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Improving the physicochemical properties of a traditional Portuguese cake – "económicos" with chestnut flour

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"Económicos" are traditional Portuguese pastry products; although their production is low-cost, their nutritional value is equally low. Since it is a widely consumed product in the Trás-os-Montes region, it is important to add value to it without making significant changes to the traditional recipe. Thus, this work has the main objective to increase the nutritional power of "económicos" through the incorporation of chestnut (*Castanea sativa*) fruit flour. The influence of the incorporation of 9% of chestnut flour as a new ingredient was analysed in terms of physical parameters (texture, colour, pH, water activity and moisture), nutritional content (according to the official AOAC methodology) and chemical parameters (sugars, fatty acids and organic acids) and the ability to control the microbial load over shelf life (32 days). Overall, the addition of the chestnut flour did not drastically change the appearance of the chemical and physical profiles of the cakes, but resulted in a lighter crumb (L^*), slight changes in the texture profile, reduction of fat, and most importantly, introduced healthier flour to this inexpensive cake. Moreover, it did not stimulate the growth of microorganisms (total aerobic mesophiles, coliforms, *Bacillus cereus*, molds, and yeasts) during the 32 days of storage.

Introduction

Currently, consumer interest in the products of regional origin with a traditional image is growing; in this sense, the issue of valuing regional and national products has been receiving a lot of attention from the population and the community.[1]

There are several species that represent a valuable natural resource for rural populations, an example of which is *Castanea sativa* Mill., which according to Fernandes *et al.*[2] is one of the main sources of income in some rural areas in the North of Portugal, its production in 2017 being 25.652 tons. Most of the production of chestnut is intended for fresh consumption; however, there are fruits that are not suitable for sale due to various factors (*e.g.* outside the marketing caliber).[3] As studies have increasingly proved the nutritional power of this fruit – rich in polysaccharides, fiber, starch, fatty acids, minerals and vitamins – it is essential to use the fruits that cannot be marketed.[4] Chestnut out of commercial caliber are currently transformed into chestnut purée, marron-glacé and chestnut flour.[5] Relative to the chestnut flour (gluten-free), it can be used in bakery products to supplement nutritional properties in foods.[6] This flour mostly composed of starch (40–60% w/w), sucrose (20–32% w/w), proteins with essential amino acids (4–8% w/w) and dietary fiber (4–10% w/w) and contains a low fat content (2–4% w/w). Besides, it is the source of vitamins (E and C), minerals (potassium, phosphorus and magnesium) and phenolic compounds (phenolic acids, flavonoids, and tannins).[6–9]

For many years, in the region of Trás-os-Montes, they have been consuming "económicos", a traditional pastry product that is highly appreciated due to its taste and consequently much consumed by the Trás-os-Montes population and by the visitors of this region.[10] This traditional pastry product is called "económicos", as it results from a mixture of economic ingredients, such as flour, sugar, margarine, olive oil, eggs, and brandy (the quantity of each ingredient varies between recipes).[11] Although the low cost of producing "económicos" is a benefit, these cakes do not have important nutrients, negatively affecting the consumer's choice for traditional pastry products.

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Consumers are increasingly aware of the labels on food products, and have been giving preference to more natural and nutritionally rich products, as they are aware of the benefits that their consumption brings to their health. Regarding the nutritional value of processed food products, this can be improved by changing the ingredients. Coello *et al.*[12] studied the effects of fortification of wheat flour with moringa sprout powder on nutritional aspects and bioactive and sensory qualities of fettuccini pasta and found that fortified flour has a high nutritional potential, increasing the values of proteins, lipids, minerals and fibers in pasta. Wang *et al.*[13] also studied the influence of functional vegetable ingredients on the nutritional quality of pasta, obtaining very promising results, such as improved nutritional value, increased antioxidant capacity and an increase in the fiber content of the pasta. Hoehnel *et al.*[14] developed fresh hybrid protein-rich pasta using a mixture of buckwheat, fava beans and lupine (to replace wheat semolina), achieving a product with a better nutritional content and a balanced amino acid profile.

Since "económicos" is a cake that is heavily consumed, it is important to increase its nutritional value, so the objective of this work was to improve the nutritional value of these cakes using flour from chestnuts which are not fit to be sold as fruits, and analysing the changes in their physical and chemical parameters.

Materials and methods

Samples

The "económicos" (Fig. 1) samples were provided by "Pão de Gimonde" (Gimonde, Braganza, Portugal), which sells typical Portuguese pastry products. Two sample batches were provided; both were produced following a traditional recipe previously published by Carocho *et al.*[10] (6 eggs, 500 g of sugar, 1.05 kg of flour (wheat), 45 g of margarine and 30 g of olive oil, 230 mL of orange juice, 35 g of orange zest, 200 mL of milk, 45 mL of brandy and 25 g of cinnamon). One of the batches contains "económicos" with 9% of chestnut flour, because through several tests of incorporation of chestnut flour (Table 1) in the traditional recipe of the "económicos", it was found that 9% of chestnut flour is the ideal percentage to incorporate in the product under study, as a higher percentage of chestnut flour alters the texture of "económicos". The samples were analyzed for 32 days at different times (T0, T12, T18, T25 and T32), and various dimensions were analyzed, namely the physical parameters, nutritional value, chemical composition, and microbial growth over shelf life. The results were expressed in g per 100 g fresh weight (fw). Both batches were stored in accordance with the rules followed in the marketing process (packaged and kept at room temperature), until further analysis. For the analysis of the physical parameters and microbial growth, fresh samples were used and for the remaining analyses the samples were lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA), reduced to a fine dried powder (~20 mesh), mixed to obtain a homogeneous sample and stored in a place protected from light and heat, until further analysis.

Fig. 1 "Económicos" with 9% chestnut flour.

Table 1 Nutritional and chemical profiles of the chestnut flour, expressed in g per 100 g of fresh weight

Nutritional value	Moisture (g per 100 g)	8.14 ± 0.02
	Proteins (g per 100 g)	4.8 ± 0.6
	Ashes (g per 100 g)	4.7 ± 0.1
	Crude fat (g per 100 g)	1.9 ± 0.2
	SFA	1.033 ± 0.001
	MUFA	0.846 ± 0.004
	PUFA	0.068 ± 0.005
	Fiber (g per 100 g)	5.6 ± 0.2
	Carbohydrates (g per 100 g)	75 ± 1
	Energy (kcal per 100 g)	370 ± 1
Chemical profile	Organic acids (g per 100 g)	1.34 ± 0.05
	Oxalic	0.0094 ± 0.0002
	Malic	0.20 ± 0.02
	Citric	1.135 ± 0.007
	Fumaric	0.003 ± 0.001
	Soluble sugars (g per 100 g)	18.4 ± 0.5
	Sucrose	18.4 ± 0.5

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Standards and reagents

All reagents were obtained from scientific retailers being of anaytical grade and also of HPLC grade when applied in the HPLC system. Acetonitrile (HPLC grade), methanol, sulfuric acid and metaphosphoric acid were purchased from Thermo Fisher Scientific (Lisbon, Portugal), toluene and ethyl ether from Sigma (St Louis, Missouri, USA) and sodium sulphate from Carlo Erba Reagents (Peypin, France). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Physical parameters

The physical analysis encompassed a full determination of the texture profile (hardness, adhesiveness, resilience, cohesiveness, springiness and chewiness), colour, pH and water activity of all samples.

Texture profile. The "económicos" were analyzed using a TA. XT Plus texturometer from Stable Micro Systems (Vienna Court, Godalming, UK). The analysis performed was a texture profile analysis (TPA) that imitates human mastication by making two compressions on the same food, managing to extract many parameters (hardness, adhesiveness, resilience, cohesiveness, elasticity, gumminess and chewability) through the use of macros. The load cell supported a maximum of 30 kg and the probe was a P/45 metal cylinder, while the strain was set to 25% and the target mode to strain. In addition, a cross-section of the food was made in order to measure the firmness of the sample. For this purpose, a metal blade set with a texture knife (HDP/BSK) was used. In both tests, a pre-test speed, test speed and post-test speed of 10 mm s^{-1}, 5 mm s^{-1} and 10 mm s^{-1}, respectively, were employed and the trigger force was set at 10 g. The results were studied using the Exponent program (Stable Micro Systems).

Colour analysis. The colour parameters were determined in three points of the "económicos", inside, outside and in the product resulting from the crumbling process, following the procedure described by Fernandes *et al.*[15] The colour was measured using a portable colorimeter Konica Minolta CR 400 (Tokyo, Japan), using the standard of the International Commission on Illumination (CIE), D65 illuminant, with 8 mm aperture and 10° observation. According to the CIE measurement space L^*, a^* and b^*, L^* represents the brightness (L = 0 black, L = 100 white), a^* represents the redness ($-a$ = 0 green, $+a$ = redness) and b^* represents the yellowness ($-b$ = blue, $+b$ = yellowness). The variation in the total colour difference (ΔE^*) was also calculated between the bread samples following eqn (1):

$$\Delta E = \sqrt{(L_2^* - L_1^*) + (a_2^* - a_1^*) + (b_2^* - b_1^*)} \quad (1)$$

Hue and chroma represent the tone and saturation of a color, respectively, and were also calculated following eqn (2) and (3).

$$h_{ab} = \arctan \frac{b^*}{a^*} \quad (2)$$

$$C_{\mathrm{ab}}^* = \sqrt{(a^{*2}) + (b^{*2})} \quad (3)$$

Digital imaging. The digital imaging process followed the procedure of Scheuer *et al.*[16] and the theory of Gonzáles-Barron *et al.*[17] with slight modifications. Images of three slices were obtained using a flatbed scanner (Canon PIXMA, MG 2550). The brightness and contrast of the images were both set to zero, and the images were converted to bitmap files with a resolution of 300 dpi and 20 × 20 mm square field of view, and finally saved in black and white using Adobe Photoshop 2020. Using the ImageJ-based Fiji 1.46 software package, the images were pre-processed and converted to 8-bit greyscale and subsequently subjected to auto threshold using the Otsu algorithm. The Analyze Particles function was used to automatically count the holes in the crumb, using a range of 50 to 1000 pixels, allowing the quantification of objects (holes), average size, and circularity.

pH. The pH was measured according to the procedure described by Ueda *et al.*,[18] using a Hanna Instruments HI 902 potentiometer (RI, USA).

Water activity. Water activity was measured using a Dew Point Water Activity Meter 4TE (Aqua Lab, Cromer, Australia).

Nutritional value

For the "económicos" of both batches, the nutritional profile was drawn from the analysis of moisture, energy and macronutrient content (fat, ash, protein, fibers and carbohydrates) according to the official AOAC methodology, 17th edition.[19] The moisture content was determined following the AOAC method 925.09, using a moisture analyzer from Adam Equipment (model PBM 163, Oxford, USA). The crude fat content was determined by the AOAC 920.85 method, which is based on fat extraction by Soxhlet, using petroleum ether as the extraction solvent. The ash content was estimated by the AOAC 923.03 method, incineration in the muffle furnace (Optic Ivymen System, N-8L, Barcelona, Spain) for 5 h at 550 °C. The crude protein (N × 5.7) content was determined by the AOAC 920.87 method using the macro-Kjeldahl method. The total dietary fiber (TDF) content was determined by the AOAC 985.29 method, using the Kit TDF100A (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), and the percentage value of the total dietary fibre was calculated using the following formula:

$$\%\mathrm{TDF} = \left(\frac{\mathrm{Residue_{sample}} - \mathrm{Protein_{sample}} - \mathrm{Ash_{sample}} - \mathrm{White}}{\mathrm{Weight_{sample}}}\right) \times 100$$

Total carbohydrates were calculated based on the difference and the energy was estimated using the following equation:

$$\mathrm{Energy} = 4 \times [\mathrm{protein(g)} + \mathrm{carbohydrates(g)}] + 2 \times [\mathrm{dietary\ fibre(g)}] + 9 \times [\mathrm{fat(g)}]$$

The results were expressed in g per 100 g of fresh weight (fw) and in Kcal and KJ for energy values.

Chemical composition

The chemical composition, encompassing free sugars, organic acids and fatty acids, was evaluated following procedures previously described by Barros *et al.*,[20] and are detailed below.

Soluble sugar analysis. Free sugars were analyzed using a high performance liquid chromatography (HPLC) system coupled with a refractive index (RI) detector (Knauer, Smartline System 1000, Berlin, Germany), using the internal standard (IS) as melezitose (PanReac AppliChem ITW Reagents Co., Darmstadt, Germany). The identification and quantification were carried out by chromatographic comparisons with authentic standards (D(−)-fructose, D(+)-glucose anhydrous, D(+)-sucrose and trehalose; Sigma-Aldrich, St Louis, MO, USA), and the data were analyzed using Clarity 2.4 software (DataApex, Podohradska, Czech Republic).

Organic acids. Organic acids were evaluated using an Ultra-Fast Liquid Chromatography system (UFLC, Shimadzu 20A series, Kyoto, Japan) coupled with a diode array detector. For the identification and quantification with organic acid standards (L(+)-ascorbic acid, citric acid, malic acid, oxalic acid, shikimic acid, succinic acid, fumaric acid, and quinic acid; Sigma-Aldrich, St Louis, MO, USA), chromatographic comparisons were performed with the peaks of the samples.

Individual fatty acids. The fatty acids were determined using a Gas Chromatography (GC) system coupled to a flame ionization detector (FID) (DANI 1000, Contone, Switzerland), and were identified by comparing their retention times to the ones of FAME (fatty acids methyl esters) peaks from the samples with commercial standards (FAME reference standard mixture, standard 47885-U; Sigma-Aldrich, St Louis, MO, USA). The results were recorded and processed using CSW 1.7 Software (DataApex 1.7, Prague, Czech Republic) and were expressed in relative percentage (%).

Microbiological analysis

To determine the microbial load in the "económicos" along the shelf life of 32 days, the following microorganisms were analyzed: total aerobic mesophiles, coliforms, *Bacillus cereus*, molds, and yeasts. The sample preparation followed the procedure described in the International Organization for Standardization.[21] These analyses were performed immediately after the "económicos" preparation (T0), after 12 days (T12), after 18 days (T18), after 25 days (T25) and after 32 days (T32) of storage at room temperature. A mass of 10 g of the "económicos" sample was mixed in 90 mL of peptone water and serial dilutions (10^{-1} to 10^{-3}) were made.

Total aerobic mesophiles (ISO 4833-2:2013): 1 mL of each dilution was placed in a Petri dish and 15 mL of Plate Count Agar (PCA) were added (incorporation technique). The procedure was performed in duplicate. The plates were homogenized, and after solidification of the medium, they were incubated at 30 °C in an inverted position for 72 h. Counting was performed on plates containing 15 to 300 colonies (Limit of Quantification (LOQ) = 1 log (Colony Forming Units) CFU per g).

Coliforms (ISO 4832:2006): 1 mL of each dilution was inoculated into a plate and 15 mL of Violet Red Bile Lactose Agar (VRBLA) were added. The plates were homogenized, solidified, and incubated at 37 °C for 48 h in an inverted position. Counting was performed on plates containing 10 to 150 colonies, in duplicate (Limit of Quantification (LOQ) = 1 log (Colony Forming Units) CFU per g).

Molds and yeasts (ISO 21527-1/2:2008): 0.2 mL of each dilution was placed on a Petri plate containing 15 mL of Dichloran Rose Bengal Chloramphenicol (DRBC) (spread plate technique). The plates were incubated in the upright position at 25 °C for 5 days, and the counting was performed on plates with less than 150 colonies (LOQ = 1.7 log CFU per g). Yeasts were counted after 3 days and molds were counted after 5 days of incubation.

Bacillus cereus (ISO 7932:2004): 0.2 mL of each dilution was inoculated on MYP (mannitol yolk polymyxin) by the spread plate technique. Incubation was performed at 30 °C for 24–48 h, in the reversed position and the counting was performed on plates containing 10 to 150 colonies (LOQ = 1.7 log UFC per g).

Statistical analysis

Throughout the manuscript, all assays were carried out with three replicates, and all data are expressed as mean ± standard deviation. Samples were analyzed through a two-way analysis of variance (ANOVA) with type III sums of squares, after verifying homoscedasticity through Levene's test. The *post hoc* tests were either Tukey's Honest Significant Difference (HSD) test (homoscedastic samples) or Tamhane's T2 test (non-homoscedastic samples) for the storage time (ST), and Student's *t*-test for the type of flour (TF). By employing a two-way ANOVA, it was possible to verify the influence of each factor ((ST) and (FT)) independently from the other factors. If a significant ($p < 0.05$) interaction (ST × FT) was detected, the potential tendencies were extracted from the plotted estimated marginal means (EMM). Inversely, if there was no significant interaction ($p > 0.05$), each factor was classified independently using the *post hoc* tests described above. All statistical analyses were performed using a *p*-value of 0.05 and IBM SPSS software, version 25 (IBM Corp.).

Results and discussion

Physical analysis

Texture profile. Regarding the physical parameters, both the texture and colors were measured. The texture analysis consisted of a TPA (texture profile analysis) and a cutting analysis. The TPA allowed to calculate the hardness, adhesiveness, resilience, cohesiveness, springiness, and chewiness, while the cutting analysis provided insight on the firmness of the cakes. These results are shown in Table 2, which are divided in two sections, corresponding to each of the two factors. The upper section refers to ST, while the lower corresponds to FT. This sectioning of the tables is derived from the two-way ANOVA employed to understand the influence of each factor individually, and thus, in the upper section of Table 2, within the ranges of the means are all the values of FT, and in the lower sections are all the values of ST, namely 0, 11, 18, 25, and 32 days of storage. This type of representation allows for a much more trustworthy interpretation of the results, for each factor change can be analyzed independently of the influence of the other. The existence of an interaction between both factors is demonstrated by ST × DT, resulting in a *p*-value under 0.05 and thus only allows for general conclusions which can be extracted from the Estimated Marginal Means plots (EMM), while ST × DT resulting in $p > 0.05$ shows that each factor can be classified independently using Tukey's HSD or Tamhane's T2 tests to classify each factor individually. Due to this type of treatment, the standard deviations should not be regarded as an accuracy measure, but rather a range of values.

Regarding the TPA analysis, a significant interaction was only sought for springiness, showing that both the storage time and type of flour had an influence on the outcome. In this way, general conclusions could be extracted from the EMM plots (Fig. 2), showing a decrease in the texture dimension over time. Only at day 32 was this difference statistically

Table 2 Texture profile analysis of the cakes

		Hardness (g)	Adhesiveness (g s^{-1})	Resilience (%)	Cohesiveness (%)	Springiness (%)	Chewiness	Firmness (g)
Storage time (ST)	0 days	8565 ± 616^{a}	−0.3 ± 0.1^{b}	0.21 ± 0.03	0.58 ± 0.05	0.85 ± 0.02	4201 ± 348^{a}	9735 ± 4832
	12 days	16 962 ± 2329^{b,c}	−1.37 ± 0.97^{a}	0.21 ± 0.02	0.57 ± 0.04	0.79 ± 0.02	7518 ± 800^{b}	10 516 ± 3265
	18 days	15 207 ± 381^{b}	−0.7 ± 0.4^{a,b}	0.21 ± 0.01	0.58 ± 0.03	0.79 ± 0.01	7068 ± 282^{b}	9785 ± 2178
	25 days	15 823 ± 2787^{b}	−0.5 ± 0.3^{a,b}	0.22 ± 0.02	0.59 ± 0.03	0.79 ± 0.03	7345 ± 1385^{b}	11 174 ± 4275
	32 days	21 799 ± 5748^{c}	−0.9 ± 0.5^{a,b}	0.21 ± 0.04	0.54 ± 0.07	0.73 ± 0.04	8647 ± 2726^{b}	10 987 ± 5483
p-Value (n = 15)	Tukey's HSD test	<0.001	0.030	0.903	0.094	<0.001	0.005	0.981
Flour type (FT)	Control	15 230 ± 4592	−0.9 ± 0.7	0.22 ± 0.01*	0.60 ± 0.03*	0.81 ± 0.03	7304 ± 1794	10 974 ± 2714
	9% Chestnut	16 556 ± 5568	−0.6 ± 0.5	0.20 ± 0.02	0.54 ± 0.04	0.77 ± 0.05	6815 ± 2154	9987 ± 4776
p-Value (n = 6)	Student's *t*-test	0.155	0.260	0.001	<0.001	<0.001	0.636	0.533
ST × FT (n = 90)	*p*-Value	0.962	0.260	0.495	0.682	0.030	0.996	0.792

Each letter in each column represents a significant difference among samples, obtained from Tukey's or Tamhane's T2 test. The asterisk (*) represents a statistically significant difference by means of Student's *t*-test. All three assays were performed using a significance of 0.05.

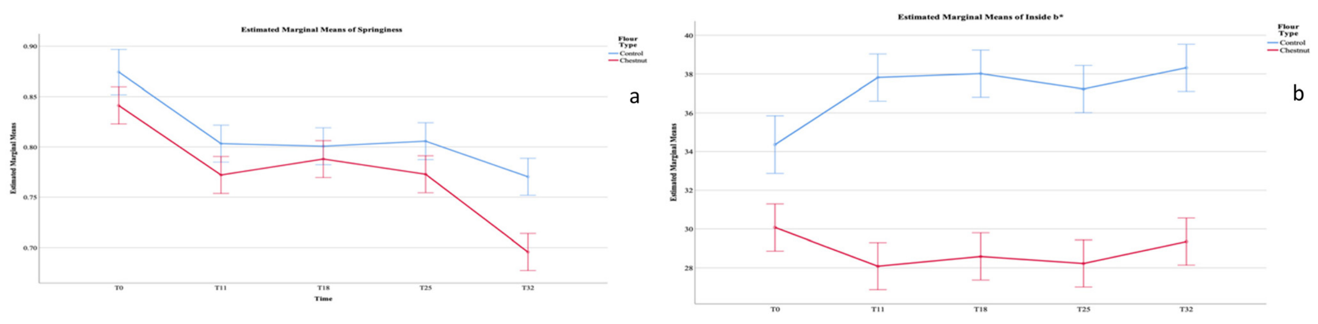

Fig. 2 EMM plots of springiness: (a) springiness (texture) and (b) the blueness-yellowness coordinates of the crumb of the "económicos".

significant. All other texture dimensions did not show a significant interaction between factors and could be classified. For hardness, the storage time showed a 70% influence on the increase of hardness over time, which was inversely correlated with adhesiveness that significantly decreased over time. Resilience and cohesiveness, both derived from hardness, were highly affected by the different flours used, in which the chestnut addition reduced bot these dimensions. For resilience, FT accounted for 45% of the variation, while for cohesiveness FT was responsible for 64%. Cohesiveness is defined as how a food can "fight" against a deformation induced by mastication; the addition of the chestnut to the flour could help make the cakes softer, although less springy. This result also shows correlation to resilience, which is the capacity of a food to resist a second deformation. Finally for the last dimension of the TPA, chewiness, once again, the factor with the highest contribution was storage time with 57%. Over time, this dimension increased, although only significantly from T0 to T11.

Firmness, which was obtained by cutting the cakes vertically in half using a texturometer special knife, showed a significant interaction, with the individual factors only accounting for 2% for each of the influence. No conclusions could be drawn from the EMM plots, and thus, this assay was quite inconclusive, meaning that both the ST and FT influence the variation, but their interaction was so strong that no meaningful conclusions could be sought.

Colour analysis. The color of the "económicos" was also measured in different parts of the cake, namely, the bottom (in contact with the heated surface during cooking), the inside (crumb), the top and finally the resulting crumb after reducing the cake to a powder, in order to understand the possible effect of the chestnut flour in all the different parts. The results of the color analysis are shown in Table 4, which followed the same scheme of presentation of the results as in Table 3.

By observing Table 3, it seems that for the bottom coordinates of color, the L^* (lightness) showed statistical differences between the control and the chestnut flour incorporated cake, meaning the incorporation showed a higher influence over the storage time. Inversely, the a^* and b^* coordinates in the bottom section were influenced mainly by the passage of time rather than the incorporation of the chestnut flour, but no statistical differences were found for either coordinates. Concerning the inside section, for L^* and a^*, both factors could be classified, showing that the incorporation of the chestnut flour showed a statistically significant difference, but the passage of time also had an influence, although with increasing and decreasing values of L^* and a^*, which could be linked to the stalling phenomena over the storage time. Considering b^* for the inside of the "económicos", a significant interaction was sought, but some considerations could be extracted from the EMM plots (Fig. 2b), in which the control

Table 3 Color analysis of the four different sections of both cakes

		Bottom			Inside			Top			Crumb		
		L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*
Storage time (ST)	0 days	41 ± 3	15.5 ± 0.7^a	31 ± 5^a	69 ± 5^b	2 ± 3^{a,b}	312 ± 3	53 ± 4	15 ± 2	36 ± 3	66 ± 7	8 ± 3	36.4 ± 3.9
	12 days	39 ± 4	14.8 ± 0.7^a	26 ± 4^a	65 ± 5^a	4.0 ± 0.5^b	33 ± 5	53 ± 4	15 ± 1	36.6 ± 2.6	65 ± 7	6 ± 2	34 ± 5
	18 days	38 ± 3	14.6 ± 0.5^a	25 ± 2^a	69 ± 4^b	2 ± 2^{a,b}	33 ± 5	52 ± 3	14.6 ± 0.8	35.7 ± 2.2	62 ± 5	4 ± 4	32.8 ± 2.7
	25 days	41 ± 5	14.5 ± 1.5^a	26 ± 5^a	68 ± 3^{a,b}	1 ± 2^a	33 ± 5	55 ± 3	15 ± 1	37 ± 3	62 ± 4	5.5 ± 3.6	34.1 ± 2.3
	32 days	41 ± 3	15.4 ± 0.4^a	28 ± 3^a	67 ± 4^{a,b}	2 ± 2^{a,b}	34 ± 5	55 ± 4	14 ± 1	36.5 ± 2.8	58 ± 3	6.3 ± 2.5	32.6 ± 3.1
p-Value (n = 15)	Tukey's HSD test	0.357	0.05	0.09	0.012	0.024	0.135	0.049	0.138	0.168	<0.001	0.051	<0.001
Flour type (FT)	Control	42 ± 3*	15.4 ± 0.5	29 ± 2	71 ± 2*	0.2 ± 0.6*	37 ± 2	57.3 ± 2.2*	14.0 ± 0.8	39 ± 1*	66 ± 5	3.7 ± 2.5	36.8 ± 2.3
	9% Chestnut	38 ± 3	15 ± 1	25 ± 5	64 ± 3	3.6 ± 0.7	29 ± 1	50.6 ± 1.4	15 ± 1	34.1 ± 0.8	59 ± 3	8.1 ± 1.5	31.1 ± 1.9
p-Value (n = 6)	Student's t-test	<0.001	0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × FT (n = 90)	p-Value	0.558	0.574	0.254	0.451	0.149	0.002	0.304	0.274	0.368	0.002	0.023	0.004

The standard deviation values should not be regarded as a measure of accuracy, but rather a range of values due to being composed of values recorded for different storage times and flour types. Each letter in each column represents a significant difference among samples, obtained from Tukey's or Tamhane's T2 test. The asterisk (*) represents a statistically significant difference by means of Student's t-test. All three assays were performed using a significance of 0.05.

samples displayed a yellower tone, while the incorporated cakes showed lower yellow and higher blue tones. Regarding the top section of the "económicos", no significant interactions were found, showing that the incorporation of the chestnut flour was responsible for the changes found on this section of the cakes. The control cakes showed a statistically significant lighter tone (lower L^*) when compared to that of the ones incorporated with the chestnut flour, while also showing yellower tones (b^*), but the difference in a^* was not significant. Finally, the crumb color was determined, which was achieved by grinding the cakes and reading its color coordinates, as a matter of holistically determining the differences between the color of each cake. All three coordinates (L^*, a^* and b^*) showed a statistically significant interaction, and thus, in this manner, general tendencies were extracted from the EMM plots (Fig. 3), in which the lightness (L^*) of the control sample gradually decreased over time reaching the same color at 32 days as the cakes incorporated with the chestnut flour. Regarding a^*, the incorporated cakes showed higher values (closer to red tones) and did not show much variation over time, while the control samples, which started at lower values tended to increase over time and achieved no differences between the samples at T32. Finally, for b^*, the higher values of the control sample showing yellower tones were maintained over time, although also tended to decrease towards the values of the chestnut incorporated cakes.

To further understand the effect of the incorporation of 9% of chestnut flour in the "económicos" cakes, the total color difference was calculated, along with the total hue and chroma values (Table 4). The color difference allows an understanding of how different two colors are, based on their color coordinates, and, in the case of the "económicos" the biggest difference was found for the inside of the cakes, while the lowest difference was registered in the bottom, probably due to the darkening by contact with the plate during baking.

The average difference, obtained by calculating the mean of all sections of the cakes, was set at 27.33, showing that in terms of difference, the chestnut flour showed significant changes compared to the control cakes. The hue of a color is the color tone, which can be calculated using the hue angle, showing different colors for the degree of the hue (hue = 0 – red; hue = 60 – yellow; hue = 120 – green; hue = 180 – cyan; hue = 240 – blue; hue = 300 – magenta). In this case, the hue was calculated for both cakes using eqn (2). The chroma is defined as the quality of the purity, intensity, and saturation of a color. Saturated (brilliant) colors show values very different from the L^* values, while pastel colors show chroma values very close to L^*. In this case, the chroma values were calculated using eqn (3). Regarding the hue values, all sections of both cakes were set near 60°, namely the yellow zone, although some did tend towards higher values of 89°. Considering the difference, in terms of the color tones, the chestnut incorporation only showed a statistical difference on the inside of the "económicos". Regarding the chroma values, considering the distance to the L^* values, the sections with a more brilliant chroma were in the inside of the cakes and their crumb, while

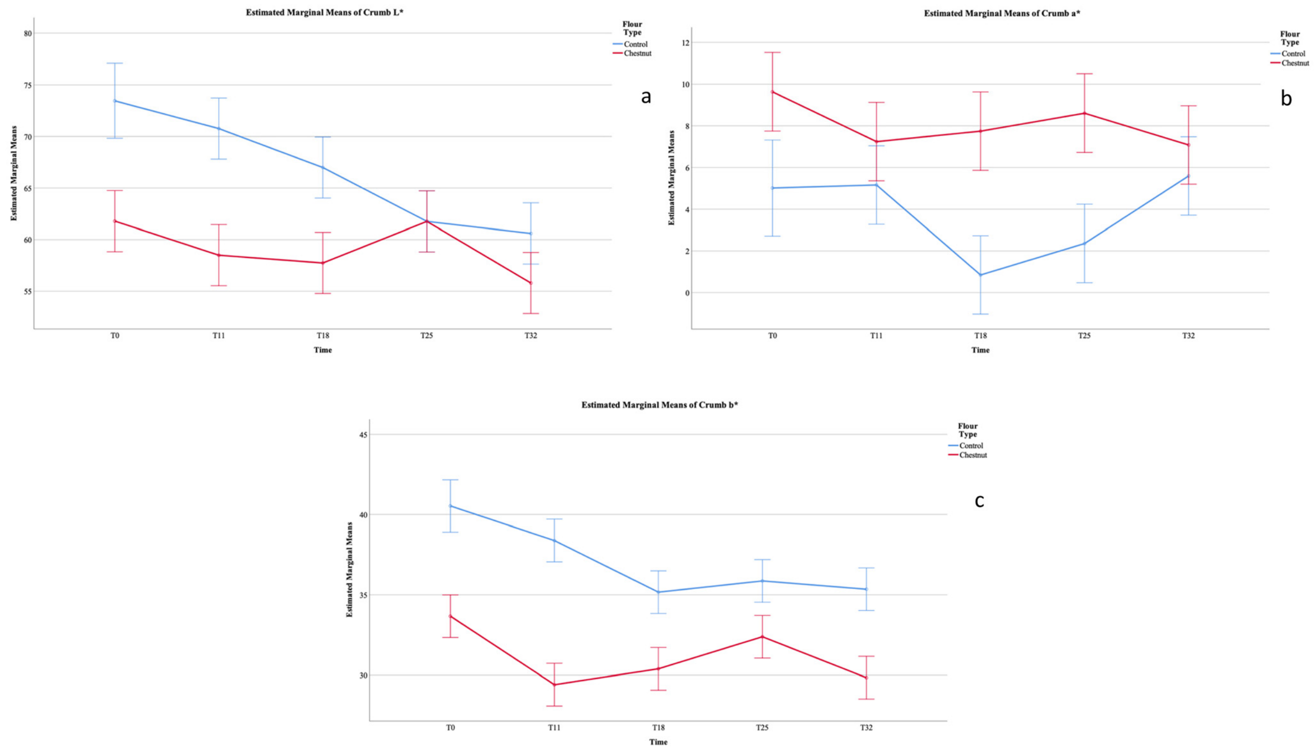

Fig. 3 EMM plots of the color coordinates of the crumb. (a) Lightness (L^*), (b) red-greenness (a^*), and (c) yellow-blueness (b^*).

Table 4 Total color difference (ΔE) and the hue values (in degrees °) and chroma values of the four sections of each cake

		Bottom	Inside	Top	Crumb
Color difference ΔE		20.01	71.78	30.78	40.75
Hue (°)	Control	62 ± 2	89.1 ± 0.8*	70.3 ± 0.6	84 ± 4
	9% Chestnut	59 ± 3	83 ± 1	66 ± 2	75 ± 1
Chroma	Control	33 ± 1(41)	34 ± 7(71)	41 ± 1*(57)	37 ± 2(66)*
	9% Chestnut	29 ± 5(38)	29 ± 1(64)	37.3 ± 0.3(50)	32 ± 2(59)

The standard deviation values should not be regarded as a measure of accuracy, but rather a range of values due to being composed of values recorded for different storage times and flour types. The asterisk (*) represents a statistically significant difference by means of Student's *t*-test, assuming unequal variances. All three assays were performed using a significance of 0.05. The values between brackets are the L^* values for each section and cake.

the other sections were set with more pastel (paler) tones. For this feature of color, the top section and the crumb showed statistically significant differences, where the control samples showed a more brilliant color.

Digital imaging. Table 5 shows three different features of the crumb of the "económicos", namely the number of holes in each cake, the average size of the holes and the circularity, allowing to understand how storage time and the incorporation of 9% chestnut flour influenced the crumb. A significant interaction was sought for both the "number of objects" and their average size, showing that both ST and ST influenced these features, although considering the partial eta squared (impact of each factor (results not shown)), it could be concluded that ST showed a higher effect than the storage time for both these features, while for the circularity, the main contributing factor for the reducing of circularity was the incorporation of chestnut flour, which reduced this parameter. This reduction could be attributed to the lack of gluten in the chestnut flour, which helps the development of uneven "holes" in the crumbs of bakery products. Still, the variation in the number and size of the "holes" over time could be linked to the stalling of the cakes, with migration of moisture from the crumb to the crust, which was not affected by the addition of the chestnut flour. Some of these results are shown in Fig. 4.

Chemical analysis

Centesimal composition. The nutritional profile analysis of the cakes was performed for all samples (Table 6), showing that the most abundant nutrients were carbohydrates, followed by crude fat. These results are in line with the ones published by Carocho *et al.*,[10] who also studied the use of natural preservatives in this type of pastry. Overall, a significant interaction

Table 5 Digital imaging features of the crumbs of the cakes, namely the number of objects, average size of "holes" and respective circularity

		Number of objects	Average size	Circularity
Storage time (ST)	0 days	30 ± 11	194 ± 23	0.40 ± 0.07
	12 days	37 ± 13	175 ± 36	0.36 ± 0.07
	18 days	28 ± 8	204 ± 41	0.4 ± 0.1
	25 days	31 ± 9	172 ± 30	0.36 ± 0.09
	32 days	31 ± 10	190 ± 32	0.38 ± 0.05
p-Value (n = 15)	Tukey's HSD test	0.650	0.095	0.849
Flour type (FT)	Control	29 ± 11	181 ± 32	0.42 ± 0.07*
	9% Chestnut	34 ± 10	198 ± 31	0.32 ± 0.06
p-Value (n = 6)	Student's *t*-test	0.253	0.075	0.001
ST × FT (n = 90)	*p*-Value	0.563	0.02	0.563

The standard deviation values should not be regarded as a measure of accuracy, but rather a range of values due to being composed of values recorded for different storage times and flour types. The asterisk (*) represents a statistically significant difference by means of Student's *t*-test, assuming unequal variances. All assays were performed using a significance of 0.05.

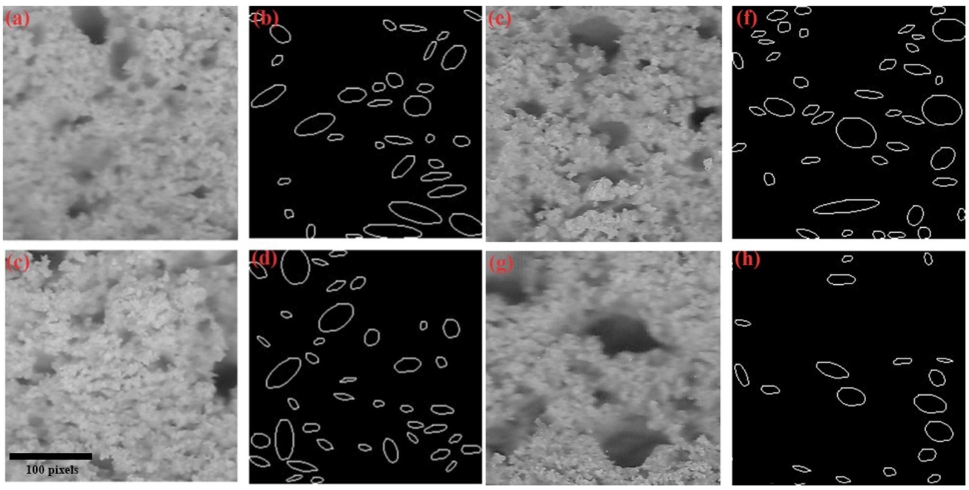

Fig. 4 Images of the cake crumbs and identified pores after the Otsu threshold conversion. (a) Control cake T0, (b) Otsu converted control cake T0, (c) control cake T32, (d) Otsu converted control cake T32, (e) chestnut flour incorporated cake T0, (f) Otsu converted chestnut flour incorporated T0, (g) chestnut flour incorporated cake T32, (h) Otsu converted chestnut flour incorporated cake T32.

Table 6 Centesimal composition of the "económicos" cakes, expressed in g per 100 g (fresh weight)

		Moisture	Fat	Protein	Ash	Fiber	Carbohydrates	Energy (Kj)	Energy Kcal
Storage time (ST)	0 days	13.6 ± 0.7	15.7 ± 0.7	6.9 ± 0.3	1.39 ± 0.09	4.3 ± 2.4	59 ± 3	1725 ± 37	412 ± 9
	11 days	12 ± 1	16.4 ± 0.9	7.1 ± 0.5	1.5 ± 0.2	4.1 ± 1.7	58 ± 3	1750 ± 16	418 ± 4
	18 days	12.5 ± 0.7	16 ± 1	7.2 ± 0.5	1.20 ± 0.07	3.3 ± 1.1	60 ± 1	1750 ± 29	418 ± 7
	25 days	12.5 ± 0.4	15.9 ± 0.8	7.2 ± 0.3	1.42 ± 0.05	4.5 ± 1.5	58 ± 1	1735 ± 26	414 ± 6
	32 days	12.5 ± 0.9	16 ± 1	7.8 ± 0.6	1.62 ± 0.09	3.1 ± 1.4	59 ± 3	1748 ± 9	417 ± 2
p-Value (n = 15)	Tukey's HSD test	0.001	0.422	0.060	<0.001	0.563	0.460	0.207	0.207
Flour type (FT)	Control	13.1 ± 0.8	16.6 ± 0.8*	7.4 ± 0.5	1.5 ± 0.2	4.2 ± 1.9	57 ± 2*	1744 ± 32	417 ± 8
	9% Chestnut	12.3 ± 0.8	15.4 ± 0.5	7.2 ± 0.5	1.4 ± 0.2	3.6 ± 1.3	60 ± 2	1740 ± 17	416 ± 4
p-Value (n = 6)	Student's *t*-test	<0.001	<0.001	0.298	0.040	0.323	<0.001	0.857	0.857
ST × FT (n = 90)	*p*-Value	<0.001	0.141	0.399	0.001	0.731	0.548	0.072	0.072

The standard deviation values should not be regarded as a measure of accuracy, but rather a range of values due to being composed of values recorded for different storage times and flour types. The asterisk (*) represents a statistically significant difference by means of Student's *t*-test. All assays were performed using a significance of 0.05.

was sought for moisture, ash and the energy value, although from the "partial eta squared", for ash and energy, the factor with the highest contribution was ST, while for moisture, the contribution was similar. Regarding fat, protein, fiber, and carbohydrates, the interaction among both factors was not significant, and thus each factor was evaluated individually. In terms of fat, significant differences were only found for FT, showing higher amounts of fat in the control cakes, being the same recorded for carbohydrates, although the highest amount was found in the cakes with chestnut flour. Proteins and fibers did not show significant differences among each factor, meaning the factors did not induce significant changes. Overall, the incorporation of 9% of chestnut flour did not result in very important changes of the nutritional profile of the cakes, however resulted in only a slight but significant increase in carbohydrates and reduction of fat, about 1 g in 100 g, which did not affect the energy value of the cakes.

Fatty acids. Table 7 shows the individual fatty acids found in the cakes, as well as the three types of fatty acids, based on the saturation of their bonds. Of the fifteen individual fatty acids identified, only the ones with at least 1% of the relative percentage were added to the table. Thus, the most abundant individual fatty acid was butyric acid (C4:0), a saturated acid with a relative short chain, followed by two unsaturated fatty acids, namely oleic and linoleic acids.

Overall, considering the ingredients of the cakes, the most abundant type of fatty acids was SFA, followed by MUFA and PUFA in ex aequo. Regarding the two factors FT and FT, a significant interaction was sought for all individual fatty acids, not allowing an understanding of the effect of each factor on the outcome, although the variations were not very high.

Chemical composition. Table 8 shows several features of the chemical analysis performed on the cakes, namely the only soluble sugar detected, sucrose, the two organic acids, oxalic and fumaric acid, as well as the pH and water activity. Sucrose, being the only soluble sugar detected is easily explained by the amount of sugar added to the batter, while only two organic acids were found. In a previous study, Carocho *et al.*[10] found quinic, malic, citric, and succinic acids, beyond oxalic and fumaric, while also finding traces of fructose and glucose. The average pH of the cakes did not suffer much change over the ST and FT, ranging from 6.3 to 7, while the aW was always under 75, revealing very little available water for microbial growth. A significant interaction was sought for all features of the chemical analysis, meaning that bot factors contributed significantly towards the outcomes. Still, through the EMM plots, the behavior of the total organic acids (mainly influenced by oxalic acid) showed that higher values of organic acids were detected in the samples with chestnut flour, and also that oxalic acid increases until the 18th day in the bot samples, but plateaus or slightly decreases after this day (Fig. 5). Regarding the pH values, through the partial eta

Table 7 Individual fatty acids of the "económicos" cakes, expressed as the relative percentage, as well as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)

		C4:0	C16:0	C18:0	C18:1n9c	C18:2n6c	SFA	MUFA	PUFA
Storage time (ST)	0 days	8 ± 1	1.6 ± 0.3	0.2 ± 0.03	2.8 ± 0.7	2.34 ± 0.07	10.5 ± 0.8	2.9 ± 0.7	2.4 ± 0.05
	11 days	9 ± 3	1.5 ± 0.5	0.3 ± 0.02	3 ± 1	2.5 ± 0.3	11 ± 2	3 ± 1	2.6 ± 0.4
	18 days	11.8 ± 0.9	0.7 ± 0.1	0.17 ± 0.03	1.57 ± 0.05	1.4 ± 0.3	12.8 ± 0.8	1.6 ± 0.05	1.5 ± 0.3
	25 days	11 ± 1	1.0 ± 0.4	0.19 ± 0.06	1.9 ± 0.6	1.6 ± 0.7	12.3 ± 0.9	1.9 ± 0.6	1.7 ± 0.8
	32 days	12.8 ± 0.7	0.59 ± 0.05	0.14 ± 0.01	1.20 ± 0.08	1.1 ± 0.2	13.6 ± 0.7	1.21 ± 0.08	1.1 ± 0.2
p-Value (*n* = 15)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Flour type (FT)	Control	11 ± 2	0.9 ± 0.4	0.22 ± 0.04	1.8 ± 0.5	1.9 ± 0.4	13 ± 1	1.8 ± 0.5	2.0 ± 0.5
	9% Chestnut	10 ± 3	1.1 ± 0.6	0.1 ± 0.06	2 ± 1	1.6 ± 0.8	11 ± 2	2 ± 1	1.7 ± 0.9
p-Value (*n* = 6)	Student's *t*-test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × FT (*n* = 90)	*p*-Value	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001

The standard deviation values should not be regarded as a measure of accuracy, but rather a range of values due to being composed of values recorded for different storage times and flour types. All assays were performed using a significance of 0.05.

Table 8 Soluble sugar (sucrose), organic acids, pH and aW of the económicos

		Sucrose (g per 100 g)	Oxalic acid (mg g^{-1})	Fumaric acid (mg g^{-1})	Total organic acids (mg g^{-1})	pH	aW
Storage time (ST)	0 days	29.9 ± 6.9	0.04 ± 0.03	0.0026 ± 0.0003	0.05 ± 0.03	7.0 ± 0.4	0.74 ± 0.04
	11 days	30.6 ± 7.7	0.05 ± 0.01	0.0012 ± 0.0007	0.05 ± 0.02	6.4 ± 0.3	0.74 ± 0.04
	18 days	33.7 ± 2.2	0.07 ± 0.01	0.002 ± 0.001	0.07 ± 0.01	6.4 ± 0.2	0.730 ± 0.003
	25 days	32.4 ± 2.4	0.06 ± 0.01	0.0012 ± 0.0005	0.06 ± 0.01	6.3 ± 0.1	0.71 ± 0.02
	32 days	31.4 ± 2.9	0.062 ± 0.009	0.0011 ± 0.0006	0.06 ± 0.01	7.0 ± 0.1	0.72 ± 0.03
p-Value (*n* = 15)	Tukey's HSD test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Flour type (FT)	Control	28 ± 4	0.04 ± 0.01	0.0010 ± 0.0007	0.04 ± 0.01	6.6 ± 0.3	0.75 ± 0.02
	9% Chestnut	35.2 ± 1.8	0.07 ± 0.01	0.0021 ± 0.0006	0.07 ± 0.01	6.6 ± 0.5	0.70 ± 0.02
p-Value (*n* = 6)	Student's *t*-test	<0.001	<0.001	<0.001	<0.001	0.495	<0.001
ST × FT (*n* = 90)	*p*-Value	<0.001	<0.001	0.002	<0.001	<0.001	<0.001

The standard deviation values should not be regarded as a measure of accuracy, but rather a range of values due to being composed of values recorded for different storage times and flour types. All assays were performed using a significance of 0.05.

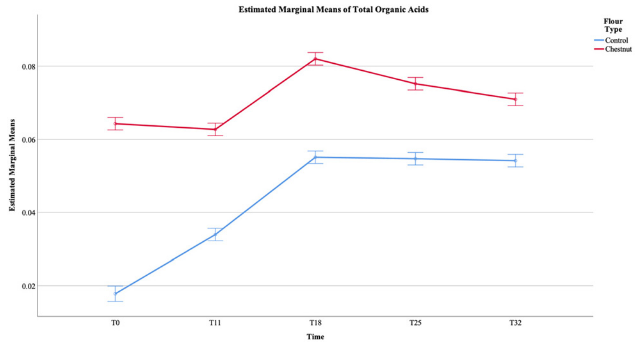

Fig. 5 EMM plots of the total organic acids.

squared, the factor with the highest contribution for variation in this parameter was ST, while the addition of chestnut flour did not show a high effect. No tendencies could be obtained for the other parameters, showing that the interaction of both factors was responsible for the outcomes.

Microbiological analysis

To ensure the quality and safety of the "económicos", it was verified whether the incorporation of chestnut flour influences the microbial growth of this pastry product, using the microorganism count over the shelf life (32 days). The growth of the tested microorganisms was not detected (aerobic mesophiles, coliforms, *B. cereus*, yeasts, and molds) during the 32 days of analysis, which means that the cooking process is able to eliminate possible microorganisms present in the ingredients of the "económicos".

Conclusion

To create innovations in the food sector, many strategies are used, namely the use of unconventional ingredients to increase the marketability and value of certain foods, and in some cases improvement or reduction of their nutritional value, improvement of bioactivity, among others. In this case, "económicos" were incorporated with gluten free flour from chestnuts to improve their aspects as much as possible. While tasting the cakes remains a future topic, the addition at 9% (maximum level without losing texture) was a success. Overall, only slight changes were found in terms of resilience and cohesiveness. The color of the crumb did show some changes, namely less lightness, which is usually linked to a lower caloric food. This fact was also corroborated with the chroma values, which were also lower, noting fewer brilliant tones of the crumb and top, while also showing lower hues, namely lighter tones. Considering the "holes" of the crumb, the only effect the chestnut flour had on them was a slight reduction in circularity. Finally, considering the nutritional profile, the flour reduced the total crude fat and increased the amount of carbohydrates, thus not changing the overall nutritional profile. Besides contibuting to the market diversification and valorization of this traditional product, this this work innovates in the improvement of the nutritional value as also in the presence of bioactive molecules in the cake. In the future, sensory analyses will be carried out to understand consumer acceptability of this innovative product.

Author contributions

Filipa A. Fernandes, Mariana C. Pedrosa and Jonata M. Ueda – investigation; Sandrina A. Heleno, Isabel C. F. R. Ferreira and Lillian Barros – conceptualization and review and editing; Márcio Carocho – data curation and writing – original draft; Elisabete Ferreira – methodology; and Paula Rodrigues and Miguel A. Prieto – methodology and data curation.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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