Osmotic dehydration effects on major and minor components of chestnut (*Castanea sativa* Mill.) slices

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Abstract The effect of osmotic dehydration (OD) conditions (temperature, time and sucrose concentration) on some nutritional parameters, soluble sugars, organic acids, fatty acids and vitamin E composition of chestnut slices was studied. Temperature at 60 °C and contact time of 7.5 h decreased significantly both protein (in 20 and 15%) and fat (in 25 and 20%) contents when compared to 30 °C and contact time of 2.5 h, simultaneously with the incorporation of sugars from the osmotic medium. An increase in temperature from 30 to 60 °C and contact time from 2.5 to 7.5 h also changed amylose percentage from 12 to 17 g/100 g of starch, suggesting modifications on starch conformation. Concerning organic acids, an increase in temperature from 30 to 60 °C induced thermal degradation of citric (54% of loss), malic (36% of loss) and ascorbic (23% of loss) acids. Temperature and sugar concentration did not affect significantly fat composition, particularly PUFA, the main fatty acid class, while contact times of 7.5 h led to the partial oxidation of linolenic acid (17% of loss when compared to 2.5 h). A 50% decrease was also observed on vitamin E content when temperature increased from 30 to 60 °C. Thus, OD might cause changes on the chemical composition of chestnut slices, requiring low temperature

and contact times to avoid loss of important bioactive components such as ω -3 fatty acids (ex. linolenic acid) and vitamin E.

Keywords *Castanea sativa* Miller · Osmotic dehydration · Proximate composition · Sugar profile · Organic acids composition · Lipid profile

Introduction

In ancient times, chestnut fruits were a staple food in Trás-os-Montes region (Portugal); however, this nut has been substituted by the potato. Nevertheless, chestnut fruits continue to be produced and consumed worldwide. One of the problems that farmers and chestnuts' processors have to face is that the production of chestnut is seasonal, being extremely important to create new products based on this nut, as a way to add economic value, increase its shelf-life and to valorise rejected fruits by the industry such as those with small size. In this way, several chestnut-products have emerged in the market such as *marrón glacé*, flours, creams, purees and jams, among others, in addition to the traditional consumption of the fruit roasted, boiled, fried or dried.

There are several post-harvest methods for long-term chestnuts preservation, being freezing and hot air convective drying the most common ones. Nevertheless, both methods promote loss of quality of the final product. For example, drying may increase hardness, while freezing needs very strict conditions to avoid partial or total spoilage of frozen products. Both processes cause flavour loss, colour changes and modify dramatically the rehydration capacity (Drouzas et al. 1999). In order to keep the quality of the product, osmotic dehydration (OD) is a post-harvest method for which interesting results have been reported

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(Rastogi et al. 2002). The popularity of OD is increasing, as an independent process or as a complementary technology in the integrated food processing chain. This method has been applied to whole chestnuts (Chenlo et al. 2006a, b, 2007; Moreira et al. 2007, 2008, 2011a, b), with particular attention to weight reduction (WR), solids gain (SG), water loss (WL), normalized moisture content (NMC), effective coefficients of diffusion for water (D_w) and solids (D_s). The possibility of using it on a sliced presentation could offer an interesting gluten-free low caloric snack, increasingly pursued by consumers. Nowadays, these are looking for healthier alternatives to the more common fat-rich snacks such as chips and fried peanuts with salt.

Osmodehydrated fruits show to have good taste, colour, texture and odour. However, loss of nutritional value has been described during OD of some fruits, as papaya and gooseberry fruits, because some hydrosoluble compounds such as organic acids, may flow from the fruit to the osmotic solution or may suffer chemical deterioration (Germer et al. 2014; Kucner et al. 2014). As OD is a method that can be affected by many factors such as concentration and composition of osmotic solution, temperature and contact time, among other factors (Chenlo et al. 2007; Moreira et al. 2007, 2011b; Singh et al. 2007; Tonon et al. 2007), it is necessary to evaluate their role on the food properties. A preliminary work on OD of chestnut slices performed by our research group pointed out that sucrose concentration, temperature and time have effect on some chestnut properties such as colour, ash, crude fat and moisture contents. Nevertheless, the role of OD on chestnut sugars composition, lipid fraction and organic acids was not evaluated.

As so far, no studies have been performed on the impact of the OD process conditions on the nutritional properties of sliced chestnuts, the aim of this work was to study the influence of temperature, time and sugar concentration on the proximate composition (major components), organic acids, sugar and lipid profiles (minor components) of chestnut slices. In more detail, the chestnut properties evaluated included protein, fat, NDF, ADF, starch, amylose, free sugars (sucrose, glucose, fructose, raffinose) and organic acids (malic, ascorbic, citric and fumaric acids) contents, as well as the lipid profile, including its individual fatty acids and vitamin E (γ -tocopherol, δ -tocopherol and α -tocopherol).

Materials and methods

Chemical

Amylose/Amylopectin Kit was obtained from Megazyme (Wicklow, Ireland). All standards used on the

quantification of sugars and organic acids, as well as for the determination of the lipid profile were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade from diversified suppliers. Concerning standards, α -, γ -, δ -tocopherols standard solutions (5 mg/mL) were prepared in ethanol and kept at -20°C . Their accurate concentrations were evaluated by UV spectrophotometry according to their molar absorptivity values. Dilutions in *n*-hexane were performed as required for calibration or other purposes. The internal standard for vitamin E quantification was tocol (2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol), obtained from Matreya Inc. (PA, USA). A 100 $\mu\text{g/mL}$ solution was prepared in *n*-hexane and kept at -20°C . Triundecanoin was used as the internal standard for fat estimation, based on the total fatty acid amounts, and was purchased from Sigma (St. Louis, MO, USA). A commercial standard solution with 37 fatty acid methyl esters (FAME) was used for the calibration of the FID signals (Supelco 37 FAME mix, Sigma, Bellefonte, USA).

Plant material

Castanea sativa Miller (European chestnut) fruits, variety Longal, were acquired in Bragança (NE Portugal) in November 2013, and stored in cold chambers ($4 \pm 1^\circ\text{C}$) until the OD experiments were carried out. Before performing these experiments, the chestnuts were carefully unshelled and sliced (approximately 4–6 mm of thickness).

Osmotic dehydration (OD)

The osmotic solutions were made with food-grade sucrose and potable water. The following parameters were tested: temperature (30, 45 and 60°C), time (2.5, 5.0 and 7.5 h) and sugar concentration (60, 70 and 80%, w/v). The temperatures at 30, 45 and 60°C were chosen because most of the studies on OD of chestnuts performed until now, have used temperatures between 25 and 65°C (Chenlo et al. 2006a, b, 2007; Moreira et al. 2007, 2008, 2011a, b). Lower temperatures will imply very long processes and the use of higher temperatures than 65°C may possibly lead to the darkening of the samples.

The OD experiments were carried out in 1L beakers. For each condition, 70 g of fresh sliced chestnuts and 700 mL of sugar solution were mixed with a magnetic stirrer at 310 rpm, being the temperature controlled in a water bath. After each experiment, chestnut slices were drained and cleaned gently with absorbent paper to remove the sugar solution in excess. For each condition, the assays were performed in triplicate. After OD, the samples were frozen, freeze-dried (ScanVac, CoolSafe, Lynge, Denmark) and ground (IKA-WERKE, M20, Staufen, Germany).

Proximate composition

The samples were analysed for proximate composition (proteins and fat), following AOAC procedures (AOAC 1995). Crude protein content of the samples was estimated by the macroKjeldahl method (VELP SCIENTIFICA, Usmate, Italy), using a conversion factor of 5.3 (de Vasconcelos et al. 2009); and crude fat was determined by extracting 5 g of sample with petroleum ether for 24 h, using a Soxhlet apparatus (P Selecta, Abrera, Barcelona). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method described by Goering and Van Soest (1970). All results were expressed on percentage (g/100 g dry matter).

Free sugar analysis

Freeze-dried samples (300 mg, in duplicate) were mixed with the internal standard (rhamnose; 50 mg/mL; 200 μ L), and let to hydrate and swell with 5 mL of water:ethanol solution (20:80, v/v) for 30 min in a vortex. To increase cell disruption and extractability, the tubes were placed in an ultrasound bath for 5 min (Elmasonic S60 h, Singen, Germany), followed by 30 min in a water bath at 60 °C. The solutions were centrifuged at 2500 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 mL of the supernatant were concentrated at 60 °C under nitrogen flushing for total ethanol removal. The solution was taken up to 1 mL with 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 μ m Nylon filters before injection. Each sample was extracted in duplicate.

The 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) (Sedere Model 75, Olivet, France). The HPLC system was equipped with a SUPELCOGEL Ca column (300 $\times$ 7.8 mm, Supelco, Bellefonte, PA, USA), 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 with that of the internal standard 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.

Starch and amylose contents

The starch and amylose contents were determined by application of the Megazyme kit (Megazyme procedure, K-AMYL 07/11, Wicklow, Ireland). The principle of this kit is that amylopectin complexes with lectin concanavalin (Con A), while the primarily linear amylose component is not able to complex with it. Moreover, total starch is extracted and hydrolysed to D-glucose and measured colourimetrically as described on the Megazyme kit, using the starch standard included in it. The results were expressed on starch percentage in the sample (by dry matter) and amylose percentage in the starch (g/100 g starch).

Organic acids analysis

The organic acids extraction was performed according to the method described by Carocho et al. (2013), with some modifications, namely the addition of internal standard and the application of a sequential extraction. In more detail, samples (500 mg, in duplicate) were mixed with 5 mL of meta-phosphoric acid (3%, w/v) and 150 μ L of gallic acid, as internal standard (1 mg/mL), for 30 min in a vortex. Then, the solutions were centrifuged (Heraeus Sepatech, Am Kalkberg, Germany) at 2500 rpm for 5 min at 20 °C. The supernatants were removed and 5 mL of meta-phosphoric acid (3%, w/v) were added to the residue, being the extraction process repeated. The supernatants were combined and approximately one mL of the supernatant was filtered through 0.22 μ m Nylon filters before high performance liquid chromatography—ultraviolet detection (HPLC–UV) analysis.

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). The HPLC system was equipped with a C₁₈ column (150 $\times$ 4.6 mm, 5 μ m, 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. The results were expressed in mg/100 g of dry weight, calculated by internal normalization of the chromatographic peak area with the internal standard and application of individual calibration curves. Organic acids identification was made by comparing the relative retention times of sample peaks with standards. The results were expressed on mg/100 g dry matter.

Lipid analysis

Fatty acids and vitamin E extraction and quantification were performed according to the method described by Delgado et al. (2016), based on cold lipid extraction,

followed by direct analysis of vitamin E by normal-phase HPLC and conversion of all glycerides to fatty acid methyl esters and their analysis by gas chromatography.

Statistical methods

The statistical analysis was performed on SPSS software (Version No. 20.0). The effect of the OD conditions on physicochemical composition of chestnut slices was evaluated by a factorial analysis of variance (Factorial ANOVA) ($p < 0.05$), 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. The level of the significance used for all statistical tests was 95%. A linear discriminant analysis (LDA) was used to identify which variables were able to discriminate the samples taking into account the temperature, contact time and sugar concentration applied during the OD of chestnut slices. A stepwise technique was performed, considering the Wilks' λ test with the usual probabilities of F (3.84 to enter and 2.71 to remove) for variable selection. This procedure uses a combination of forward selection and backward elimination steps, where the inclusion of a new variable is preceded by verifying the significance of all variables selected previously (Maroco 2010). To verify the significance of the canonical discriminating functions, Wilk' λ test was used. A leaving-one-out cross validation procedure was carried out to assess the model performance. All statistical tests were performed at a 5% significance level.

Results and discussion

The changes on the nutritional composition of chestnut slices subjected to OD due to temperature (30, 45 and 60 °C), time (2.5, 5.0 and 7.5 h) and sugar concentration (60, 70 and 80%, w/v), as well as the interaction of these main factors, are represented in Table 1. Almost in all situations, the factor interactions were insignificant ($p \geq 0.05$). On contrary, the effect of temperature was significant on protein, fat and NDF contents, but not for ADF. Contact time had also a significant effect on protein and fat content. The lowest protein and fat contents were observed in the samples osmodehydrated at the highest temperature (60 °C) and in those subjected to the highest contact time (7.5 h). This decrease (20 and 15% for protein, and 25 and 20% for fat, when compared to 30 °C and 2.5 h) could be related with the diffusion of sucrose to the interior of chestnut or to the output of water from the fruit to the osmotic medium, increasing the dry matter and

Table 1 Nutritional composition of the osmotic dehydrated chestnut slices (g/100 g dry matter) (n = 27)

	Protein	Fat	NDF	ADF
Temperature (°C)				
30	3.5 ± 0.3 ^b	2.0 ± 0.2 ^b	10.1 ± 2.1 ^a	2.8 ± 0.3 ^a
45	3.2 ± 0.4 ^b	1.7 ± 0.2 ^b	13.0 ± 3.3 ^b	3.0 ± 0.3 ^a
60	2.8 ± 0.3 ^a	1.5 ± 0.3 ^a	15.3 ± 3.4 ^b	3.0 ± 0.4 ^a
<i>p</i>	<0.001	<0.001	<0.001	0.157
Time (h)				
2.5	3.4 ± 0.4 ^b	2.0 ± 0.2 ^b	13.4 ± 5.5 ^a	2.9 ± 0.4 ^a
5.0	3.2 ± 0.4 ^b	1.7 ± 0.2 ^a	13.7 ± 3.8 ^a	3.0 ± 0.3 ^a
7.5	2.9 ± 0.5 ^a	1.6 ± 0.4 ^a	12.0 ± 1.7 ^a	2.8 ± 0.2 ^a
<i>p</i>	0.007	0.001	0.360	0.063
Sugar concentration (%)				
60	3.1 ± 0.5 ^a	1.9 ± 0.3 ^a	12.1 ± 2.9 ^a	2.9 ± 0.2 ^a
70	3.2 ± 0.4 ^a	1.7 ± 0.2 ^a	13.1 ± 3.4 ^a	3.0 ± 0.3 ^a
80	3.2 ± 0.5 ^a	1.7 ± 0.4 ^a	13.1 ± 4.6 ^a	2.9 ± 0.4 ^a
<i>p</i>	0.566	0.054	0.660	0.363
Interactions (<i>p</i> value)				
T × t	0.539	0.055	<0.001	<0.001
T × sugar	0.350	0.243	0.239	0.033
t × sugar	0.246	0.463	0.529	0.005
T × t × sugar	0.604	0.830	0.479	0.071

Mean ± SD. Different small letter (a, b) superscripts on the same column are significantly different ($p < 0.05$)

decreasing the relative proportion of fat and protein. On the other hand, the effect of sugar concentration was insignificant and did not change the proximate composition of chestnut slices in terms of protein, fat and fiber.

The results obtained for soluble sugars and starch fractions are presented in Table 2. Temperature caused significant changes on all sugar contents. Free sugars, sucrose, glucose, fructose and raffinose contents increased with temperature, while starch decreased. Sucrose was the major compound in total sugars due to its incorporation from the OD solution, achieving as much as 30 g/100 g dry matter in chestnuts, corresponding to an increase of approximately 50% when compared with fresh chestnut (15 g/100 g dry matter). The observed decrease on starch content with temperature increase could be also a direct consequence of the increased relative proportion of sucrose, complemented by some potential enzymatic hydrolysis (Correia et al. 2009). Also, for increased temperatures, both glucose and fructose increased, indicative of sucrose hydrolysis. Raffinose, a trisaccharide formed by galactose, fructose and glucose, was slightly affected by temperature. The high amylose percentage (16.9 g/100 g of starch) observed at the highest temperatures tested is in line with Attanasio et al. (2004) and Correia and Beirão-da-Costa (2012).

Table 2 Sugar composition of the osmotic dehydrated chestnut slices (g/100 g dry matter) (n = 27)

	Starch	Amylose*	Total sugars	Sucrose	Glucose	Fructose	Raffinose
Temperature (°C)							
30	36.7 ± 4.1 ^b	12.4 ± 2.3 ^a	29.3 ± 2.3 ^a	27.8 ± 2.5 ^a	0.7 ± 0.3 ^a	0.7 ± 0.3 ^a	0.17 ± 0.02 ^a
45	31.6 ± 4.0 ^a	15.6 ± 3.2 ^{a,b}	31.6 ± 2.0 ^b	28.9 ± 2.1 ^{a,b}	1.3 ± 0.6 ^b	1.1 ± 0.7 ^{a,b}	0.24 ± 0.07 ^b
60	31.3 ± 2.4 ^a	16.9 ± 3.1 ^b	33.4 ± 2.0 ^c	30.0 ± 2.5 ^b	1.6 ± 0.7 ^b	1.6 ± 0.8 ^b	0.27 ± 0.08 ^b
<i>p</i>	0.006	0.015	<0.001	0.031	0.001	0.003	<0.001
Time (h)							
2.5	33.5 ± 2.0 ^a	12.5 ± 2.5 ^a	30.7 ± 2.1 ^a	28.7 ± 2.2 ^a	1.0 ± 0.7 ^a	1.0 ± 0.7 ^a	0.22 ± 0.08 ^a
5.0	30.3 ± 4.6 ^a	16.4 ± 4.0 ^b	31.9 ± 1.6 ^a	29.9 ± 1.6 ^a	1.0 ± 0.3 ^a	0.9 ± 0.3 ^a	0.22 ± 0.08 ^a
7.5	34.5 ± 5.9 ^a	16.8 ± 3.0 ^b	32.0 ± 3.6 ^a	29.1 ± 3.2 ^a	1.4 ± 0.7 ^a	1.3 ± 0.8 ^a	0.23 ± 0.07 ^a
<i>p</i>	0.063	0.023	0.162	0.147	0.097	0.127	0.925
Sugar concentration (%)							
60	32.8 ± 4.0 ^a	14.6 ± 3.0 ^a	32.4 ± 1.9 ^b	30.8 ± 1.6 ^c	0.8 ± 0.2 ^a	0.7 ± 0.2 ^a	0.18 ± 0.02 ^a
70	30.4 ± 5.0 ^a	15.5 ± 4.1 ^a	31.5 ± 1.8 ^{a,b}	29.0 ± 2.0 ^b	1.2 ± 0.7 ^{a,b}	1.1 ± 0.8 ^{a,b}	0.23 ± 0.10 ^{a,b}
80	35.2 ± 4.5 ^a	14.7 ± 4.1 ^a	30.3 ± 3.5 ^a	27.0 ± 2.3 ^a	1.5 ± 0.8 ^b	1.6 ± 0.8 ^b	0.26 ± 0.09 ^b
<i>p</i>	0.054	0.819	0.048	<0.001	0.012	0.002	0.044
Interactions (<i>p</i> value)							
T × t	0.037	0.571	0.013	0.004	0.221	0.004	0.135
T × sugar	0.489	0.259	0.006	0.810	<0.001	<0.001	<0.001
t × sugar	0.359	0.983	0.961	0.630	<0.001	<0.001	0.544
T × t × sugar	0.576	0.407	0.012	0.009	0.444	0.360	0.509

Mean ± SD. Different small letter (a–c) superscripts on the same column are significantly different (*p* < 0.05)

*The results are expressed in g/100 g of starch

Contact time (2.5, 5.0 and 7.5 h) showed to cause less significant modifications than temperature, only affecting amylose percentage (from 12.5 to 16.8 g/100 g of starch), suggesting changes on starch conformation. As expected, sugar concentration affected all the individual sugars analysed. The variations on glucose content could probably be due to the partial starch enzymatic hydrolysis or to sucrose hydrolysis into glucose and fructose, explaining the last phenomenon the increase of both sugars. The slight but significant decrease on sucrose concentration on chestnuts observed when using the highest sugar concentration, complemented by glucose and fructose increase, is a direct consequence of its hydrolysis, but the occurrence of a case-hardening effect that may induce some rigidity of the external cell layers and form a barrier to sucrose transfer is not to be excluded (Lee et al. 2014).

Table 3 details the organic acid composition of the osmotically dehydrated samples. Citric and malic acids were the major organic acids present on chestnuts. Regarding temperature, the highest values of citric, malic and ascorbic acids were obtained when applying the lowest temperature (30 °C), suggesting a high instability of these compounds for higher temperatures (45 and 60 °C), with 54, 36 and 23% of losses, respectively. Contact time also originated significant losses on malic and ascorbic acids,

whose contents decreased at higher contact times (5.0 and 7.5 h). Besides their thermal degradation, partial mass diffusion to the osmotic medium cannot be excluded. Concerning sugar concentration, only malic and fumaric acids were significantly affected; however, the highest values of malic acid were observed at 60 and 80% of sugar concentrations, without any particular trend. On the other hand, for fumaric acid, the highest contents were obtained at the highest sugar concentrations (70 and 80%), suggesting that sucrose concentration affects differently the organic acids in chestnut slices.

The main fatty acids composition of chestnut slices is present in Table 4. As previously discussed, high temperatures and times caused a relative lower content of fat on a chestnut dry mass basis, due to sucrose incorporation, explaining the observed lower contents of SFA, MUFA and PUFA. On the other hand, sucrose concentration did only affect MUFA contents, being also the highest values obtained with the lowest sucrose concentration (60%). Regarding the relative percentages of fatty acids in the fat, PUFA represented the highest fraction, with the main contribution of linoleic and α -linolenic acids (C18:2 and C18:3, respectively), two essential fatty acids. Temperature and sugar concentration did not affect significantly these compounds, while

Table 3 Organic acid composition of the osmotic dehydrated chestnut slices (mg/100 g dry matter) (n = 27)

	Citric acid	Malic acid	Fumaric acid	Ascorbic acid
Temperature (°C)				
30	611 ± 87 ^c	218 ± 65 ^b	17.6 ± 3.4 ^a	14.8 ± 3.3 ^b
45	394 ± 151 ^b	133 ± 56 ^a	22.2 ± 50 ^a	12.6 ± 2.6 ^a
60	283 ± 136 ^a	140 ± 55 ^a	20.4 ± 10 ^a	11.4 ± 0.1 ^a
<i>p</i>	<0.001	<0.001	0.069	0.001
Time (h)				
2.5	483 ± 221 ^a	202 ± 77 ^b	23.7 ± 7.3 ^b	14.2 ± 3.6 ^b
5.0	405 ± 174 ^a	132 ± 52 ^a	21.1 ± 6.1 ^b	12.1 ± 1.4 ^a
7.5	424 ± 181 ^a	156 ± 58 ^a	14.1 ± 5.3 ^a	11.9 ± 1.0 ^a
<i>p</i>	0.386	0.001	<0.001	0.001
Sugar concentration (%)				
60	413 ± 216 ^a	157 ± 67 ^{a,b}	16.4 ± 8.5 ^a	13.5 ± 3.8 ^a
70	394 ± 174 ^a	147 ± 68 ^a	20.6 ± 4.3 ^{a,b}	12.7 ± 2.8 ^a
80	490 ± 184 ^a	201 ± 70 ^b	21.9 ± 6.5 ^b	12.7 ± 1.4 ^a
<i>p</i>	0.225	0.035	0.024	0.675
Interactions (<i>p</i> value)				
T × t	<0.001	<0.001	<0.001	<0.001
T × sugar	0.011	0.010	0.427	<0.001
t × sugar	<0.001	<0.001	0.029	<0.001
T × t × sugar	<0.001	<0.001	0.009	<0.001

Mean ± SD. Different small letter (a–c) superscripts on the same column are significantly different ($p < 0.05$)

high contact times led to lower percentages, suggesting some oxidation. Nevertheless, C18:3 was the only PUFA that decreased significantly (17% of loss when contact time increased from 2.5 to 7.5 h). MUFA represented the second main class, being the oleic acid (C18:1) the major contributor, while for SFA palmitic acid (C16:0) was the main component. Both MUFA and SFA increased on a relative proportion in the fatty acids, suggesting their stability in the tested conditions and also showing the variability that exists among the fruits used in the different experiments performed, even they were of the same variety (Table 4).

Concerning the tocopherol profile (Table 5), a significant decrease (50%) on the total vitamin E content, particularly on γ -tocopherol and δ -tocopherol contents, was observed when temperature increased from 30 to 60 °C. This loss is again proportional to the apparent fat loss, but some oxidation cannot be excluded. Contact time caused significant changes on γ -tocopherol and α -tocopherol, decreasing the former with contact time, in line with the reduction of total fat content. On the other hand, α -tocopherol showed a variable behaviour. Furthermore, sugar concentration used on the osmotic treatments did not affect the vitamin E main vitamins (γ - and δ -tocopherols).

In general terms, when performing OD, temperature, contact time and sugar concentration might affect the

proximate composition, as well as sugar, organic acids, fatty acids and tocopherol profiles of chestnut slices.

Linear discriminant analysis (LDA)

With the aim to better understand the effect of the OD factors on chestnut chemical composition a linear discriminant analysis was performed. The significant independent variables were selected following the stepwise method of the LDA, according to the Wilks' λ test. Only variables with a statistically significant classification performance ($p < 0.05$) were kept in the analysis. Concerning contact time (Fig. 1a), two significant functions were extracted, which allowed to classify correctly 100% of the original grouped cases. From the chemical variables, the model selected fat, protein, malic acid, fumaric acid, C18:3, MUFA (%) and α -tocopherol as the significant variables. The most outstanding contribution to discrimination in the first function was obtained from fat, fumaric acid and C18:3, while for the second function it was obtained from protein, malic acid, MUFA (%), and α -tocopherol.

Regarding the temperature effect (Fig. 1b), two significant functions were extracted, which allowed to classify correctly 91.7% of the original grouped cases. From the chemical variables, the model selected amylose, MUFA

Table 4 Fatty acids profile of the osmotic dehydrated chestnut slices ($n = 27$)

	SFA (mg/100 g dry weight)	MUFA	PUFA	SFA (%)	C16:0	C18:0	MUFA	C18:1	PUFA	C18:2	C18:3
Temperature (°C)											
30	360 ± 29 ^c	560 ± 42 ^b	994 ± 121 ^c	18.3 ± 1.6 ^a	15.3 ± 1.2 ^a	1.76 ± 0.30 ^a	28.0 ± 2.1 ^{a,b}	26.7 ± 2.0 ^b	49.5 ± 4.3 ^a	43.1 ± 3.4 ^a	5.96 ± 0.94 ^a
45	323 ± 34 ^b	444 ± 46 ^a	820 ± 98 ^b	19.5 ± 1.4 ^b	16.2 ± 1.1 ^b	1.88 ± 0.35 ^a	26.6 ± 1.8 ^a	25.1 ± 1.7 ^a	49.1 ± 4.4 ^a	42.5 ± 3.6 ^a	6.08 ± 0.86 ^a
60	291 ± 33 ^a	441 ± 67 ^a	718 ± 172 ^a	19.5 ± 1.8 ^{a,b}	16.2 ± 1.4 ^{a,b}	1.92 ± 0.31 ^a	29.1 ± 2.0 ^b	27.4 ± 1.8 ^b	46.6 ± 4.7 ^a	40.5 ± 3.8 ^a	5.64 ± 0.90 ^a
<i>p</i>	<0.001	<0.001	<0.001	0.032	0.027	0.325	<0.001	<0.001	0.123	0.105	0.253
Time (h)											
2.5	347 ± 30 ^b	536 ± 50 ^b	967 ± 140 ^b	18.6 ± 1.0 ^a	15.5 ± 0.8 ^a	1.82 ± 0.32 ^a	28.0 ± 1.9 ^{a,b}	26.7 ± 1.8 ^{a,b}	50.2 ± 2.6 ^a	43.4 ± 2.2 ^a	6.34 ± 0.56 ^b
5.0	330 ± 36 ^{a,b}	455 ± 46 ^a	805 ± 108 ^a	19.8 ± 1.9 ^a	16.4 ± 1.3 ^a	1.96 ± 0.42 ^a	27.0 ± 2.0 ^a	25.4 ± 1.8 ^a	47.7 ± 5.4 ^a	41.3 ± 4.6 ^a	5.92 ± 0.91 ^b
7.5	305 ± 52 ^a	464 ± 92 ^a	746 ± 197 ^a	19.2 ± 2.4 ^a	16.0 ± 1.8 ^a	1.86 ± 0.32 ^a	29.0 ± 2.2 ^b	27.4 ± 2.1 ^b	45.9 ± 5.3 ^a	40.2 ± 4.4 ^a	5.27 ± 0.91 ^a
<i>p</i>	0.011	<0.001	<0.001	0.073	0.103	0.403	0.005	0.001	0.052	0.082	0.003
Sugar concentration (%)											
60	325 ± 40 ^a	516 ± 81 ^b	916 ± 132 ^a	18.0 ± 0.9 ^a	15.0 ± 0.6 ^a	1.78 ± 0.31 ^a	27.9 ± 1.3 ^{a,b}	26.5 ± 1.3 ^{a,b}	49.6 ± 1.6 ^a	43.1 ± 1.3 ^a	6.09 ± 0.43 ^a
70	331 ± 34 ^a	454 ± 46 ^a	808 ± 121 ^a	19.9 ± 1.9 ^b	16.4 ± 1.3 ^b	1.97 ± 0.42 ^a	26.9 ± 2.3 ^a	25.3 ± 2.0 ^a	47.7 ± 5.6 ^a	41.3 ± 4.7 ^a	5.90 ± 0.97 ^a
80	327 ± 54 ^a	484 ± 82 ^{a,b}	796 ± 245 ^a	19.7 ± 2.1 ^b	16.5 ± 1.5 ^b	1.90 ± 0.32 ^a	29.2 ± 2.5 ^b	27.6 ± 2.3 ^b	46.5 ± 6.1 ^a	40.5 ± 5.0 ^a	5.52 ± 1.2 ^a
<i>p</i>	0.864	0.006	0.050	0.003	0.001	0.242	0.002	0.001	0.246	0.233	0.221
Interactions (<i>p</i> value)											
T × t	0.171	0.001	0.212	0.199	0.166	0.587	0.265	0.175	0.892	0.916	0.670
T × sugar	0.624	0.744	<0.001	0.107	0.107	0.392	0.022	0.038	0.005	0.007	0.002
t × sugar	0.680	0.572	0.039	0.120	0.235	0.126	0.060	0.077	0.081	0.115	0.023
T × t × sugar	0.389	0.208	0.967	0.580	0.438	0.903	0.448	0.346	0.710	0.585	0.607

Mean ± SD. Different small letter (a–c) superscripts on the same column are significantly different ($p < 0.05$)SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; C16:0, palmitic; C18:0, stearic; C18:1, oleic; C18:2, linoleic; C18:3, α -linolenic

Table 5 Tocopherols composition of the osmotic dehydrated chestnut slices (mg/100 g dry matter) (n = 27)

	Total vitamin E	γ -tocopherol	δ -tocopherol	α -tocopherol
Temperature (°C)				
30	14.3 ± 4.9 ^b	13.4 ± 4.8 ^b	0.72 ± 0.20 ^c	0.19 ± 0.02 ^{a,b}
45	10.6 ± 3.9 ^a	9.8 ± 3.8 ^a	0.57 ± 0.10 ^b	0.22 ± 0.03 ^b
60	7.17 ± 6.6 ^a	6.7 ± 6.4 ^a	0.33 ± 0.16 ^a	0.18 ± 0.04 ^a
<i>p</i>	<0.001	0.001	<0.001	<0.001
Time (h)				
2.5	13.0 ± 7.3 ^a	12.4 ± 7.0 ^b	0.51 ± 0.22 ^a	0.18 ± 0.02 ^a
5.0	10.7 ± 4.4 ^a	10.0 ± 4.2 ^{a,b}	0.56 ± 0.17 ^a	0.21 ± 0.03 ^b
7.5	8.5 ± 5.5 ^a	7.7 ± 5.3 ^a	0.55 ± 0.31 ^a	0.19 ± 0.04 ^{a,b}
<i>p</i>	0.062	0.048	0.742	<0.001
Sugar concentration (%)				
60	12.3 ± 5.1 ^a	11.6 ± 5.0 ^a	0.52 ± 0.12 ^a	0.18 ± 0.03 ^a
70	10.2 ± 5.3 ^a	9.4 ± 5.1 ^a	0.54 ± 0.17 ^a	0.22 ± 0.02 ^b
80	9.2 ± 8.0 ^a	8.5 ± 7.7 ^a	0.53 ± 0.36 ^a	0.19 ± 0.04 ^a
<i>p</i>	0.306	0.274	0.957	<0.001
Interactions (<i>p</i> value)				
T × t	<0.001	<0.001	<0.001	0.655
T × sugar	<0.001	<0.001	<0.001	0.336
t × sugar	<0.001	<0.001	0.648	<0.001
T × t × sugar	<0.001	<0.001	0.009	<0.001

Mean ± SD. Different small letter (a–c) superscripts on the same column are significantly different ($p < 0.05$)

Fig. 1 Linear discriminant analysis obtained for the osmotic dehydration conditions: **a** contact time and **b** temperature

(%), fumaric acid, and δ -tocopherol as the significant variables. The most outstanding contribution to discrimination in the first function was obtained from amylose percentage, while for the second function it was obtained from MUFA (%), fumaric acid and δ -tocopherol.

Concerning the sugar concentration effect, two significant functions were extracted (data not shown); however, they only classified correctly 75.0% of the original grouped cases. Thus, sugar concentration shows to have lower discriminant ability than temperature or contact time.

Overall, considering the effect of temperature, contact time and sucrose concentration, a high number of correctly classified cases by LDA for proximate, sugar, organic acids, fatty acids and tocopherol composition on chestnut slices was obtained, mainly when considering the first two factors. Thus, the present study showed that the application of different conditions during the OD of chestnuts introduce differences on their chemical composition.

Conclusion

Temperature, contact time and sugar concentration applied on the OD of chestnut slices have influence on their nutritional composition, starch, free sugars, organic acids, fatty acids and tocopherol profiles. Temperature at 60 °C and contact time of 7.5 h decreased the major components, protein and fat, when compared to 30 °C and 2.5 h. Regarding some of the minor components, temperature at 60 °C promoted significant loss in citric, malic and ascorbic acids, as well as vitamin E, when compared to 30 °C. Besides temperature, high contact times caused losses on α -linolenic acid. Globally, OD should be carried out during the shortest period possible and at low temperatures in order to maintain chestnut components, while sucrose concentration was less important to preserve chestnut bioactive components.

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