution. On the other hand DATTPIXE code reports elemental concentration data both, for the As-Kα and As-Kβ lines as well as for the Pb-Lα and Pb-Lβ lines that allows checking for data reliability.

The same overlapping problem can be found between the Br-Kβ (13.296 keV) and Rb-Kα (13.375 keV) lines and, to a certain extent, between the As-Kβ (11.726 keV) and Br-Kα (11.924 keV) lines, problems that are managed in a way similar to the previously reported one.

Although PIXE technique is a standard-less compositional analysis, in order to control and validate the methodology used in this study NBS Citrus Leaves 1572 Standard Reference Material was also added to the set of pellets to be measured, results are shown in Table 1.

3. Results and discussion

In Fig. 2 are shown the PIXE spectra obtained for the Judia chestnut (shells and fruit) when using the high energy proton beam (2.220 MeV).

Table 1
Elemental concentrations, certified and measured values for NBS Citrus Leaves 1572. (n.c. means non certified and n.d. means not detected).

Z	Element	Certified value (μg/g)	std%	Measured value (μg/g)	std %
14	**Si**	–	–	624	25.4
15	**P**	1300	15.3	945	1.6
16	**S**	4070	2.2	3376	2.6
17	**Cl**	414.0	n.c.	351	2.0
19	**K**	18,200	3.3	17,400	3.0
20	**Ca**	31,500	3.2	24,700	5.0
24	**Cr**	0.8	25.0	n.d.	
25	**Mn**	23.0	8.7	33.0	15.2
26	**Fe**	90.0	11.0	115.0	5.0
28	**Ni**	0.6	50.0	n.d	–
29	**Cu**	16.5	6.0	21.0	16.7
30	**Zn**	29.0	6.9	29.0	22.8
33	**As**	3.1	9.7	4.0	12.0
35	**Br**	8.2	n.c.	9.0	6.2
37	**Rb**	4.8	1.2	7.0	13.0
38	**Sr**	100.0	2.0	84.0	13.4
56	**Ba**	21.0	14.0	17.0	15.0
82	**Pb**	13.3	18.0	12.0	9.0

In the spectra are labelled the X-ray lines of some of the elements identified. Similar spectra were recorded for the Longal variety. Elemental quantification values (mean average concentration, in wt %) are presented in Tables 2 and 3.

It can be observed that calcium (Ca) is the major element in shells (outer and inner) for both varieties, being quite similar in the outer and inner shells for the Judia variety (0.25%) and much higher in the inner shell (0.37%) than in the outer shell (0.20%) for the Longal variety. On the other hand, potassium is the major element in both varieties of edible fruits.

The elements concentration for outer shells, in descendent order, is Ca > K > Fe ≈ S ≈ Mn for the Judia variety, and Ca > K > S > Mn ≈ Fe for Longal variety; where the Fe is the third prevalent element in chestnuts shells from Judia cultivar and the fifth from Longal.

For chestnuts inner shells the concentration order of major elements, from the higher to the lower, is Ca > K > S > Mn > Si > P for Judia variety, and Ca > P > K > Si > S > Mn for Longal variety. The concentration of phosphorus in Longal inner shells (0.11%) point out to be considerable higher than in Judia variety (0.01%). Other main major minerals present in the shells are sulphur and manganese, with similar concentrations for both varieties, being lower in the inner shell than in the outer. The other minor elements detected (Ba, P, Si, Zn, Cl, Cu, Sr, Pb, Ti, Rb) have higher concentration in pericarp than in episperm. When both shells are compared, barium and zinc concentrations is about three times higher for Judia and about six times higher for Longal, while copper (Cu) is about ten times higher for Judia and fourteen times higher for Longal. Chromium (Cr) and nickel (Ni) were detected only in outer shells.

Regarding the fruits, the elemental concentration, from the highest to the lowest, is: K > P > Ca > S > Cl > Si, for Judia fruits; and K > Si > P > Ca > S, for Longal fruits; where calcium is the third element in Judia and the fourth in Longal variety.

Other elements were detected in fruits (Mn, Fe, Zn, Cu), being some of them for the first time reported in this work: S, Cl, Si, Al, Rb, Pb, Ba, Ti, Sr, Br, As, Ni, Cr (see Table 4). Even in minor concentration, they could have relevant physiological functions in human body.

Some of these elements have different concentrations in shells and fruits. For example, rubidium is higher in Longal and Judia outer shells (34 and 8 $\mu g\,g^{-1}$, respectively) than in inner shells (1 and 2 $\mu g\,g^{-1}$, respectively), with Longal and Judia fruits presenting the same value (15 $\mu g\,g^{-1}$).

For some elements (Ba, Pb, Cu, Ti, Sr) the concentration is higher in outer shells than in inner shells, and lower in fruits (pericarp > episperm > fruits), for both varieties.

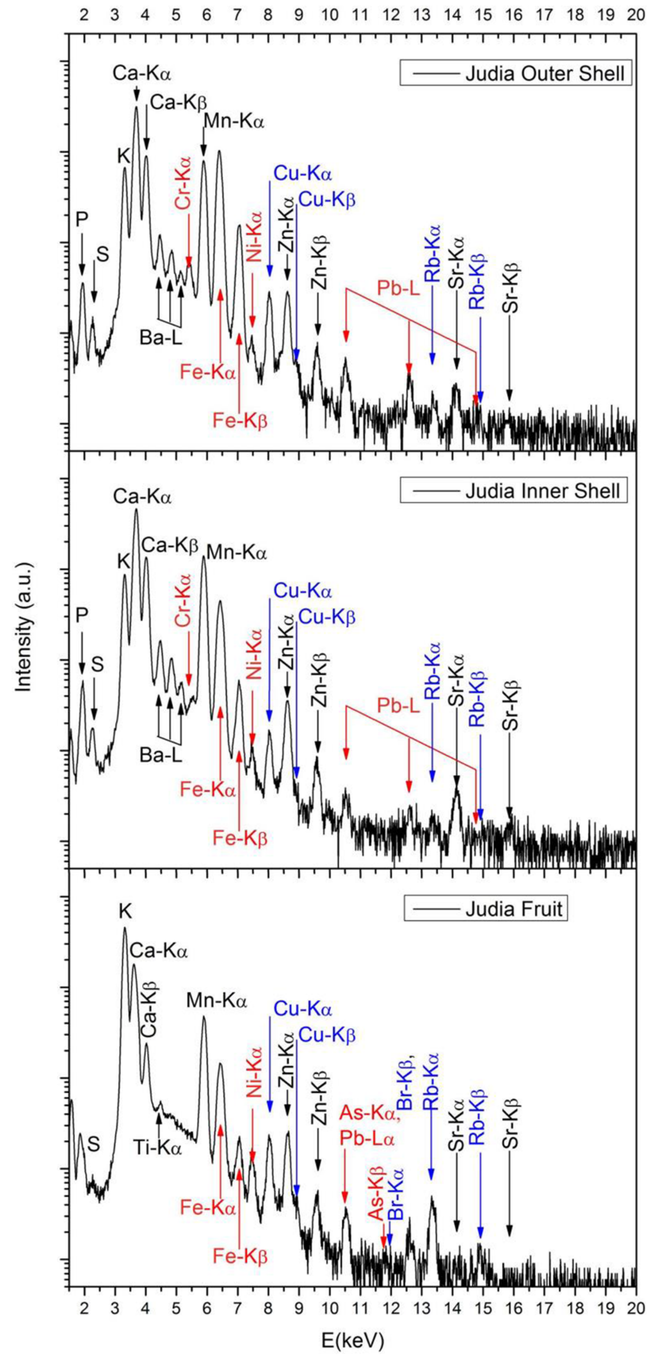

Fig. 2. PIXE spectra for the Judia chestnut variety (shells and fruit) using a proton beam energy of 2.220 MeV.

Regarding zinc, the lower concentration was observed in inner shells (13 and 12 $\mu g\,g^{-1}$, for Longal and Judia varieties, respectively) and the higher value in outer shells (84 and 43 $\mu g\,g^{-1}$, for Longal and Judia, respectively), with the fruits presenting lower values of Zn (15 and 27 $\mu g\,g^{-1}$, for Longal and Judia). Similarly, manganese concentration is higher in outer shells (456 and 522 $\mu g\,g^{-1}$, for Longal and Judia) than in fruits (55 and 67 $\mu g\,g^{-1}$, for Longal and Judia). The presence of arsenic and bromine was only detected in the fruits, with similar low concentrations for both varieties.

Only results obtained for the edible fruits can be compared with those obtained by other authors, which have analyzed these varieties of chestnut fruit minerals content using other techniques. In Table 4 are

Table 2
Elemental concentration of the outer (pericarp) and inner (episperm) shells for Longal and Judia varieties (* means statistical error. Only one pellet considered).

		PERICARP				EPISPERM			
		Longal		Judia		Longal		Judia	
Z	Element	Conc. (μg/g)	std%	Conc. (μg/g)	std%	Conc. (μg/g)	std%	Conc. (μg/g)	std%
14	**Si**	134	18.4*	96	13.7	270	26.2	150	36.8
15	**P**	112	79.6	143	4.2	1130	6.1	140	21.3
16	**S**	590	4.9*	530	3.1	230	11.1	270	11.2
17	**Cl**	74	22.1*	32	29.0	57	40.1	34	30.5
19	**K**	810	4.3	1650	2.1	530	7.2	1440	10.5
20	**Ca**	1950	0.5	2490	4.4	3700	5.8	2500	14.0
22	**Ti**	39	9.6*	11	14.4	6	12.0	5	14.3
24	**Cr**	4	70.9	12	100.7	–	–	–	–
25	**Mn**	460	0.1	520	3.5	70	3.2	200	13.0
26	**Fe**	410	24.5	530	51.8	59	6.6	45	5.1
28	**Ni**	2	30.8	3	10.2	n.d.	–	n.d.	–
29	**Cu**	28	15.8	29	3.0	2	3.3	3	12.9
30	**Zn**	84	1.6	43	8.4	13	7.7	12	11.5
37	**Rb**	34	51.3	8	30.6	1	2.3	2	12.2
38	**Sr**	72	25.3	27	11.1	11	6.4	8	5.5
56	**Ba**	202	2.1	155	6.0	30	7.9	57	13.9
82	**Pb**	225	3.9*	16	93.4	1	11.3	1	20.0

Table 3
Elemental concentration of the edible fruits: Judia and Longal varieties (* means statistical error. Only one pellet considered. n.d. means not detected).

		Judia		Longal	
Z	Element	Conc. (μg/g)	std%	Conc. (μg/g)	std%
13	**Al**	90	19.5*	170	16.3*
14	**Si**	100	12.4*	5000	94.3
15	**P**	650	11.0	870	8.3
16	**S**	550	8.1	500	17.8
17	**Cl**	130	19.6	55	40.6
19	**K**	6700	2.6	8110	4.9
20	**Ca**	600	5.5	580	5.8
22	**Ti**	5	58.4	n.d.	–
24	**Cr**	1	22.1*	2	13.7*
25	**Mn**	67	10.6	120	2.4
26	**Fe**	51	80.6	39	76.2
28	**Ni**	2	12.6	4	12.7
29	**Cu**	7	8.2	10	17.0
30	**Zn**	27	11.6	15	5.8
33	**As**	2	28.9*	3	118.5
35	**Br**	2	30.2	1	32.2*
37	**Rb**	15	13.1	15	7.5
38	**Sr**	3	18.4*	3	27.9*
56	**Ba**	5	9.1*	7	4.4
82	**Pb**	10	97.3	6	6.0*

presented the results for the 20 elements detected in our study and the values for seven of these elements measured by other authors. It should be noticed that under the experimental conditions used in this work Na and Mg concentration could not be calculated with the PIXE technique (LOD of 0.36%.for Na and 0.02% for Mg).

For the major elements (K, P, Ca) the results could be considered of the same order. For the minor elements (Mn, Fe, Zn), our results for manganese are slightly higher for Judia variety and double for the Longal variety, when compared with the results for the same varieties from different procedence. For zinc, the value in Judia is higher and the same for Longal, except when compared with the same variety from "Soutos da Lapa", also from the north of Portugal. Our result for iron is about half for Judia and in the middle for Longal, considering the maximum value from "Soutos da Lapa" and the minimum for "Galicia" region.

Taking into account the different origin of chestnut samples and limitations of each technique used by the authors, PIXE or atomic absorption and UV–VIS, it could be highlighted the similarity of the results. As previously referred, the main advantage of using PIXE was the possibility of simultaneously detect and quantify a higher number of elements with relevant physiological functions, and to detect the presence of contaminants or toxic elements.

Concerning the shells (outer and inner), some authors have measured and reported their bioactivity and biochemical profile [29,30] but, from our knowledge, nothing was reported before in the literature related with shells mineral content for *Castanea sativa* Miller varieties. Therefore, it was not possible to compare our results due to the lack of available data in the literature. The different concentrations of elements in shells compared with fruits could be explained by the different physiological processes, during fruit growth, and their different role in the plant, where the shells should protect fruits growing, more the outer than the inner.

Till the moment, shells are not used directly for human consumption but may be incorporated, after processed, in other food supplements, which makes relevant data on their composition. Moreover, the fruits, including the shells, have been used for animal feeding, and this compositional information is also important, since the presence of heavy metals in these types of food is also regulated [31].

With the limitation of fruits species, variety and origin, our results with PIXE for chestnuts have some similarities with the results obtained by other authors using the same technique for biological samples. In the analysis of soybeans with PIXE, Medeiros et al. [26] were able to identify several elements in some conventional and genetically modified products and observed the same elemental composition for all food samples (Br, Ca, Cl, Cr, Fe, K, Mg, Mn, Na, Ni, P, Rb, S and Zn), but the comparative analysis suggested that products genetically modified have higher concentration values for the majority of the elements that could also be due to different cellular mechanisms that are involved in these plants for the absorption of heavy metals [32].

Some of the elements detected by PIXE in these fruits are classified as potentially toxic heavy metals for humans as As, Sb, Cd, Hg, Pb and Al. Inorganic arsenic can produce toxicological effects in human even at low concentrations, being food the main contributor to human arsenic intake [33]. Similarly, lead can induce biochemical effects as neurological problems or haematological effects among others [34]. Nevertheless, the concentrations found are very low, can be either compatible or below the LOD of the PIXE system and below the considered toxicological level [35]. The relation between elements concentration and toxicity is not direct, since it should be taken into account several metabolic mechanisms, the absorption by human body after ingestion, its elimination and the interaction with other elements. This is an object of concern of the regulatory food agencies, namely the European Food Safety Agency which regularly publishes and updates the daily adequate intakes.

Further studies with other chestnuts varieties will contribute to better understand the observed differences and its relation with external factors, such as atmospheric or soil contamination, industrial pollution or inadequate use of fertilizers.

4. Conclusion

By PIXE it was possible, for the first time, to detect and quantify up to 20 elements in two *Castanea sativa* Miller varieties (pericarp and episperm shells and fruits). The highest concentration for both fruit cultivars is potassium (K), and for the shells is calcium (Ca). The higher Ca and P concentration was measured in Longal variety inner shell, while the higher value of K was measured in Judia variety outer shell. K and P concentrations are higher in Longal fruits, with Ca concentration similar for both varieties. The use of PIXE also allowed detect and quantify the presence of other minor and trace elements, which were not referred in previous studies: Rb, Pb, Ba, Ti, Sr, Br, As, Ni, Cr.

Table 4
Elemental composition values of chestnuts varieties compared with data available in the literature (§ our data).

	Cultivar	wt.% K	P	Ca	S	Cl	Si	Al	Mg	Na	Ref.
Portugal	**Judia**	**0.670**	**0.065**	**0.060**	**0.055**	**0.013**	**0.010**	**0.009**	–	–	§
Padrela	Judia	0.905	0.130	0.041	–	–	–	–	0.067	0.095	[5]
Portugal	**Longal**	**0.811**	**0.087**	**0.058**	**0.050**	**0.006**	**0.5**	**0.017**	–	–	§
Galicia	Longal	0.915	0.068	0.026	–	–	–	–	0.049	0.005	[7]
Soutos da Lapa	Longal	0.673	0.132	0.045	–	–	–	–	0.072	0.076	[5]

	Cultivar	µg/g Mn	Fe	Zn	Rb	Pb	Cu	Ba	Ti	Sr	Br	As	Ni	Cr	Ref.
Portugal	**Judia**	**67**	**51**	**27**	**15**	**10**	**7**	**5**	**5**	**3**	**2**	**2**	**2**	**1**	§
Padrela	Judia	56	109	14	–	–	19	–	–	–	–	–	–	–	[5]
Portugal	**Longal**	**122**	**39**	**15**	**15**	**6**	**10**	**7**	**n.d.**	**3**	**1**	**3**	**4**	**2**	§
Galícia	Longal	57	23	14	–	–	9	–	–	–	–	–	–	–	[7]
Soutos da Lapa	Longal	68	68	31	–	–	16	–	–	–	–	–	–	–	[5]

n.d. not detected.

PIXE was confirmed as a useful tool for the analysis of biological samples, with the detailed information added by this study, giving a contribute for a better characterization of a food item that is part of the human diet in several countries and highlighting the potential add-value benefits of by-products such as chestnuts inner and outer shells.

This work opens new questions for the future that need to be investigated. The use of a broad proton beam and powdered samples allows establishing fine average concentration results on account of losing more detailed information related to the elemental distribution in the different tissues of the fruit (embryo, endosperm, etc.). This distribution can be obtained by PIXE when using a proton beam with micrometer dimensions. Another open question is the origin of the heavy metals in the fruit, which can be partially resolved by a compositional analysis of the water, soil or the fertilizers used, although the atmospheric contamination role should not be discarded, particularly for the external shell.

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