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Organic acid profile of chestnut (*Castanea sativa* Mill.) as affected by hot air convective drying

Drying influence on chestnut organic acids

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ABSTRACT

The objective of the present work was to evaluate the effect of hot air convective drying on the organic acid profile of chestnut. An extractive method, with internal standard, followed by HPLC-UV was validated, allowing the quantification of malic, ascorbic, citric, and fumaric acids with good precision and accuracy. Fresh chestnuts presented differences between varieties. The thermal process caused equivalent losses of ascorbic acid on both varieties, but higher losses of malic acid in Longal. Furthermore, fumaric acid contents increased on both varieties. Therefore, hot air convective drying affected the organic acid composition of chestnut slices, and this effect was varietal dependent.

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Introduction

Chestnut fruit is widely consumed worldwide since ancient times, being *Castanea sativa* Miller the most common species. "Trás-os-Montes" region is the main Portuguese producing area, with sociocultural and economic values contributing greatly to the trade balance of the region. Even though chestnuts are generally sold fresh in autumn, a significant part is stored under refrigeration and processed afterwards, mostly by freezing and drying. Different works had previously focused on the nutritional properties of raw chestnut fruits[1–6], but the effect of the most common preservation methods is not well established. Most studies performed on hot air drying of chestnuts have evaluated different technological aspects, including drying kinetics[7–10], energetic requirements[11], temperature effect on morphological and rheological properties of chestnut flours, rehydration effect[12–14], as well as on some chemical parameters (reducing sugars, starch, amylose, and damaged starch).[15–18]

Among chestnut bioactive components, organic acids' nature and concentration affect chestnut's nutritive value. Some organic acids such as citric, succinic, fumaric, and malic acids are essential for human metabolism. For example, malic acid shows antimicrobial activity against *Listeria monocytogenes, Salmonella Enteritidis*, and *Escherichia coli* O157:H7[19], while fumaric acid derivatives are effective against psoriasis[20] and in the prevention of diabetic cardiovascular diseases.[21] Ascorbic acid is a powerful antioxidant and essential against scurvy. Good results have also been achieved in hypertension treatment by Duffy et al.[22], after supplementing hypertensive patients with ascorbic acid. Oral administration of citric acid may also ameliorate ketosis and protect against the development of diabetic complications.[23] Nevertheless, until now no studies have detailed the effect of hot air drying on chestnut organic acids, with only some references to the cooking effect (roasting, boiling, and frying)[24–26], where higher temperatures

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are generally applied. Therefore, the aim of our work was to evaluate the effect of hot air convective drying of chestnuts at 50°C on the organic acid profile and their amounts.

Material and methods

Chemicals

Standards of L(+)-ascorbic acid, malic acid, citric acid, fumaric acid, and gallic acid were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade from diverse suppliers.

Plant material

Chestnut fruits (*Castanea sativa* Mill.) used in this study were acquired in November 2012 and stored in cold chambers (4 ± 1°C) until the drying experiments were carried out. Two varieties were used: Longal and Judia, acquired directly to chestnut producers of Macedo de Cavaleiros and Vinhais (Bragança, Northeast of Portugal), respectively.

Drying experiments

Chestnut exterior shell was carefully removed, and the fruits were sliced approximately with 4–6 mm thickness. Around 150 g of chestnut sliced portions were dried in a tray dryer (Armfield, Ringwood, England) at 50°C, at an air velocity of 1.2 ± 0.1 m s^{-1} for 1, 2, 4, 6, 8, and 10 h, until achieving the final moisture content of 100 g kg^{-1} of dry matter. After drying, samples were frozen, freeze-dried (ScanVac, CoolSafe, Lynge, Denmark), ground (IKA-WERKE, M20, Staufen, Germany), and stored (in the dark) until analysis. Fresh chestnuts were used as control, being directly frozen and freeze-dried. All drying experiments were performed in triplicate.

Organic acid extraction

The organic acid extraction was performed with meta-phosphoric acid (3%, w/v), according to the method described by Carocho et al.[27], with some modifications in what regards the addition of an internal standard and the application of a sequential extraction. In more detail, samples (500 mg, in duplicate) were mixed with 150 µL of gallic acid solution (1 mg mL^{-1}; internal standard) and 5 mL of meta-phosphoric acid (3%, w/v), being mixed for 30 min in a vortex. The supernatants were separated after centrifugation (Heraeus Sepatech, Am Kalkberg, Germany; 2500 rpm for 5 min at 20°C), and the solid residues were further extracted with 5 mL of meta-phosphoric acid (3%, w/v). Both supernatants were combined and a portion was filtered through 0.22 µm Nylon filters before analysis.

Organic acid identification and quantification

Organic acids were determined in a Jasco integrated system (Easton, USA) equipped with an autosampler (AS-2057 Plus), a PU-980 intelligent pump, coupled to an UV detector set at 215 nm (UV-975). Separation was performed on a C_{18} column (150 × 4.6 mm, 5 µm, Gemini NX, Phenomenex, Torrance, USA) operating at room temperature. The mobile phase was sulfuric acid (3.6 mM) at a flow rate of 0.7 mL min^{-1}. The organic acid identification was made by comparing the retention times of the sample peaks with those of standards, supported by literature data. The results were expressed on g kg^{-1} of dry matter, calculated by individual calibration curves for each organic acid with internal standard.

Statistical methods

The statistical analysis was performed on SPSS software (Version No. 20.0). Normality and variance homogeneity were evaluated by the Kolmogorov–Smirnov and Levene's tests, respectively. As the data set was well modelled by a normal distribution and the variances of the groups were identical, the effect of drying time and the influence of cultivar over organic acid contents were evaluated by the two-way analysis of variance ($p < 0.05$), followed by the Tukey HSD *post-hoc* test. A principal component analysis (PCA) was performed to the organic acid results of the two chestnut cultivars along drying time. The PCA score plot was used to differentiate cultivars and verify the effect of drying time on chestnut organic acids. Moreover, Pearson correlation coefficients (r) were determined to evaluate the relationships between compounds. The level of the significance used for all of the statistical tests was 95%.

Results and discussion

Peak resolution

Good separation between the peaks of the organic acid standards was observed (Fig. 1a). Malic acid was the first standard to elute, followed by ascorbic acid, citric acid, and fumaric acid. The HPLC-UV analysis of organic acids of both varieties (Longal and Judia) showed a similar qualitative profile (Fig. 1b), with the four organic acids referred above.

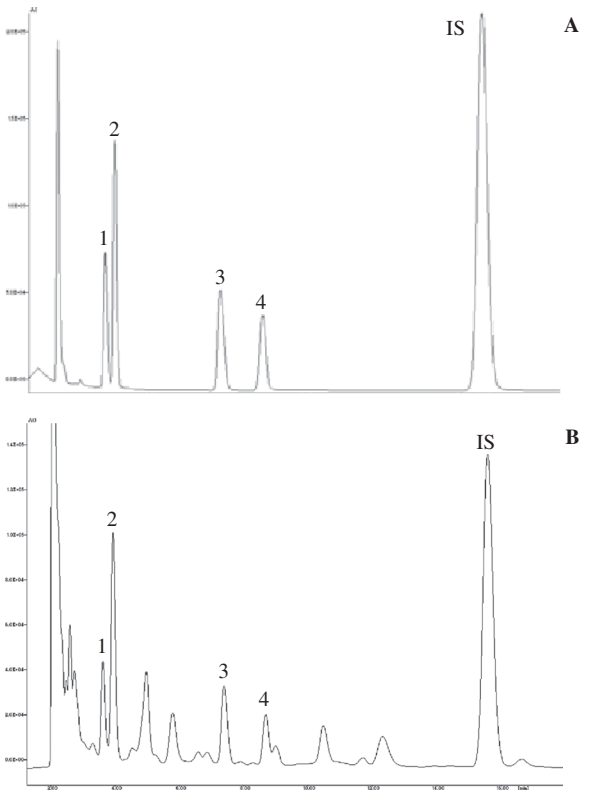

Figure 1. Chromatograms of organic acid standard solution (A) and of a chestnut sample extract (B). Peaks: (1) – malic acid; (2) – ascorbic acid; (3) – citric acid; (4) – fumaric acid; and (IS) – internal standard (gallic acid).

Linearity

Under the experimental conditions described, good linear relationships between organic acid concentrations and peak areas (determined taking into account the internal standard peak area) were obtained, with correlation coefficients (r) greater than 0.9997 for all organic acids (Table 1) under the tested concentrations range, adapted to sample concentration, of 0.1–2.0 mg mL^{-1} for malic and citric acids, 0.01–0.15 mg mL^{-1} for ascorbic acid, and 0.001–0.015 mg mL^{-1} for fumaric acid.

Intraday and inter-day precisions

The coefficient of variation (CV) for six repeated analyses of the same sample on the same day (intraday precision) is shown for each organic acid in Table 1. The inter-day experiments (intermediate precision) were performed for the same sample in two different days and repeated six times. The method showed intraday CVs in the range of 0.7–2.1% and inter-day CVs between 1.1% and 2.7%.

Recovery studies

The accuracy of the method was confirmed by recovery experiments. The recoveries were calculated based on the difference between the amounts quantified in the samples spiked before extraction at a 10% level and that observed in the non-spiked samples. All analyses were carried out in quadruplicate with recoveries ranging from 97.0 ± 1.5% to 101.0 ± 2.3%, demonstrating the accuracy of the proposed methodology.

Effect of drying on organic acid composition of chestnut slices

The results obtained for malic, ascorbic (vitamin C), citric, and fumaric acid contents for the two chestnut varieties along drying are shown in Fig. 2. In all conditions, significant interactions between variety and drying time were observed ($p < 0.001$).

Citric acid

Significant differences on the main organic acid were found between both varieties at time = 0 h, with a mean of 5.22 ± 17 g kg^{-1} dry matter for Longal and 9.55 ± 12 g kg^{-1} dry matter for Judia variety, persisting through all sampling times (Fig. 2a). These results were in accordance with Gonçalves et al.[25] (1.46–8.79 g kg^{-1} dry matter), also reporting differences between varieties. Small variations were observed along the drying time, stabilizing after 4 h and with a cumulative increase of 36% and 16% (by dry matter) for Longal and Judia varieties, respectively, after 10 h of drying. Gonçalves et al.[25] also reported an increase in the citric acid content (4.8–318%), especially after roasting at high temperatures, being this increase explained by probable occurrence of heat-induced reactions between nitrogen-free carboxylic acids and sugars that might cause an increase in organic acid content.

Table 1. Figures of merit of the proposed methodology.

			Precision (CV (%), $n = 6$		10% Standard added
Organic acid	Retention time (min)	r	Intraday	Inter-day	Recovery (mean ± SD)
Malic acid	3.6	0.9999	0.7	2.6	98.2 ± 1.5
Ascorbic acid	3.9	0.9997	0.7	1.1	97.0 ± 1.5
Citric acid	7.3	1.000	1.4	1.7	99.7 ± 1.7
Fumaric acid	8.6	0.9999	2.1	2.7	101.0 ± 2.3

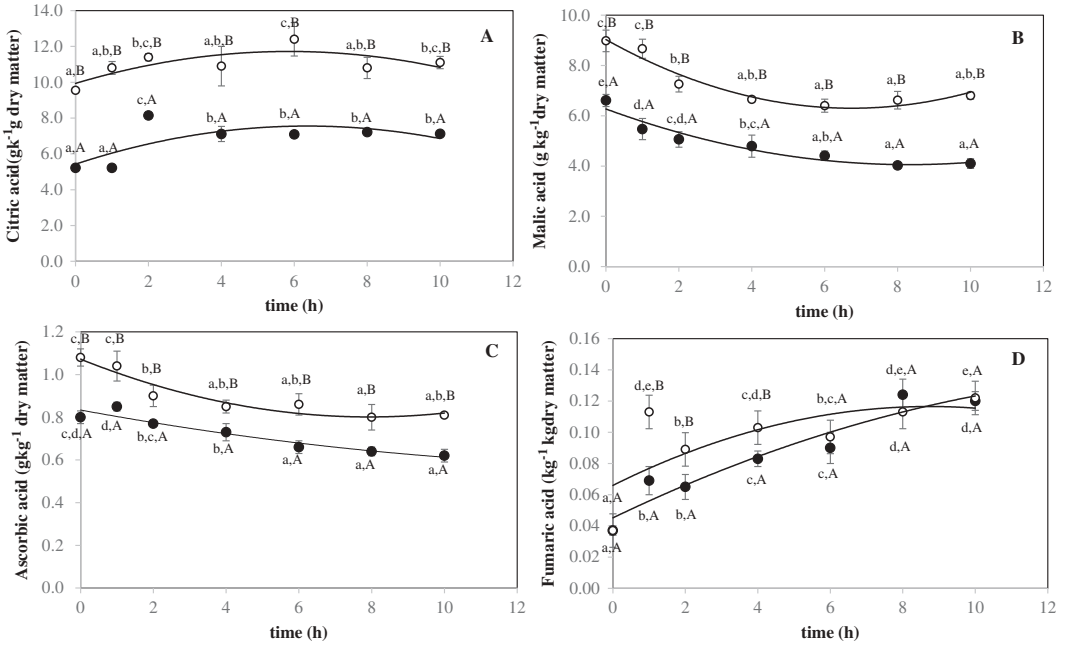

Figure 2. Drying behavior in terms of citric (A), malic (B), ascorbic (C), and fumaric (D) acids along drying time for Longal (·) and Judia (o) varieties.

Malic acid

Again, significant differences on malic acid contents were found between both varieties before drying, with lower average amounts in Longal (6.61 ± 24 g kg^{-1} dry matter) than in Judia variety (8.98 ± 43 g kg^{-1} dry matter). These results were slightly higher than those reported by Gonçalves et al.[25] (1.47–5.32 g kg^{-1} dry matter) and Neri et al.[28] (1.52–3.30 g kg^{-1} dry matter) for chestnut fruits from Portugal and Italy, respectively. These differences might be explained by edaphoclimatic conditions[29], ripening stage, etc.[30] Ribeiro et al.[24] also reported slight differences on organic acid profile for different chestnut cultivars harvested in the same region and year. Differences induced by the analytical methodology used are also not to be excluded.

Concerning the thermal process, Judia presented always higher amounts than Longal, but a similar decreasing trend was observed along drying (Fig. 2b), stabilizing after 4 h for Judia, and after 6 h for Longal. The decrease pattern presented a similar trend that could be approximated to a parabolic equation, yielding good determination coefficients (0.920 and 0.948 for Longal and Judia varieties, respectively). Total losses after 10 h of drying achieved 38% and 24% (by dry matter) for Longal and Judia, respectively. Ribeiro et al.[24] and Gonçalves et al.[25], when comparing raw and cooked chestnuts (after roasting, boiling, and frying), also observed a decrease on this organic acid content.

Ascorbic acid (vitamin C)

Significant differences were found between the two varieties through all sampling times, with Judia showing always higher amounts than Longal variety. Our initial contents (0.80 ± 3 g kg^{-1} for Longal and 1.08 ± 4 g kg^{-1} for Judia variety, dry matter) were in accordance with Neri et al.[28] (0.28–1.28 g kg^{-1} dry matter) and higher than those reported by Barros et al.[26] (0.40–0.693 g kg^{-1} dry matter) and Ribeiro et al.[24] (0.045–0.164 g kg^{-1} dry matter). These differences can probably be explained by the effect of several factors such as sunlight, maturity stage, postharvest processing, cultivar and storage[31,32], as well as the time delay between harvest and analysis.

Concerning drying, some fluctuation was observed on the mean values along time, again with a decrease and stabilization trend after 4 and 6 h for Judia and Longal, respectively. As observed in Fig. 2c, the two varieties presented again a similar behavior along drying time that could be approximated to a parabolic equation with good determination coefficients (0.929 for Longal and 0.898 for Judia), showing good fits. Significant differences were observed between the beginning (t = 0 h) and after 10 h of drying, with losses equal to 22% and 25% (by dry matter) for Longal and Judia varieties, respectively. Ribeiro et al.[24] and Barros et al.[26] also reported a decrease on the ascorbic acid contents when chestnuts were roasted, boiled, or fried. Barros et al.[26] reported losses on vitamin C contents around 25–54% for boiling and from 2% to 77% for roasting. Both processes correspond to more intense heating treatments than the one applied on the present work, also with increased losses on ascorbic acid contents.

Fumaric acid

Fumaric acid was the minor organic acid identified in both varieties, with no significant differences observed between them (0.037 g kg^{-1} dry matter). Along drying, an increase was observed on both varieties (Fig. 2d), following a similar parabolic behavior with good R-squares for Longal (R^2 = 0.956) and Judia (R^2 = 0.679). In more detail, Judia presented higher fumaric acid contents than Longal until 4 h of drying, but afterwards no significant differences were observed between varieties. In general terms, a similar increase was stated, corresponding to 224% and 230% (by dry matter) for Longal and Judia varieties, respectively. Some authors have referred that during heat processing (evaporation, pasteurization, and sterilization) of apple juices, the content of fumaric acid may increase slightly due to malic acid dehydration.[33] This process might have also occurred during chestnut drying with significant negative correlations observed between fumaric and malic acids for Longal (−0.916, $p < 0.001$) and Judia (−0.655, $p < 0.001$) varieties, corroborating this hypothesis.

Principal component analysis

After performing a PCA to the organic acid data for the two varieties (Fig. 3), two principal components were extracted (PC1 and PC2) that accounted for 92.6% of the total variation. According to the PCA score,

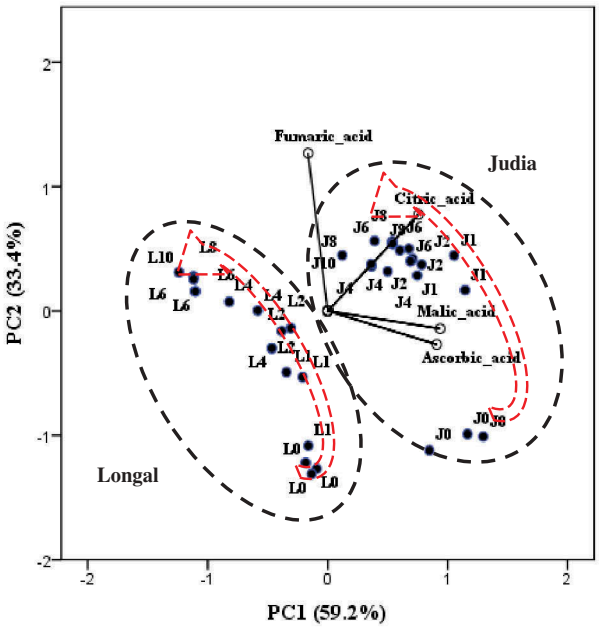

Figure 3. Loadings and scores plot resulting from the principal component analysis of organic acids for both varieties.

the two varieties were clearly differentiated from each other, whereas their organic acid profiles along drying time were not so well differentiated; however, at the beginning of the drying process (0 and 1 h), higher contents of malic and ascorbic acids were determined on both varieties, while after 10 h of drying, the fumaric acid increase had a predominant role. These results indicated that chestnut variety had a more important role than the drying process on the organic acid profile, even though clear differences along drying were observed.

Conclusions

The present study describes for the first time the effect of convective drying on organic acids of sliced chestnut, using two common varieties in Portugal: Longal and Judia. Chestnut variety had a more important role than the drying process on the organic acid profile, even though clear differences along drying were observed. In more detail, Judia always presented higher values of citric, malic, and ascorbic acids than Longal, while no differences on the fumaric acid (minor organic acid identified in both varieties) content were detected between varieties. Concerning drying, after 10 h at 50°C, there was an increase in citric and fumaric acid contents, being much higher in the last compound (a cumulative increase >200% (by dry matter) for both varieties). On the other hand, total losses between 22% and 38% were observed for ascorbic and malic acids on both varieties.

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