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Effect of hot air convective drying on sugar composition of chestnut (*Castanea sativa* Mill.) slices

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Abstract

The main objectives of the present work were as follows: (a) to develop an analytical procedure to quantify free sugars on chestnuts based on green-chemistry principles and (b) to evaluate the effect of hot air convective drying (50 °C along 10 hr) on the carbohydrate profile of sliced chestnuts (Longal and Judia varieties). The analytical method allowed the quantification of several sugars in chestnut extracts, with low detection limits and good precision. In fresh, Judia variety had higher sucrose, fructose, and glucose contents than Longal, together with amylose. Nevertheless, when applying hot air convective drying to chestnut slices, a significant increase in fructose contents was observed with time, particularly after 10 hr drying in Longal variety. Nonetheless, a significant increase in glucose content was observed on both varieties, probably related to starch thermal hydrolysis.

Practical applications

An environment-friendly methodology for free sugars quantification, based on ethanol/water extraction (green solvents), ion-exchange high-performance liquid chromatography separation with water as mobile phase, and evaporative light scattering detection, was developed. The method allowed the quantification of raffinose, sucrose, glucose, and fructose in chestnut extracts, with detection limits of 6 μg for sucrose and 0.3 μg for other sugars. Good precision was achieved with intra-day and inter-day coefficients of variation below 5%. Regarding chestnuts' drying, hot air convective drying might be applied to chestnut slices but it may induce modifications on the carbohydrate composition of the samples, particularly when long drying periods are applied, requiring some attention because it might induce both visual and sensory changes.

1 | INTRODUCTION

Portugal is one of the major worldwide producers of chestnut (*Castanea sativa* Mill.) with 19100 MT (FAO, 2015), with high importance for the country's trade balance. Carbohydrates are the major nutrients of chestnuts, especially starch, followed by sugars, namely sucrose, glucose, and fructose (Desmaison & Adrian, 1986). Indeed, free sugars are one of the most important parameters for the commercial quality assessment of this fruit, since consumers prefer the sweetest fruits. However, free sugar content and composition can vary along storage due to the effects of temperature, relative humidity, harvesting time, oxygen level or packaging (Kazantzis, Nanos, & Stavroulakis, 2003). Moreover, the free sugar composition can be influenced by variety, genotype, ecological conditions, or technical and cultural practices (Barreira, Pereira, Oliveira, & Ferreira, 2010).

Previous studies published on chestnut sugar quantification used diversified methodologies (Supporting Information Table S1). Generally, the most common extraction solvents are aqueous solutions of ethanol and methanol, followed by high-performance liquid chromatography (HPLC) with refractive index detection (RID). Separation is usually achieved with amino (NH_2), CarboPac PA20, and Sugar-Pak columns, using isocratic acetonitrile:water mixtures as mobile phase. Even though the application of green solvents such as water has been exploited in some matrices, none was found on chestnuts.

Concerning postharvest technologies, drying is one of the most traditional preservation techniques for fruits, including chestnuts. Therefore, several studies dealing with chestnut drying are found in the literature but most have focused on the drying process itself, with few reporting the effect on chestnuts chemical properties. Still, the study performed by Fernandes, Guiné, and Correia (2005) presented some

evidence of drying effects (at 70, 80, and 90 °C) on total protein and reducing sugars. Also, Attanasio, Cinquanta, Albanese, and Di Matteo (2004) and Correia, Leitão, and Beirão-da-Costa (2009) studied the effect of drying at different temperatures (40/60 and 40–70 °C, respectively), with reported modifications at the end of the drying process, mainly in total protein content, starch fraction, and some sugars. However, little information on chemical modifications along chestnuts drying time is available in the literature. Moreover, all studies have been performed in whole fruits or chestnut flours. Therefore, this work had two main objectives: (a) to develop an analytical methodology for free sugars quantification in chestnuts based on green chemistry principles and (b) to evaluate in detail the effect of hot air convective drying on free sugars and starch composition of sliced chestnuts, along the drying process, with the aim of producing a low caloric and gluten free appetizer in the future.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Sucrose, fructose, glucose, raffinose, and rhamnose were obtained from Sigma (St. Louis, MI). Water was treated in a water purification system (Pro90CN; Seralpur, Ransbach-Baumbach, Germany). The amylose/amylopectin kit was obtained from Megazyme (Wicklow, Ireland). All other chemicals were of analytical grade from diversified suppliers.

2.2 | Sampling

Two varieties were used in this study, Longal and Judia, acquired directly to chestnut producers of Macedo de Cavaleiros and Vinhais (Bragança, NE Portugal), respectively, in November 2012, and stored in cold chambers (4 ± 1 °C) until the drying experiments.

2.3 | Drying experiments

The exterior shell was removed with a knife, followed by slicing to approximately 4–6 mm thickness. Then, around 150 g of chestnut slices portions were dried in a tray dryer (Armfield, Ringwood, England), at 50 °C, under an air velocity of 1.2 ± 0.1 m/s. After drying for 1, 2, 4, 6, 8, and 10 hr, always in triplicate for each variety, the samples trays were cooled, reweighted for water loss calculation, frozen, freeze-dried (ScanVac; CoolSafe, Lynge, Denmark), and ground (WERKE, M20; IKA, Staufen, Germany). Fresh chestnuts were used as control, corresponding to time 0. Based on the moisture content, all results were expressed on dry basis.

2.4 | Color

The color of chestnut slices was determined during the drying experiments by a Minolta CR-400 colorimeter in CIELab color space, through the coordinates: L^*, a^*, and b^*, using the Spectra Magic Nx software (version CM-S100W 2.03.0006; Konica Minolta Company, Tokyo, Japan) described in a previous study (Delgado, Pereira, Baptista, Casal, & Ramalhosa, 2014). In order to analyze the color changes along the drying process, this parameter was determined at the beginning (the color of fresh chestnuts was considered as reference) and after the drying process on 60 slices. So, the ΔL^*, Δa^*, and Δb^* were determined by the difference between the values at the end and the beginning of the drying process. Moreover, the total color difference (ΔE) was calculated according to

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

2.5 | Sugar extraction

Dry samples (300 mg, in duplicate) were mixed with the internal standard (rhamnose; 50 mg/mL; 200 μL) and let to hydrate and swell with 5 mL of water:ethanol solution (20:80, vol/vol) for 30 min in a vortex. For increased cell disruption and extractability, the tubes were placed in an ultrasound bath for 5 min (S60h; Elmasonic, Singen, Germany), followed by 30 min in a water bath at 60 °C. The solutions were centrifuged at 2,500 rpm for 5 min at room temperature. The supernatants were transferred to a second vial, and the residue was further extracted with 5 mL of the same solution. Both supernatants were mixed together. Two milliliters of the supernatant were concentrated at 60 °C by nitrogen flushing until total ethanol removal. The solution was taken up in 1 mL of ultra-pure water, mixed in a vortex, transferred to an Eppendorf, centrifuged at 13,000 rpm for 15 min at 0 °C, and filtered through 0.22 μm Nylon filters before injection. Each sample was extracted in duplicate.

2.6 | Sugars identification and quantification by HPLC-evaporative light scattering detection (ELSD)

Extracted free sugars were analyzed in a Jasco integrated high performance liquid chromatographic system (Tokyo, Japan), equipped with an autosampler (AS-2057 Plus), a PU-980 intelligent pump, coupled to an evaporative light scattering detector (ELSD) (Model 75; Sedere, Olivet, France). The HPLC system was equipped with a SUPELCOGEL Ca column (300 mm ×7.8 mm; Supelco, Bellefonte, PA) operating at 80 °C. The mobile phase was ultra-pure water at a flow rate of 0.7 mL/min. The optimized detector temperature and gas pressure were 40 °C and 2.4 mbar, respectively. The results were expressed on g/100 g (dry matter), calculated by internal normalization of the chromatographic peak area and application of individual calibration curves. Each sample extract was injected twice. Sugar identification was made by comparing the relative retention times of sample peaks with those of standards, standard addition, and literature data.

2.7 | Starch and amylose contents

The starch and amylose contents were determined by application of the Megazyme kit (K-AMYL 07/11; Megazyme). The principle of this kit is that amylopectin complexes with lectin concanavalin (Con A), while the primarily linear amylose component is not able to complexes with it. Moreover, the total starch is hydrolyzed to D-glucose and measured colorimetrically by glucose oxidase/peroxidase. In

parallel, the total starch was extracted and determined by the procedure also described on the Megazyme kit, using the starch standard included in it.

The results were expressed as starch percentage (by dry matter), amylose percentage in the starch (g/100 g starch) and amylose percentage (g/100 g dry matter).

2.8 | Statistical methods

The effect of drying time and the influence of cultivar on sugar and starch contents were evaluated by the two-way analysis of variance (ANOVA) ($p < .05$), followed by the Tukey HSD post hoc test, because normality was observed and the variances of the groups were identical. The normality and variance homogeneity were previously evaluated by the Kolmogorov–Smirnov and Levene's tests, respectively. A principal component analysis (PCA) was also performed to evaluate the effect of the drying time on the sugar composition of both varieties, as well as to differentiate them. Moreover, Pearson correlation coefficients (r) were determined to evaluate the relationships between the analysed properties. The level of the significance used for all of the statistical tests was 95%.

3 | RESULTS AND DISCUSSION

3.1 | Figures of merit of the proposed analytical methodology for sugars quantification

3.1.1 | Peaks resolution

Four sugars were separated and quantified, namely, sucrose, raffinose, fructose, and glucose. Good baseline separation between the sugar peaks was observed on both standards (Figure 1a) and sample solutions (Figure 1b), eluting from the largest molecule (6.7 min; raffinose - trisaccharide), followed by the main disaccharide sucrose (7.5 min), and then the monosaccharides glucose (9.0 min) and fructose (10.7 min).

The internal standard (rahmnose) eluted between glucose and fructose.

3.1.2 | Linearity

Under the described experimental conditions, good linear relationships between sugars concentration and peak area were obtained, with correlation coefficients (r) greater than 0.99 for all sugars (Table 1). This linearity was maintained over the concentration range tested of 1.0–75 mg/mL for sucrose and 0.06–7.50 mg/mL for raffinose, glucose, and fructose.

3.1.3 | Detection limits (LOD) and quantification limits (LOQ)

The detection limits (LODs), based on a signal-to-noise ratio of three ranged from 0.3 mg/mL for sucrose to 0.015 mg/mL for the other sugars, corresponding to 6 and 0.3 μg of injected compound. The quantification limits (LOQs), corresponding to 10 times the baseline noise, ranged from 1.0 mg/mL for sucrose to 0.06 mg/mL for raffinose, glucose, and fructose (equivalent to 20 and 1 μg of injected compounds). Our LODs and LOQs are similar to those reported by other authors when using refractive index detection and other chromatographic separation mechanisms (Supporting Information Table S1).

3.1.4 | Intra-day and inter-day precisions

The precision of the method was assessed by determining the repeatability (intra-day variability) and intermediate precision (inter-day variability). The coefficient of variation (CV) for six repeated analyses of the same sample in the same day (intra-day precision) is shown for each sugar in Table 1, together with the inter-day variability (intermediate precision) performed in two different days. The results showed that both the intra- and inter-day CVs were <5%. Our results were similar to other authors, who used more complex extraction methodologies, including fat clean-up. Also, there was no necessity to regenerate the column through the entire work, with peak shape, retention time, and column response being constant through time.

3.1.5 | Recovery studies

The accuracy of the extraction method was confirmed by recovery experiments. All analyses were carried out in quadruplicate at two different sugar standard additions (10 and 20%), before the entire analytical sequence. The recoveries were calculated based on the difference between the total sugars amount determined in the spiked samples and the amount observed in the nonspiked samples, ranging from 100.8 ± 2.0 to 104.9 ± 0.8% and 99.9 ± 1.5 to 104.5 ± 0.5% for the additions of 10 and 20% of the sugar standards, respectively. These results are generally better or within those reported for the same matrix (Barreira et al 2010; Bernárdez, Miguélez, & Queijeiro, 2004). The use of an internal standard, as proposed previously by other authors, contributed to the results achieved, including the uniformization of the detector response through time.

3.2 | Effect of drying on sugar composition of chestnut slices

The results obtained for starch, amylose, and free sugar contents of the two chestnut varieties, Longal and Judia, along the drying time, are detailed in Figure 2. Significant interactions between variety and drying time were observed ($p < .05$), except for the starch content. Each component will be discussed separately.

3.2.1 | Starch

Starch is the predominant organic component of chestnuts. Fresh chestnuts ($t = 0$ hr) contained 56.0 g starch/100 g and 51.6 g starch/100 g dry matter, respectively, for Longal and Judia varieties. These results were in agreement with those published by Künsch et al. (2001), Ertürk, Mert, and Soylu (2006), Pereira-Lorenzo, Ramos-Cabrer, Díaz-Hernández, Ciordia-Ara, and Ríos-Mesa (2006), Míguelez, Bernárdez, and Queijeiro (2004), Borges, Gonçalves, Soeiro de Carvalho, Correia, and Silva (2008), and de Vasconcelos, Bennett, Rosa, and Ferreira-Cardoso (2009) (38.6–81.7 g starch/100 g dry matter).

Along drying small variations on starch content were observed but, when compared with $t = 0$ hr, no significant differences were observed

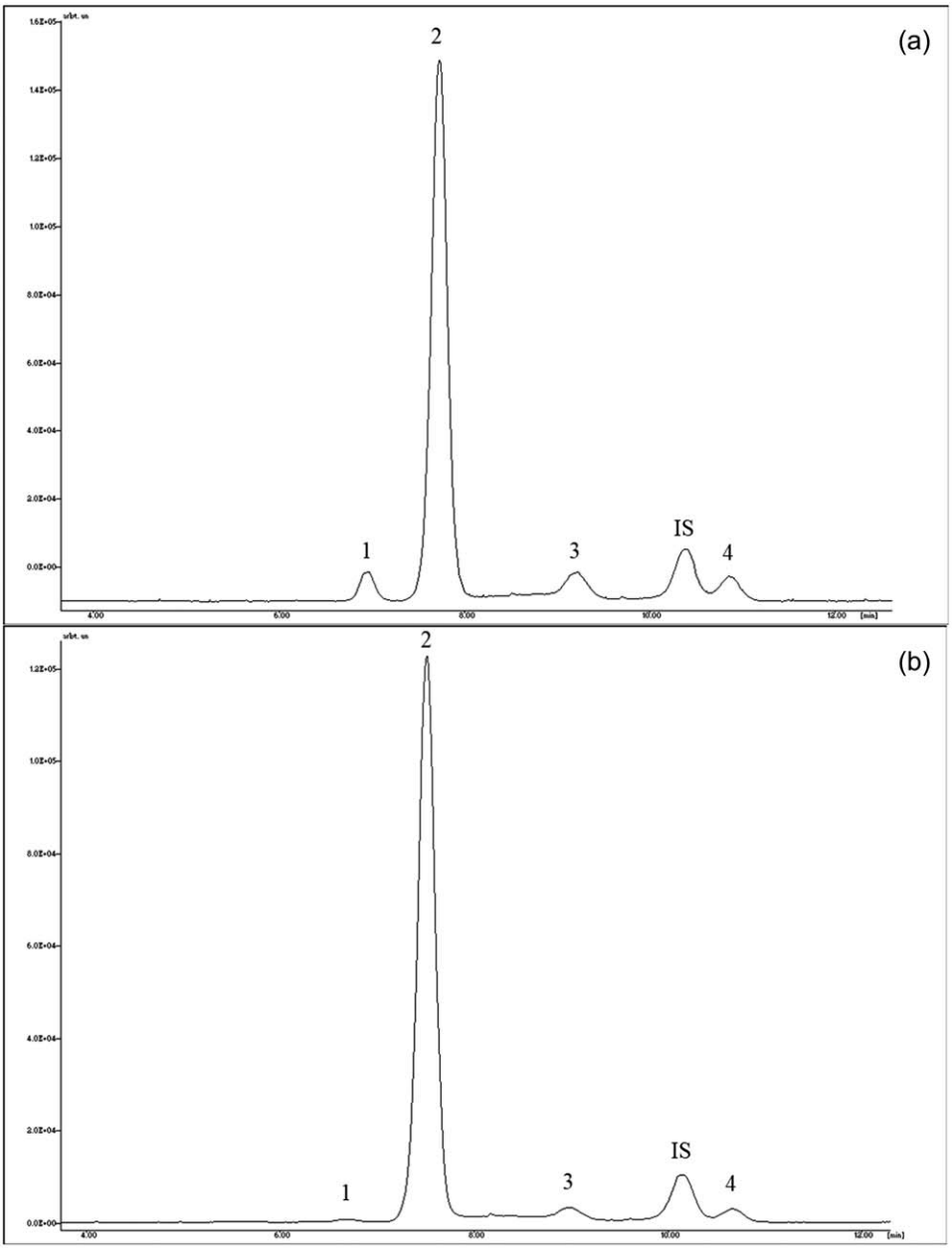

FIGURE 1 Chromatogram of sugars standards (a) and of a chestnut sample (b). Peaks: 1, raffinose; 2, sucrose; 3, glucose; 4, fructose; IS, internal standard (rahmnose)

for both varieties. Still, Longal variety had slightly higher starch contents than Judia through the entire process (with the exception of 4 hr of drying), again with no statistical significance.

Regarding amylose, significant differences were found between both varieties along the drying time, having Judia variety most of the time higher amylose contents than Longal. At the beginning (t = 0 hr),

TABLE 1 Figures of merit of the proposed methodology

Sugars	Retention time (min)	r	LOD (mg/mL)	LOQ (mg/mL)	Precision (CV [%], n = 6)	
					Intra-day	Inter-day
Raffinose	6.7	0.998	0.015	0.06	2.1	2.8
Sucrose	7.5	0.995	0.31	1.0	2.7	4.4
Glucose	9.0	0.999	0.015	0.06	3.3	5.4
Fructose	10.7	0.998	0.015	0.06	3.9	3.0

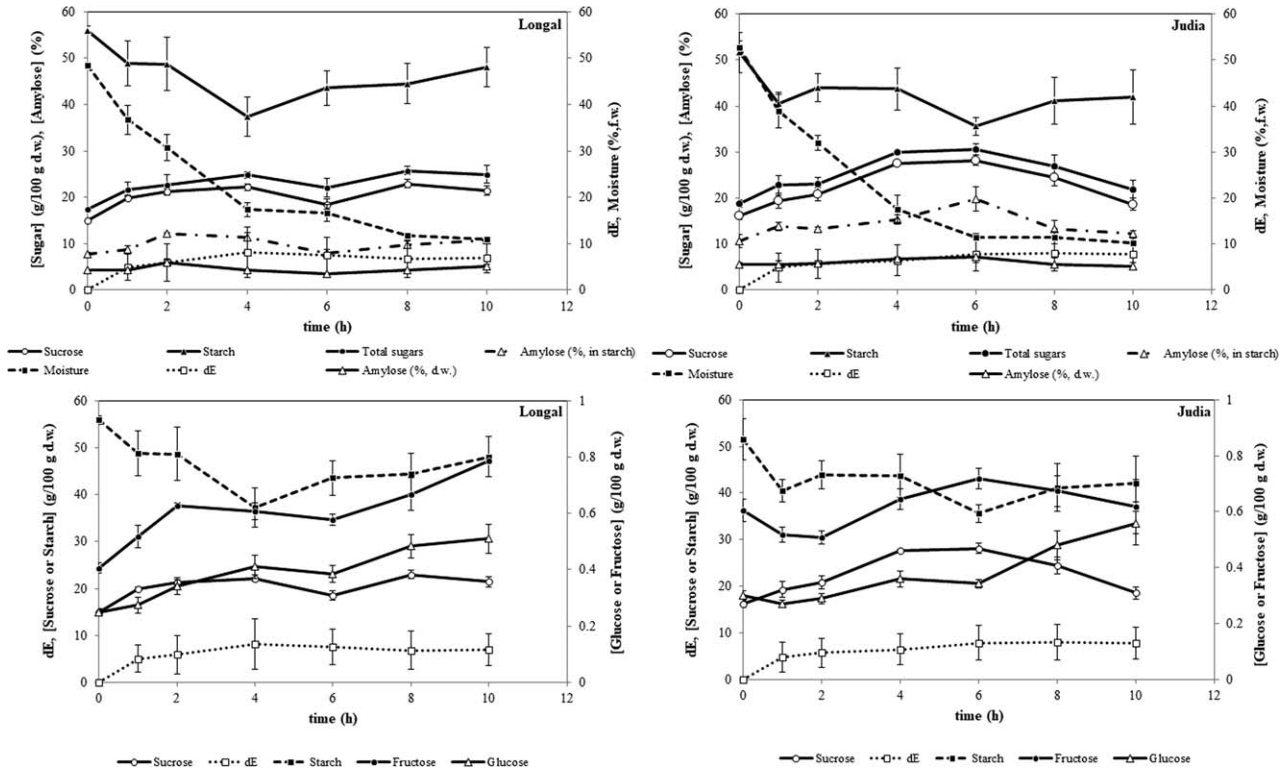

FIGURE 2 Evolution of sugar contents, color variation, and moisture of chestnuts of two varieties, Longal and Judia, during drying

Longal accounted with 4.33 g of amylose/100 g dry matter (or 7.7 g amylose/100 g starch), while Judia had 5.46 g of amylose/100 g dry matter (or 10.6 g amylose/100 g starch). These values were lower than those published by Attanasio et al. (2004) (32.9 g amylose/100 g starch) and Correia and Beirão-da-Costa (2012) (32.4–33.4 g amylose/100 g dry starch); however, these differences can be related with plant variety or cultivar, but mostly to growth conditions and distinct harvesting periods (Copeland, Blazek, Salman, & Tang, 2009; Kaur, Singh, & Singh, 2006). On the present work, even though some variation was observed on the amylose percentage regarding starch along drying, no significant modifications were detected in starch functionality after 10 hr of thermal treatment, supported by the inexistence of significant variations of amylose/starch ratio at the end when compared with the beginning.

3.2.2 | Sucrose

Sucrose is the main free sugar in chestnut, being therefore highly implicated in its perceived sweetness. Fresh chestnuts ($t = 0$ hr) had slight differences on sucrose contents between both varieties, with 15.0 g/100 g dry matter for Longal and 16.2 g/100 g dry matter for Judia. These contents were within the range of other authors (Barreira et al., 2010; Bernárdez et al., 2004; de Vasconcelos et al., 2010; Ertürk et al., 2006; Künsch et al., 2001; Míguelez et al., 2004) for different varieties and origins, but a high variability is found (3.7–24.2 g/100 g). The differences on sucrose concentrations observed in those studies and our work are probably explained by the influence of edaphoclimatic conditions that might affect the biochemical properties of chestnuts (Dinis et al., 2011) or due to other factors such as different origins of chestnuts of the same variety and differences in ripening stage (Bernárdez et al., 2004). Moreover, as shown by de Vasconcelos et al. (2010), slight differences may be found on sugar composition of chestnuts harvested in different years. As our samples were from similar locations and were harvested at the same time, the influence of edaphoclimatic conditions and maturity was similar, justifying the reduced variability observed between the two different varieties.

Along drying time, a significant increase in sucrose content was verified until 4 hr for Longal, remaining constant thereafter, and until 6 hr for Judia, followed by a small decrease in the remaining time for the last variety up to a value similar to that from time 0 hr. On the other hand, a significant increase (43%) on the sucrose content was observed in Longal variety from the 4th hour forward. This increase was probably due to the contribution of maltose that would elute at the same retention time of sucrose, as it was expected a decrease in the sucrose content along drying due to its hydrolysis to glucose and fructose. Beyond glucose, maltose is a product of the starch catabolism (de Vasconcelos et al., 2010).

3.2.3 | Fructose

Regarding fructose, significant differences between the two varieties were only perceived for some drying times. At the beginning ($t = 0$ hr), fructose content was significantly lower for Longal (0.41 ± 0.02 g/100 g dry matter) than for Judia (0.60 ± 0.04 g/100 g dry matter). Both amounts were in agreement with those published by Künsch et al. (2001) (0.37–0.69 g/100 g dry matter) and Barreira et al. (2010) (0.57–5.32 g/100 g dry matter), although slightly higher than those published by Bernárdez et al. (2004) and Míguelez et al. (2004) (0.04–

TABLE 2 Correlations (linear correlation coefficients) between the analyzed parameters

	Moisture	ΔE^*	Starch	Amylose	Sucrose	Glucose	Fructose	Raffinose	Total sugars
Moisture	–	−0.894**	0.659*	−0.334	−0.667**	−0.820**	−0.728**	0.220	−0.734**
ΔE^*	−0.894**	–	0.531	0.130	0.293	0.710**	0.589*	−0.119	0.374
Starch	0.659*	−0.531	–	−0.669**	−0.660*	−0.333	−0.407	0.623*	−0.711**
Amylose	−0.334	0.130	−0.669**	–	0.753**	0.011	0.424	−0.695**	0.744**
Sucrose	−0.667**	0.293	−0.660*	0.753**	–	0.265	0.636*	−0.488	0.985**
Glucose	−0.820**	0.710**	−0.333	0.011	0.265	–	0.696**	−0.126	0.344
Fructose	−0.728**	0.589*	−0.407	0.424	0.636*	0.696**	–	−0.284	0.681**
Raffinose	0.220	−0.119	0.623*	−0.695**	−0.488	−0.126	−0.284	–	−0.508
Total sugars	−0.734**	0.374	−0.711**	0.744**	0.985**	0.344	0.681**	−0.508	–

*Correlation is significant at the .05 level.
**Correlation is significant at the .01 level.

0.31 g/100 g dry matter), and de Vasconcelos et al. (2010) (0.05–0.27 g/100 g dry matter). Concerning drying, an increasing trend was perceived for Longal variety, while no significant differences were obtained for Judia, showing again that Judia variety seemed to be less affected by the thermal process than Longal variety. The fructose increase might result from sucrose hydrolysis.

3.2.4 | Glucose

Concerning glucose, slight differences were found between both varieties on fresh chestnuts ($t = 0$ hr), with a mean of 0.25 ± 0.01 g/100 g dry matter for Longal and 0.30 ± 0.02 g/100 g dry matter for Judia. These results were in accordance with de Vasconcelos et al. (2010), Míguelez et al. (2004), and Bernárdez et al. (2004) (0.04–0.30 g/100 g dry matter). Along drying, the two varieties presented similar amounts for most sampling times, but the accumulated increases were highly significant for both varieties (104% for Longal and 87% for Judia when compared with the beginning), being these also highly correlated with drying time.

The fructose/glucose proportions are also in accordance with Bernárdez et al. (2004) for Longal variety and with de Vasconcelos et al. (2010) for both varieties (Longal and Judia). In more detail, an increased activity of hydrolytic enzymes during the drying process might be expected (Correia et al., 2009), mainly, α-amylase, β-amylase, and glucoamylase, with optimum temperatures between 55 and 60 °C (Matherwson, 1998), partially hydrolyzing starch. Even though significant differences were not detected on the starch content when comparing the beginning with after 10 hr of drying, a decrease in the mean values was observed for both varieties, corroborating the hypothesis of starch catabolism. The increase in the glucose content could be directly correlated with this, as would probably be maltose, not separated from sucrose under our chromatographic conditions.

3.2.5 | Raffinose

Regarding raffinose (data not shown), fresh chestnut ($t = 0$ hr) had significant differences between varieties, with a mean of 0.87 vs. 0.51 g/100 g dry matter for Longal and Judia, respectively. These differences were preserved along drying, despite the small variations observed with time, with no pattern or significant differences for Judia variety, while for Longal, a significant decrease was apparent after 10 hr of drying (−23%).

3.3 | Relationship between sugars

In general terms, on what concerns sugar profiles a similar behavior was observed between both varieties along drying, as can be stated in Figure 2. The starch content slightly decreased due to starch catabolism, explaining the observed increase in glucose content. However, it was also expected an increase in maltose content. Nevertheless, as this compound elutes at the same retention time than sucrose, it might have contributed to the increase in sucrose content during drying. In fact, it was expected that sucrose might be hydrolyzed to glucose and fructose, explaining the observed increases on both compounds. These results were similar to the reported by de Vasconcelos et al. (2010) after industrial peeling by flame or fire (brûlage) at high temperatures (800–1,000 °C) during a small period of time (1–2 s). However, during roasting at 210 °C during 40–80 min, no significant variation was observed on fructose and glucose contents probably due to enzyme denaturation (Künsch et al., 2001).

Moreover, in the present work, as glucose and fructose contents increased, a higher color variation was stated due to the occurrence of Maillard reactions between reducing sugars and amino groups of amino acids and proteins. Significant correlations were found between color variation and the content of both monosaccharides (0.710 for glucose [$p = .01$] and 0.589 for fructose [$p = .05$]) (Table 2).

Regarding water loss, a fast decrease in moisture content was observed at the first hours of drying, requiring around 6–8 hr (Judia and Longal, respectively) to achieve a low and constant moisture content that will guarantee the stability of the product. Furthermore, very significant negative correlations were found between moisture content and color variation (–0.894), as well as between moisture content and glucose (–0.820) and fructose (–0.728), suggesting an increase in color variation and on glucose and fructose contents along drying, when the moisture content decreased.

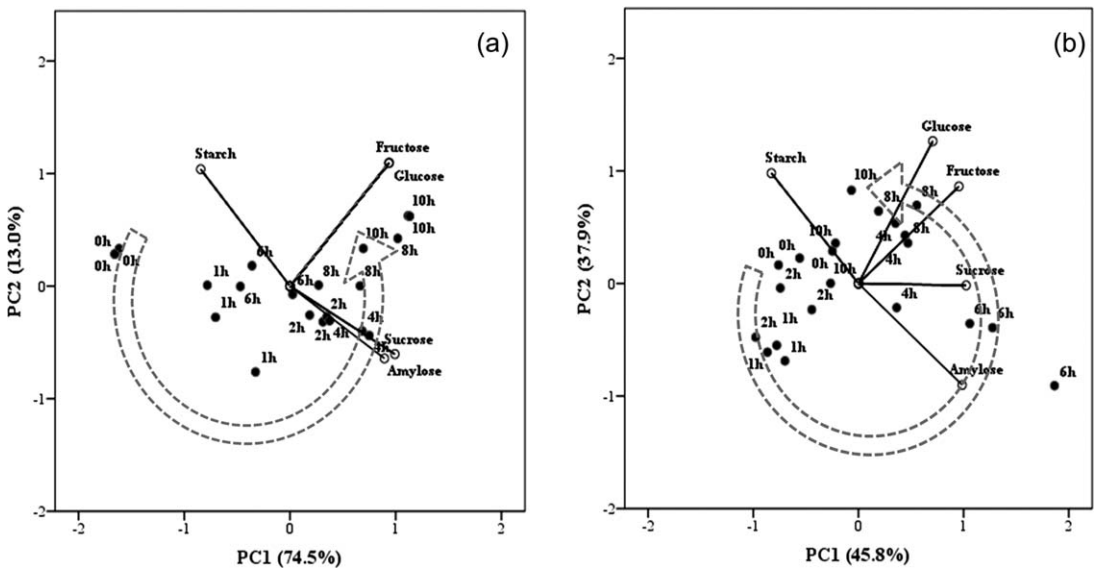

FIGURE 3 Loadings and scores plot resulting from the principal component analysis (PCA) of starch, amylose, and sugar contents: (a) Longal variety and (b) Judia variety

These facts were reinforced after performing a PCA to the free sugars, starch, and amylose contents, for both varieties (Figure 3a and b), where two principal components were extracted (PC1 and PC2) that accounted globally for 87.5 and 83.7% of the total variation for Longal and Judia varieties, respectively. For both varieties, it was observed that at the beginning of drying (0 and 1 hr) the samples presented the highest content of starch. With increasing time, it became more evident the strength of amylose and sucrose, while the monosaccharides were increased at the highest drying times. When both varieties (data not shown) were analysed together, their separation became less evident, indicating that the drying effect at 50 °C had a slight more important role than chestnut variety on these parameters.

4 | CONCLUSIONS

The present study described a greener analytical procedure of HPLC-ELSD for sugars determination on chestnuts, as no organic solvents were used in the mobile phase and only ethanol was used in the extraction procedure. The proposed methodology showed to be sensitive, reproducible and accurate, being a suitable method for routine determination of chestnut sugars. Additionally, this work presents for the first time the effect of convective drying on sugars profile of slices of two chestnut varieties, Longal and Judia. Even though significant differences were found at the beginning on sugar profile of both varieties, no significant effect on starch contents was observed. The drying process showed to have some effect on sugars profile on chestnuts because an increase in glucose and fructose contents was observed, probably due to starch hydrolysis that can also explain the color variation detected due to the occurrence of Maillard reactions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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