Original Research Article

Traditional pastry with chestnut flowers as natural ingredients: an approach of the effects on nutritional value and chemical composition

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Abstract

Portuguese traditional pastry known as *económicos* with satiating qualities, have been elaborated with chestnut (*Castanea sativa* Mill.) flowers and their decoctions. The complete nutritional profile, mineral content, free sugars, organic and fatty acids, and tocopherols were determined immediately after baking and also after 15 and 30 days of storage. The results were processed through a 2 way ANOVA, followed by a linear discriminant analysis to conclude that only slight effects were detected even in the assayed parameters, after 30 days. The amount of water decreased with time, resulting in a raise of ash, carbohydrates, energy and insoluble fiber over time. In terms of organic acids, succinic acid was the most abundant molecules, with the samples incorporated with the decoction showing the highest amounts of these acids. Sucrose was the highest sugar, although a decrease was detected overtime. Sodium and potassium were the most abundant minerals while zinc was the least. Finally, α-tocopherol was the most abundant isoform of tocopherols and palmitic acid the most abundant fatty acid. Polyunsaturated fatty acids tended to decrease along storage time. The use of dried flowers seemed to better preserve the original profile (control) of the *económicos* in comparison with the decoctions.

Keywords: Food analysis; Food composition; Traditional pastry; Castanea sativa; Nutritional/chemical profile

1. Introduction

The food industry is, not surprisingly, one of the most important markets in the world, which struggles to get food from producer to consumer in the best possible conditions at the least expense (Cheng et al., 2010). To maintain food products in conditions that are acceptable for consumption, the industry relies on different treatments (heat, pasteurization, water immersion, irradiation, among others) and on the introduction of food additives, which are used especially to maintain the best conditions during the final steps of preparation, expedition and shelf-life (Carocho et al., 2014a).

Nowadays, centuries after the exclusive use of salt, the oldest preserver, the conservation relies mainly on 3 principal types of additives: antimicrobials, like benzoates, sulphates and propionates; antioxidants, like ascorbic acid or butylated hydroxytoluene (BHT); antibrowning agents, like sulphites, ascorbic acid and cysteine (Carocho et al., 2014a). Some of these additives have legal constraints which vary among countries; in fact, it is possible to indicate specific additives allowed in the European Union and simultaneously forbidden in the United States of America (USA). Examples of these ambiguities are cyclamates, some color additives, *p*-hidroxybenzoate, or sodium sorbate (Fennema, 1987; Sindelar and Mikowski, 2012).

Due to the mentioned setbacks, many companies have searched for alternatives to chemical preservers, namely natural extracts from plants, which can display the same properties, often presenting the additional advantage to possess various bioactive properties or even synergistic effects (Si et al., 2005; Rasooli, 2007; Ye et al., 2013). Although there are many examples of food products in which incorporated plant extracts have been used as preservers and/or functionalization agents (McCarthy et al., 2001; Stojković et al., 2013; Reihani et al., 2014), none of those deal with the effects on the nutritional profile of the pastry, which is studied in this work. In Portugal, traditional cakes known as *económicos*, are quite appreciated for their taste. These products are

offered in groceries and food retailers and their preparation still follows the original recipe: flour, sugar, margarine, olive oil, eggs, brandy, cinnamon, orange juice and zest. In this work, dried chestnut flowers and flower decoctions were added to the original recipe aiming to preserve their nutritional and chemical properties, and extending their shelf life. Chestnut flowers were chosen for their high antimicrobial and antioxidant capacity (Carocho et al., 2014b, c), which could delay fat oxidation, rancidity and growth of moulds and/or bacteria, during storage. In addition, using chestnut flowers might boost the local agriculture with an increasing demand for these presently discarded by-products. From the consumers' point of view, the functionalized *económicos* might also deliver beneficial molecules (Carocho et al., 2014c), besides eliminating the use of chemical preservers. In fact, there have been studies claiming that infusions of chestnut flowers have beneficial effects against various illnesses, namely as mucolytic, antispasmodic and anti-dysenteric treatments, among others, due to the high antioxidant and antimicrobial properties of this matrix (Neves et al., 2009). They also proved to bring antioxidant potential to *económicos* after their use as natural ingredients (Carocho et al., 2014d), but until now there were no studies evaluating the effects of their incorporation on nutritional and chemical characteristics of the final product.

2. Materials and Methods

2.1. Standards and Reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade, acquired from Fisher Scientific (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, standards of sugars, tocopherols, and organic acids, and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-

carboxylic acid). Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA). Micro (Fe, Cu, Mn and Zn) and macroelements (Ca, Mg, Na and K) standards (> 99% purity), as well as $LaCl_2$ and CsCl (> 99% purity) were purchased from Merck (Darmstadt, Germany). Anthrone was obtained from Panreac (Barcelona, Spain). All other reagents were purchased from specialized retailers. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Flower collection and sample preparation

Chestnut (*Castanea sativa* Mill.) flowers of the cultivar Judia were collected near Bragança (Oleiros) in the north-eastern region of Portugal in June of 2013 (41º51'02''N, 6º49'54''W). After lyophilisation (FreeZone 4.5, Labconco, KS, USA) they were milled and stored at -5 ºC until further analysis. Lyophilisation removes water through sublimation to avoid degradation of the samples. The decoctions were prepared following the procedure previously described (Carocho et al., 2014b). For the decoctions preparation, the flowers were added to cold water and heated on a laboratorial hotplate at 105 ºC until the solution reached boiling point. It was left at this state for 5 minutes and another 5 minutes without heating before filtration. After freezing the solution, it was lyophilized and stored at -5 ºC until needed for the assays. This manuscript continues a study by Carocho et al. Initially a review was conducted regarding controversies surrounding food additives and the benefits of natural ones (Carocho et al., 2014a). Then, an exhaustive study was conducted on decoctions of chestnut flowers (Carocho et al., 2014b, c) namely on their antioxidant, antimicrobial and antitumor activities as well as individual molecules like polyphenols, sugars and organic acids. Finally, after incorporating the flowers in the cakes, the antioxidant activity of these samples was studied to detect the impact of the functionalization of the

chestnut flowers (Carocho et al., 2014d). The samples used in this study are the same ones used in the manuscript by Carocho et al., 2014d).

2.3. Preparation of the pastry

To prepare the cakes, a traditional recipe was followed: 6 eggs were thoroughly mixed with 500 g of sugar, 1.05 kg of flour, 45 g of margarine and 30 g of warm olive oil. Then, 230 mL of pure orange juice, 35 g of orange zest, 200 mL of milk, 45 mL of brandy and 25 g of cinnamon were sequentially added to the mixture while mixing vigorously. When the dough had the right consistency (when the dough was placed on a tray without losing its shape), it was divided into 5 lots of 500 g each.

2.4. Incorporation of dried flowers and decoctions

Of the 5 lots *i)* one was used as the control sample; *ii)* 2 lots were incorporated with the decoctions of the dried flowers at different concentrations: 50 mg according the EC_{50} value (0.099 mg/mL) achieved through the DPPH (2,2-diphenil-1picril-hydrazil) scavenging assay for chestnut flowers decoction (Carocho et al., 2014b), and 100 mg in the other lot; *iii)* the remaining 2 lots were incorporated with dried flowers, also at different concentrations: taking into consideration the yield of the decoction extraction of 20% for 1 g of flowers, 200 mg of flowers were added to 1 lot and 400 mg to the second one.

In both cases, the final concentrations of decoction in the prepared *económicos* were approximately 16 and 32 mg/mL of dough, since the used decoctions might be considered as an enriched extract in comparison to the raw flowers.

The control sample was labelled “C”, the decoctions “D50” and “D100” corresponding to the quantity of extract added, and finally “F200” and “F400” for the dried flowers.

After incorporation, each portion was divided into 6 cakes that were all baked at the same temperature of 170 ºC for 15 minutes on a tray in an oven.

2.5. Storage

Immediately after baking, two cakes of each lot were frozen and immediately lyophilized (Labconco FreeZone 4.5, Kansas City, MO, USA). After this, they were milled and subjected to the assays, which are further detailed. The rest of the cakes were left for 15 and 30 days in sealed plastic bags, protected from sunlight, at room temperature ranging from 18 to 23 ºC, and afterwards subjected to the same assays to determine the changes along the storage period.

2.6. Proximate composition

The nutritional value was calculated based on moisture, proteins, fat, ash, carbohydrates, and fiber, relying on the AOAC procedures (AOAC, 2012). Moisture was determined by desiccation to constant weight at 100 ± 2 °C. Total protein content (N × 6.25) was calculated as nitrogen content by the Kjeldahl method. Crude fat was determined by extraction of a known weight of powdered samples with petroleum ether using a Soxhlet apparatus. The ash content was determined by incineration at 550±15 ºC. Total available carbohydrates (TAC) were calculated through the Anthrone method, described previously (Osborne and Voogt, 1986). After treating the samples with $HClO_4$ at 52% for 18 hours away from light, distilled water was added and the volume was adjusted to 100 mL. Further dilutions were performed until it reached 10%, and the anthrone solution at 0.1% in H_2SO_4 was added. The samples were placed in boiling water for 12 minutes and the developed green coloration was measured at 630 nm in a UV/Vis Spectrophotometer (EZ210 Perkin Elmer, Waltham, Ma, USA). A calibration

curve of glucose (10-100 µg/mL) was prepared to be compared to the samples. TAC values are expressed as g glucose/100g of fresh cake.

The AOAC enzymatic-gravimetric methods (993.19 and 991.42) were employed to determine soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) respectively, as reported before (Latimer, 2012). The total dietary fiber results (g/100 g of fresh cake) were obtained from the addition of the soluble and insoluble fiber. Energy was calculated according to the equation of the European Parliament and Council Regulation, No. 1169 (EU Regulation): Energy (kcal) = 4 × (g protein + g total available carbohydrate) + 2 × (g fiber) + 9 × (g fat). Energy was expressed in kcal/100g of fresh cake.

2.7. Mineral composition: Macro and microelements

To determine the total mineral content (ashes) and mineral elements, the 930.05 AOAC method was used. Initially the samples were subjected to dry-ash mineralization at 550ºC. The resulting residue was extracted with HCl and HNO_3, and the volume was adjusted with distilled water. The microelements Fe and Zn were directly measured, while macroelements (Ca, Mg, Na and K) depended on an additional 1/10 dilution to avoid interferences. The methodology followed, previously reported (Fernández-Ruiz et al., 2004), relied on an atomic absorption spectroscope (AAS) with air/acetylene flame in an Analyst 200 Perkin Elmer equipment (Perkin Elmer, Waltham, MA, USA), comparing the absorbance responses with >99.9% pure analytical standard solutions for AAS made with $Fe(NO_3)_3$, Zn $(NO_3)_2$, NaCl, KCl, $CaCO_3$ and Mg. The results were expressed in mg/100 g of fresh cake.

2.8. Free sugars

Free sugars were determined through High Performance Liquid Chromatography (HPLC). The equipment was composed of a pump (Knauer, Smartline system 1000, Berlin, Germany), a degasser system (Smartline manager 5000) and an auto sampler (AS-2057 Jasco, Easton, MD, USA), coupled to a refraction index (RI) detector (Knauer Smartline 2300, Berlin Germany). The methodology has been previously described (Barros et al., 2010). Sugars were identified by comparing their peak retention times with commercial standards, with the data being analysed through the Clarity 2.4 software (DataApex, Prague, Czech Republic). Quantification was based on the RI signal response of each standard, relying on the internal standard (melezitose) method and using calibration curves of the standards. The results were expressed in g/100 g of fresh cake.

2.9. Organic acids

Organic acids were detected through Ultra-Fast Liquid Chromatography (UFLC). The chromatograph (Shimadzu 20A series, Shimadzu Corporation, Kyoto, Japan) was coupled to a photodiode array detector following the procedure previously described (Pereira et al., 2013). The preferred wavelengths were set at 215 and 245 nm. The quantification compared the area of the peaks with calibration curves of commercial standards of each compound. The results were expresses in g/100 g of fresh cake.

2.10. Fatty acids

Fatty acids were determined by gas chromatography. The equipment consisted of a gas chromatograph (GC) (DANI 1000, Contone, Switzerland) coupled to a split/splitless injector and a flame ionization detector (FID) as previously reported (Barros et al., 2010). The identification was carried out by comparing the relative retention times of

the fatty acid methyl esters of the samples (FAME) to commercial standards. The quantification was achieved through CSW 1.7 (DataApex 1.7, Prague, Czech Republic). The results were expressed in relative percentage of each fatty acid.

2.11. Tocopherols

Tocopherols were also determined by HPLC, coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA) using the methodology described previously (Barros et al., 2010). The detector was programmed for excitation at 290 nm and emission at 330 nm. The detected peaks were compared with commercial standards, with quantification relying on fluorescence signal, using the internal standard (tocol) method. The results were expressed in mg/100 g of fresh cake.

2.12. Statistical analysis

The three types of cakes, control, cakes incorporated with dried flowers and cakes incorporated with decocted flowers, were labelled, and two cakes of each group were used for the assays, which were carried out in triplicate. Data was expressed as mean±standard deviation, maintaining the decimal places allowed by the magnitude of the standard deviation. An analysis of variance (ANOVA) with type III sums of squares was performed using the general linear model (GLM) procedure using SPSS software. The dependent variables were analyzed using 2-way ANOVA with the factors "storage time" (ST) and "concentration" (C). When a statistically significant interaction was detected for these two factors, they were evaluated simultaneously by the estimated marginal means plots for both levels of each factor. Furthermore, if no statistically significant interaction was found, the means were compared using Tukey's multiple comparison test, with a previous assessment of the equality of variances through

Levene's test. A linear discriminant analysis (LDA) was used to compare the effect of ST and C over all the assayed parameters. A stepwise technique was applied, 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 (Zielinsky et al., 2014). The basic purpose of a discriminant analysis is estimating the relationship between a single categorical dependent variable (the cake formulation) and a set of quantitative independent variables (the values obtained in all the assays). Through this method, it is possible to determine which of the independent variables account most for the differences in the average score profiles of the different cakes. To verify the significance of the canonical discriminating functions, Wilk's 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 (López et al., 2008).

3. Results and Discussion

3.1. Nutritional and chemical parameters of the prepared cakes

The outstanding antioxidant and antimicrobial effects of chestnut flowers as well as their functionalizing capacity in cakes have been previously reported (Carocho et al., 2014b, c, d).

Tables (**1-4**) presenting the proximate composition, hydrophilic compounds, micro and macro elements, and lipophilic compounds, were divided in two distinct parts to simplify the interpretation of the results. The upper section refers to the samples incorporated with the decocted flowers, while the lower one concerns the samples incorporated directly with the dried flowers. In all cases, the results are presented as the mean value of each storage time (ST) of all concentrations, including different

functionalizing concentrations, and also the mean value of each concentration (C) of all storage times. With this approach, it was intended to identify the optimal ST independently of the used C of dried or decocted flowers, and also the best concentration of each functionalizing agent, irrespectively of the ST. Therefore, the standard deviations should not be regarded as a measure of accuracy of the applied methodologies, since they reflect results obtained from samples prepared in different conditions (variation of the non-fixed factor: ST or C). The interaction among both effects was also evaluated. Every time that a significant interaction was found (p<0.050), no multiple comparisons could be performed. In those cases, the influence of each factor was assessed by interpreting the estimated marginal means (EMM) plots.

Nutritional and chemical profiles were given in fresh weight (fw) to allow a more realistic notion about the dietary dose of each reported component. As it could be expected, the water content decreased along time (0 days: 14.3-18.7 g/100 g fw; 15 days: 14.7-18.0 g/100 g fw; 30 days: 11.0-13.0 g/100 g fw). The proximate composition, as well as the fiber profiles, are displayed on **Table 1**. As it can be deduced from the C×ST p-values, the interaction was significant in all cases. Furthermore, the effect of each individual factor was significant in most cases, except energy (decoctions), ash and carbohydrates (dried flowers), concerning the effect of C. From the EMM (data not shown) it was possible to obtain some tendencies. Samples functionalized with decoctions submitted to a ST of 30 days showed higher contents in ash and carbohydrates, higher energy values and lower protein levels. In terms of C effect, the only observable tendency was the higher content in fat presented by non-functionalized samples. Regarding fiber profiles, it is clear that insoluble fiber is present at higher concentration. Once again, ST and C proved to act cooperatively (p<0.050) in all cases; thereby, the next conclusions were obtained from the EMM plots. Cakes

added with 100 mg/g of decocted flower showed the highest contents in insoluble fiber and simultaneously the lowest in soluble fiber; concerning the ST effect, the 30 days period favoured the insoluble fiber level, probably due to the water reduction over time. The same effect (and the only one identifiable) was also observed in samples prepared with incorporation of dried flowers.

On **Table 2**, the composition in hydrophilic compounds, specifically free individual sugars and organic acids, is shown. Regarding the organic acids, different compounds were detected: oxalic, quinic, malic, citric, succinic and fumaric acids. The most abundant organic acid was succinic acid, although all organic acids are present in a very low quantity. The interaction between ST and C was significant in almost all cases, except for oxalic in samples functionalized with decocted flowers, and quinic acid in both types of functionalizated cakes. In samples added with decocted flowers, ST had a higher effect than that produced by C, which maintained malic (p = 0.486) and citric (p = and 0.981) acids unaffected. On the other hand, the effect of both factors was essentially the same in the *económicos* prepared with dried flowers, as it was significant (p<0.050) in all cases except for quinic acid. The main tendency observed for ST was the higher amount of quinic, malic, citric, succinic, fumaric and total organic acids in non-stored samples of cakes functionalized with decocted samples. Actually, this same trend was also verified for malic, succinic and total organic acids in samples prepared with dried flowers. Regarding the effect of C, the only detected differences were those resulting from Tukey's test classification, *i.e.*, the higher amounts of oxalic and quinic acids in samples functionalized with 50 mg/g and 100 mg/g of decocted flowers, respectively. In all the remaining, it seems apparently irrelevant adding 50 or 100 mg/mL, when using decocted flowers, or 200 or 400 mg/g, when using dried flowers.

Only three free sugars were detected: fructose, glucose and sucrose, with a high prevalence of the disaccharide, which was one of the food ingredients used in cake preparation. In addition, the monosaccharides were only detected after 15 days of storage and in a very low quantity, which implies a possible breakdown of sucrose into its two constituents. For all samples, once again, the interaction between ST and C was significant (p<0.050), so the tendencies of the sugars' behaviour were drawn through the EMM. Independently of functionalization type, sucrose tended to present the lowest values after 15 days of storage, tendency also observed for total sugars content.

Table 3 reports the minerals composition of the samples, namely Ca, Mg, Na, K as macrolements, and Fe and Zn as microelements. Sodium and potassium were the most abundant macroelements in all the samples, while zinc was the least. The interaction among ST and C was significant (p<0.050) in all mineral elements. Considering the EMM plots, it might be concluded that in samples incorporated with decoctions, ST induced some apparent changes, which might be due to the decrease in water content along time, or to some heterogeneity in the batter, which is quite normal in these types of cakes, that include various ingredients of different solubility.

Two different types of lipophilic compounds were studied: fatty acids and tocopherols, both presented in **Table 4**. A total of 23 fatty acids were detected (although many of them in trace amounts), among which the most abundant, along with the relative percentage of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were tabled (**Table 4**). The most abundant were palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:1n9c), linoleic acid (C18:2n6c), and linolenic acid (C18:3n3). For all samples, the interaction between C and ST was significant. In general, PUFA tended to decrease along time, which is in agreement with its higher propensity to oxidation processes. This variation caused an

increase in the relative percentages of SFA, which is nothing more than a direct consequence of their interlinked amounts. In terms of C effect, the only identifiable tendencies were observed in samples functionalized with decocted flowers, which shown higher levels of C16:0 and SFA when using 100 mg/g.

In terms of vitamin E, α-, β- and γ-tocopherol were detected, with prevalence of α-tocopherol. Once again, the interaction among the ST and C was significant for all samples, therefore the EMM were used to determine general tendencies. The incorporation of decocted flowers did not seem to exert a positive effect, since tocopherols content tended to be higher in control samples. In all remaining cases, no particular differences could be observed, either for C, as well as for ST effects.

Overall, the analysis of the results for each individual compounds did not allow to obtain relevant conclusions, such as the best storage time, the best functionalization type or the occurrence of significant profiling changes. Accordingly, all the obtained data were evaluated in an aggregative manner by applying linear discriminant analysis, in order to finally obtain the proposed conclusions.

3.2. Linear Discriminant Analysis

Aiming to acquire a comprehensive knowledge about each type of formulation, two different linear discriminant analysis were 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.

Initially, only the results for *económicos* functionalized with decocted flowers were assessed. Regarding the C effect, the discriminant model selected 2 significant functions, which included 100.0% of the observed variance. From the assayed 37

variables, the model selected 18 (fat, protein, soluble fiber, oxalic acid, quinic acid, fumaric acid, glucose, sugars, Ca, Mg, K, Fe, Zn, C16:0, PUFA, α-tocopherol, β-tocopherol and γ-tocopherol). The graph representation (**Figure 1**) indicates that function 1 separated primarily control and functionalized samples. Among the variables selected by the model, the highest correlation coefficients with function 1 were obtained for PUFA, α- and γ-tocopherol, which presented higher values in the control samples. The higher levels of tocopherols in the control samples could be due to overproduction of these metabolites (namely from the orange juice) due to the stress conditions that were attenuated in the samples with chestnut flowers (Sharma et al., 2012). The higher amounts of PUFA might be a direct consequence of the tocopherols quantity, since these are particularly active lipophilic antioxidant agents, which might have prevented more effectively the oxidation of PUFA. Regarding differences resulting from using 50 or 100 mg/mL, the separation among markers was only achieved through function 2, which showed the highest correlations with zinc, iron, quinic acid and soluble fiber, components detected in higher amount in samples added with 50 mg/mL of decoction.

Concerning the ST effect, function 1 (more correlated to glucose and fructose) separated mainly non-stored samples, a result that could be expected, since these monosaccharides were only detected in stored samples. In this case, the model selected also 18 (protein, ash, insoluble fiber, soluble fiber, succinic acid, fumaric acid, fructose, glucose, sucrose, Ca, Mg, Fe, Zn, C16:0, C18:1n9c, C18:2n6c, β-tocopherol and γ-tocopherol) variables. Differences among 15 and 30 days of storage affect mainly the variables more highly correlated with function 2, *i.e.*, ash, protein, insoluble fiber, sucrose and β-tocopherol, which were all (except protein) higher after a 30 days ST.

Considering samples prepared with direct incorporation of dried flowers, the results were different, since samples functionalized with the highest concentration (**Figure 1C**)

and stored for the longer period (**Figure 1D**) were placed close to the respective controls. Regarding the effect of C, function 1, which basically separated markers corresponding to the 400 mg/mL dose, was more highly correlated to potassium, sodium and fat contents. The selected variables were fat, protein, insoluble fiber, total fiber, quinic acid, fumaric acid, sucrose, sugars, Ca, Na, K, Fe, Zn, SFA and γ-tocopherol. Function 2, which allowed the discrimination among functionalized and control samples, correlated better with fat and Ca contents (higher in control samples), fumaric acid and Fe (higher in control samples). The interpretation of results for ST effect showed the major contribution of sucrose, fructose and C18:0 to separate the markers corresponding to the 15 days period and also the relevancy of the lower contents of ash, fructose, glucose and Ca in control samples, which discriminate these samples from those incorporated with dried flowers (function 2). In this case, the selected variables were fat, protein, ash, insoluble fiber, fumaric acid, fructose, glucose, sucrose, sugars, Ca, Na, C18:0, C18:2n6c and SFA.

For all the performed LDA, the classification performance was 100% accurate, either for original grouped cases, as well as for the cross-validated grouped cases. Nevertheless, the identified differences are not likely to affect the overall quality of the prepared *económicos*, since most of the variables were not considered to have discriminant ability, indicating their similarity.

Another interesting analysis was comparing the effects of incorporating plant decoctions or dried flowers. The results for this analysis are depicted in **Figure 2**. Starting by the ST effect (**Figure 2A**), three individual groups corresponding to the assayed periods were completely individualized, indicating that changes induced by ST are independent of the type of formulation. Function 1 was more highly correlated with fructose, malic acid, organic acids and Ca, while ash, fructose, sucrose and sugars were

the variables with highest correlation coefficients with function 2. In the case of C effect, four significant functions were defined, from which the first three are represented in **Figure 2B**. Function 1, more correlated with fat, C18:3n3, γ-tocopherol and total tocopherols, separated markers corresponding to samples functionalized with direct incorporation of dried flowers, maintaining those prepared with the decoctions close to the control. Function 2 was more effective in discriminating the 200 mg/mL decoction, mainly with the contribution of sucrose, quinic acid and γ-tocopherol, while function 3 allowed the complete separation of markers corresponding to control samples and to the 400 mg/mL decoction, mainly due to the contributions of fat, organic acids, Fe and Mg contents.

4. Conclusion

Overall, the results obtained in this work proved that both types of natural ingredients have slight effect on the chemical profiles of *económicos*. This is a very interesting result, since consumers might benefit from the bioactive compounds present in the decoctions or in the flowers without compromising the organoleptic quality, chemical and nutritional profile of this highly appreciated pastry, even when assayed after 30 days of storage time. Despite the suitability of both natural ingredients in maintaining the chemical profiles, the obtained results advice in favor of incorporating dried flowers directly, since this approach allowed the highest similarity with the control samples. It is relevant to highlight that these cakes were prepared following common practices; thereby, these findings will have an advantageous practical application, due to the fact that the incorporation may confer benefits without having to change their manufacturing.

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Conflict of interest

The authors state no conflict of interest regarding this manuscript.

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