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RESEARCH ARTICLE

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Active packaging to prevent lipid oxidation on Brazil nuts (*Bertholletia excelsa* HBK) stored under varying temperatures

Camila Maria Marinho Leme[1] | Amarilis Santos de Carvalho[1] | Vanessa de Carvalho Rodrigues[1] | Adriele Rodrigues dos Santos[1] | Ailey Aparecida Coelho Tanamati[1] | Odinei Hess Gonçalves[1,2] | Patrícia Valderrama[1] | Fernanda Vitória Leimann[1,2]

[1]Post-Graduation Program of Food Technology (PPGTA), Federal University of Technology, Paraná, Brazil

[2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

Correspondence
Patrícia Valderrama, Fernanda Vitória Leimann and Ailey Aparecida Coelho Tanamati, Post-Graduation Program of Food Technology (PPGTA), Federal University of Technology, Paraná, Campus Campo Mourão (UTFPR-CM), Rosalina Maria dos Santos Street, 1233, 87301-899, Campo Mourão, Paraná, Brazil.
Email: patriciav@utfpr.edu.br; fernandaleimann@utfpr.edu.br and aileytanamati@gmail.com

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Abstract

Brazil nuts are largely consumed either as a whole or as an ingredient in different food products. These seeds are rich in lipids that are susceptible to oxidation, which is the most common cause of deterioration in their sensory and nutritional quality. Active food packagings are a potential strategy to prevent food lipid oxidation. In this work, thermoplastic starch/poly (butylene adipate–*co*–terephthalate) (TPS/PBAT) containing water-soluble curcumin and pinhão (*Araucaria angustifolia*) extract as natural antioxidants were used to package Brazil nuts (*Bertholletia excelsa*). Packaged nuts were stored under different temperatures and analysed for up to 30 days of storage. The lipid profile of the oil extracted from the nuts before packaging showed that it is rich in unsaturated fatty acids. The UV–Vis spectra were organized into an augmented matrix and then into a principal component analysis (PCA). Results showed that for 10°C, the control TPS/PBAT film and the film containing pinhão extract resulted in the best preservation. When evaluated at 25°C, the nuts packed in films that contained water-soluble curcumin presented the best oxidative stability until the 15th day of storage. After this period, the oxidation reactions were significant for all samples, indicating a possible compromise on the sensory and nutritional quality of Brazil nuts.

KEYWORDS
active packaging, antioxidant packaging, *Araucaria angustifolia*, augmented matrix, curcumin

1 | INTRODUCTION

Nuts are dry, one-seeded fruit that do not open when they reach maturity and can be classified as tree nuts (such as almonds, walnuts, pecans, pistachios, cashews, hazelnuts, macadamia nuts and Brazil nuts) and groundnuts (peanuts that belong to the legume family).[1] The Brazil nut is the fruit of the *Bertholletia excelsa* HBK nut tree, which has received increasing interest in the last decade for the potential positive effects on human health.[2] Among the health benefits associated with Brazil nuts consumption are the atherogenic risk reduction in obese women,[3] the prevention of oxidative DNA damage in type 2 diabetes patients,[4] and the promotion of satiety without increasing blood glucose and insulin response.[5]

Many of Brazil nuts' health benefits are associated with its rich lipid composition (60%–70%) with a high content of unsaturated fatty acids (UFAs).[6] These UFA are vulnerable to oxidation leading to quality deterioration if stored inadequately or for too long. Oxidative rancidity is one of the major causes of quality deterioration in foods and is often caused by the oxidation of lipids by atmospheric oxygen.[1] Briefly, lipid oxidation is characterized by three phases: initiation,

propagation and termination. Initiation begins when hydrogen is abstracted from a fatty acid, thereby generating an alkyl radical (R•). The higher the degree of fatty acid unsaturation, the greater its susceptibility to lipid oxidation due to increasingly lower bond dissociation energies of methylene-interrupted carbons. Also, the formation of deleterious sensory products increases with increasing unsaturation.[7]

Oxidative rancidity in nuts is affected by external factors, for example, concentration of oxygen, temperature, light and relative humidity, and by intrinsic factors, for example, lipid composition, degree of unsaturation, free fatty acids, trace metals and antioxidants; the physical characteristics of the nut and the packaging material used.[1]

The active packaging materials are promising alternatives for preserving food quality. These materials interact with the packaged product or with the atmosphere inside the packaging to ensure the quality, protect the valuable nutritional components, prevent quality degradation and prolong shelf life.[8] Plant extract-based antioxidants can release the active compounds from the packaging to protect foods from free radicals, oxidative intermediates and secondary breakdown products.[9] Usually, the polyphenolics undergo single-electron transfer and hydrogen transfer mechanisms in edible films to protect the food product from lipid oxidation. In a single-electron transfer mechanism, the active compound donates an electron to the oxidant molecule of the food, resulting in the formation of a safe and stable compound.[10] The biodegradable packaging material composed of thermoplastic starch (TPS) and poly (butyl adipate-*co*-terephthalate) (PBAT) was already evaluated as active packagings for food application. Silva et al.[11] incorporated the *Araucaria angustifolia* (Bertol.) Kuntze extract (pinhão seeds boiling or cooking water rich in phenolic compounds, mainly [+]-Catechin) into this material and concluded that it presents potential antioxidant capacity and good mechanical properties. In another study, Mücke et al.[12] applied curcumin (in a modified composition to promote water solubility) in the same packaging material (TPS/PBAT) and concluded that it was able to protect chia oil from oxidation during the Schaal Oven Test.

In the case of Brazil nuts, some packaging strategies were already evaluated such as the application of active edible coatings,[13] vacuum packaging,[14] and packagings with O_2 absorbent pads, modified atmosphere (CO_2) and O_3 treatment.[15] This indicates that the application of active packaging added with natural antioxidants to preserve the oxidative stability of Brazil nuts is a promising alternative that deserves to be better investigated. This is particularly the case when they are commercialized after being crushed as a powder. In fact, Brazil nuts are commercialized either crushed to form a powder or as a whole. The challenge here is to keep the properties of the nuts after crushing since oxidation is potentialized by the increase of the surface area of the powder when compared to the whole nut.

Concatenate different data sets is emerging as a branch in chemometrics since the possibility to join results of different analytical methods (conventional and alternative) for a set of samples can enhance the quantity and quality of the information, which can be extracted.[16] Here, the concatenation was considered from the same analytical technique (UV–Vis spectroscopy) to simultaneously analyse different mixtures of the same compounds under different conditions (in this case, the time in days). This approach is a bright and reliable way of extracting information about the individualities of the systems.[17,18]

In the present work, TPS/PBAT packaging containing *A. angustifolia* (Bertol.) KKuntze seeds extract or water-soluble curcumin was used to pack Brazil nuts. The oxidative stability of the nuts was also evaluated during 30 days at different temperature conditions (10, 25 and 50°C) using UV–Vis spectroscopy and principal component analysis (PCA) coupled with augmented matrices approach.

2 | MATERIALS AND METHODS

2.1 | Materials

The Brazil nuts were purchased by e-commerce (Viva Salute, São Paulo, SP, Brazil, which is located 700 km from the university where the experiments were done) and transported in a vacuum package protected from light. Isooctane (Sigma-Aldrich) and *n*-heptane were used to dilute the oil sample for chromatographic analysis. Methyl tricosanoate (C 23:0) (Sigma-Aldrich, chromatographic standard) was used as a standard in gas chromatography (GC). The standards used to identify the fatty acids present in the samples Brazil nut oil were: methyl myristate (C 14:0), methyl palmitate (C 16:0), methyl stearate (C 16:0 18:0), oleic acid methyl ester (C 18:1), elaidic acid methyl ester (C 18:1), linoleic acid methyl ester (C 18:2), methyl linolelaidate (C 18:2), methyl linolenate (C 18:3), methyl arachidate (C 20:0) and methyl behenate (C 22:0) (Sigma-Aldrich, F.A.M.E. mix C 14-C 22).

The active packages composed of TPS/PBAT were produced using the reactive extrusion process based on the works of Silva et al.,[11] who incorporated in it the pinhão aqueous extract (cooking water, CW films) at 0.5% w/w, and described by Mücke et al.,[12] who incorporated into the polymeric matrix a water-soluble form of curcumin (WSC films) at 0.5% w/w. A control film formulation was also used without the addition of pinhão extract or curcumin (CF).

2.2 | Brazil nuts proximate composition

The proximate composition of Brazil nuts was carried out as described by Instituto Adolfo Lutz[19] as follows. The gravimetric method was used to determine the moisture at 105°C until obtaining constant weight.[19] To evaluate the ash content, the Brazil nuts were incinerated in muffle at 550°C. The lipids were determined by the Bligh & Dyer method and the protein content was obtained by the Micro-Kjeldahl method with a correction factor of 6.25. Carbohydrates content was calculated by difference. All values are presented in dry basis and as mean ± standard deviation.

2.3 | Brazil nuts preparation and packaging

The nuts were coarsely crushed in a domestic processor (Britânia Mixer) for 20 s to expose the oil from the nuts and immediately packed. The films (CW, thickness = 98 ± 9 μm; WSC, thickness = 116 ± 14 μm, and CF, thickness = 115 ± 9 μm) were cut in the form of 25 × 10 cm bags (diameter of the blown bag equal to 25 cm) and used to pack 100 g of the crushed nuts. A sealing machine (Tecfag, Brazil) was used to close two sides of the packages. Packages were filled under air conditions to simulate the way they are often commercialized. In Figure 1, it is possible to observe the Brazil nuts in their whole form, crushed and the packages filled with the crushed Brazil nuts. There was no oxygen remotion.

The packages were stored inside hermetic pots, and these were taken to a refrigerator (Consul, Brazil, 10 ± 1°C), to a BOD (Tecnal, Brazil, 25 ± 1°C), or to an oven (Labstore, Brazil, 50 ± 1°C). Samples were collected for analysis at 0, 5, 10, 15 and 30 days. The hermetic pots were used to preserve packaging mechanical properties since in previous tests it was detected a fragilization behaviour for the packaging stored at 50 ± 1°C. Thus, to guarantee that all samples were submitted to the same storage conditions, the hermetic pots were applied for all cases. All analyses in this study were performed in triplicate.

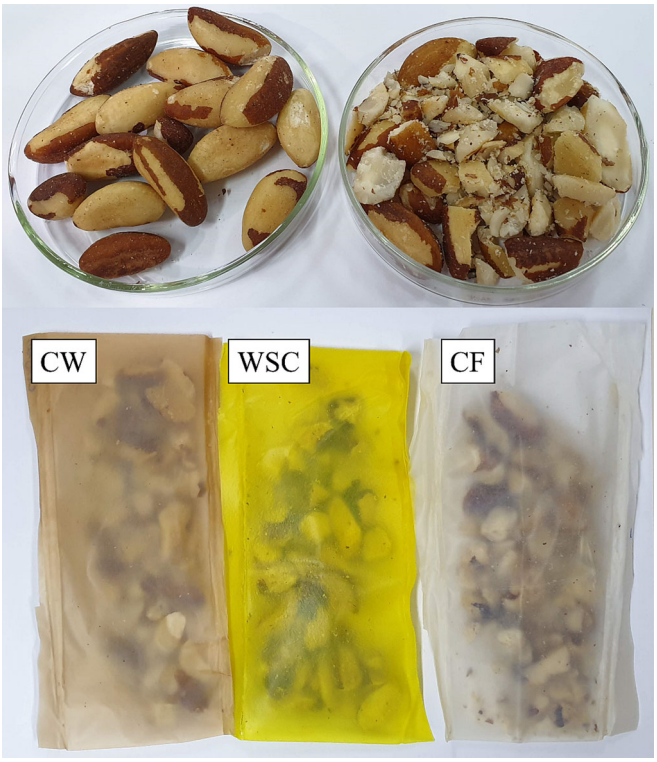

FIGURE 1 Brazil nuts in their whole form and crushed to be packaged. Packages of TPS/PBAT incorporated with pinhão aqueous extract (CW film, 0.5% w/w), incorporated with a water-soluble form of curcumin (WSC films) at 0.5% w/w, and the control film formulation without the addition of antioxidants (CF).

2.4 | Oil extraction from Brazil nuts packed with TPS/PBAT films

The oil from Brazil nuts was extracted by cold pressing using a hydraulic press (Bovenau, P15 ST), followed by oil centrifugation (refrigerated centrifuge, NT 815, Novatécnica) to ensure that the oil did not contain any suspended particles. The oil samples were frozen in an ultra freezer (−90°C) until the analysis.

2.5 | Esterification and GC

The quantification of fatty acids in the sample without contact with the packagings was performed by GC using methyl tricosanoate (23:0) as an internal standard according to the transesterification method of Kartman and Lago,[20] described in Milinsk et al.[21] Fatty acid methyl esters (FAMEs) were separated on the chromatograph and identified by comparison of retention time and addition of standards (Sigma-Aldrich, F.A.M.E. Mix C14-C22). A gas chromatograph (Shimadzu, GC-2010 Plus AF) equipped with a Split/Splitless capillary injector, flame ionization detector (FID), automatic flow and pressure controller, and capillary column with 100% dimethyl-polysiloxane phase, model Rtx-1 (30 m × 0.25 mm × 0.25 μm), was used. Other conditions of esters injection, as well as gas flows (hydrogen, nitrogen and hydrogen/synthetic air, White Martins), were performed according to Milinsk et al.[21] Initially, 1000 μL of the 1 mg/mL standard in isooctane were added to a test tube, and the solvent was evaporated under a stream of nitrogen. Next, 20 to 30 mg of oil were weighed, and 4 mL of 0.5 mol/L NaOH aqueous solution in methanol were added, stirring for 30 s, and heated under reflux for 5 min. Subsequently, 5 mL of the esterification reagent were added, and the mixture was stirred for 30 s and heated again under reflux for another 5 min. Then, 4 mL of saturated NaCl solution was added and stirred for 30 s. The organic phase was collected, the solvent evaporated on a rotary evaporator and the residue was removed with a stream of nitrogen. The methyl esters were solubilized in n-heptane for subsequent injection into the gas chromatograph. The transesterifications were performed in triplicate.

2.6 | Oxidative stability of Brazil nut oil

The oil extracted from the nuts packed with the active films was evaluated by UV–Vis Spectroscopy (Ocean Optics model USB-650-UV–VIS) in a 1 mm quartz cuvette. Oil absorbance was measured from 200 to 800 nm with a resolution of 1 nm.

The spectra were pre-processed with the baseline correction made by the algorithm available in PLS-Toolbox 5.2 (Matlab software version R2007b), and the PCA was applied with mean-centre pre-processing. For PCA, all data were coupled into an augmented matrix (hypermatrix). The spectral data from the initial day of evaluation (Day 0) were the first one in all cases, and it was combined with the spectral data obtained on Days 5, 10, 15 and 30 for each package system.

After that, the hypermatrix with the fused data was analysed with PCA.

PCA is an exploratory data analysis tool that searches for trends or similarities in a data set as well as highlights and extracts information from a data set.[22] The PCA basis concerning in decompose an **X** matrix (UV–Vis spectra in this case) into a product of two other matrices: scores (**T**) and loadings (**P**).[23] Through the scores, it is possible to verify trends among samples. On the other hand, the loadings indicate which variables are responsible for those similarities observed in the scores.[24,25]

3 | RESULTS AND DISCUSSIONS

3.1 | Brazil nuts proximate composition and fatty acid profile

The proximate composition determined for the Brazil nuts was: 2.03% ± 0.03% of moisture, 3.66% ± 0.06% of ashes, 14.90% ± 0.27% of proteins, 38.62% ± 3.82% of lipids and 40.79% ± 3.51% of carbohydrates. These results are in accordance with the findings of Sartori et al.[26] and Freitas et al.[27] Still, according to Freitas et al.,[27] edible seeds and nuts, such as the Brazil nut, have high contents of lipids, dietary fibre, ashes (minerals) and proteins with a good essential amino acids profile, usually with a slight lysine deficiency; however, for a typical Brazilian diet, this is not a nutritional problem, since this amino acid is found in beans.

The fatty acid composition of the Brazil nuts sample used in this study is shown in Table 1. It can be noted that Brazil nut oil is composed mainly of UFAs, approximately 74%. The main UFAs found were oleic acid (31.31% ± 0.33%) and linoleic acid (44.43% ± 0.69%). Palmitic acid (13.03% ± 0.04%) and stearic acid (10.68% ± 0.38%) were the saturated fatty acids (SFAs) determined in higher concentrations on the Brazil nut oil sample.

In the present work, linoleic acid was quantified as the FA present in the major concentration, followed by oleic acid. In the work developed by Cornelio-Santiago et al.,[28] who extracted the oil of Brazilian nuts by pressurized liquid extraction (isopropyl-alcohol and ethyl alcohol as solvents) the major concentration of FA found was for oleic acid (~38%), followed by linoleic acid (~30%). On the other hand, the same trend observed in the present work was found by Sartori et al.,[29] for cold-pressed Brazil nut oil, 39.52% for linoleic acid and 32.79% for oleic acid. These differences must be a result of the different extraction methods applied, as well as of different climate conditions and geographic regions where these nuts were cultivated.[30]

The products derived from lipid oxidation are classified as primary and secondary. Primary products comprise all intermediate products formed early in the oxidation process, including hydroperoxides. The decomposition products of these compounds belong to the secondary oxidation class. The products formed in lipid oxidation strongly depend on the fatty acid composition of the vegetable oil.[31] The highly polyunsaturated nature of Brazil nut lipids makes them susceptible to oxidative instability during their processing and shelf-life.[32]

TABLE 1 Fatty acid composition for the Brazil nuts (before packaging). Results expressed as mean ± standard deviation of three transesterifications.

Fatty acids (FAs)	Concentration (mg_{FA}/g_{oil})	FA percentage (%)
C16:0 (palmitic acid)	136.61 ± 6.41	13.03 ± 0.04
C16:1n-9c (palmitoleic acid)	1.87 ± 0.17	0.18 ± 0.01
C17:0 (heptadecanoic acid)	0.7 ± 0.04	0.07 ± 0.00
C18:0 (stearic acid)	110.28 ± 1.56	10.68 ± 0.38
C18:1n9c (oleic acid)	321.19 ± 12.70	31.31 ± 0.33
C18:2n6c (linoleic acid)	450.18 ± 29.40	44.43 ± 0.69
C18:3n3 (α-linoleic acid)	0.71 ± 0.06	0.07 ± 0.00
C20:0 (eicosanoic acid)	1.78 ± 1.03	0.17 ± 0.10
C20:1n-9C (9-eicosenoic acid)	0.62 ± 0.03	0.06 ± 0.01
Saturated fatty acids (SFAs)	249.37 ± 6.68	23.95 ± 0.50
Monounsaturated fatty acids (MUFAs)	323.68 ± 12.70	31.55 ± 0.33
Polyunsaturated fatty acids (PUFAs)	450.89 ± 29.40	44.50 ± 0.69
n-6	450.18 ± 29.40	44.43 ± 0.69
n-3	0.71 ± 0.06	0.07 ± 0.00
n-6/n-3	630.33 ± 69.01	631.40 ± 31.18
Unsaturated fatty acids (UFAs)/SFA	1.81 ± 0.13	1.86 ± 0.05

The high levels of fatty acids in Brazil nuts make this product and its derivatives susceptible to deterioration since oxidation processes can occur and lead to a reduction in their nutritional value. In addition, lipid oxidation can give the product undesirable odours and flavours, especially when they are stored for a long time at high temperatures and relative humidity.[33] By considering those possibilities, Brazil nut oil seems to be a feasible food model to investigate differences among package materials.

3.2 | Influence of the active packaging and temperature on the oxidative stability of Brazil nuts

The UV–Vis spectra obtained for Brazil nut oil samples collected on Days 0, 5, 10, 15 and 30 of storage, from different package materials, are shown in Figure 2. The augmented matrix scheme is also presented. For all the storage conditions and types of packaging used, the nut oils with up to 15 days of storage had similar spectra to the analysed nut oil on Day 0, while the oil spectrum on Day 30 differed from the other spectra. However, the UV–Vis spectroscopy is a non-selective analysis, making it difficult to conclude concerning the oil oxidation just by observing these spectra. Then, the augmented matrices approach, with normalization in the hypermatrix, was evaluated by PCA to improve the interpretation of the results.

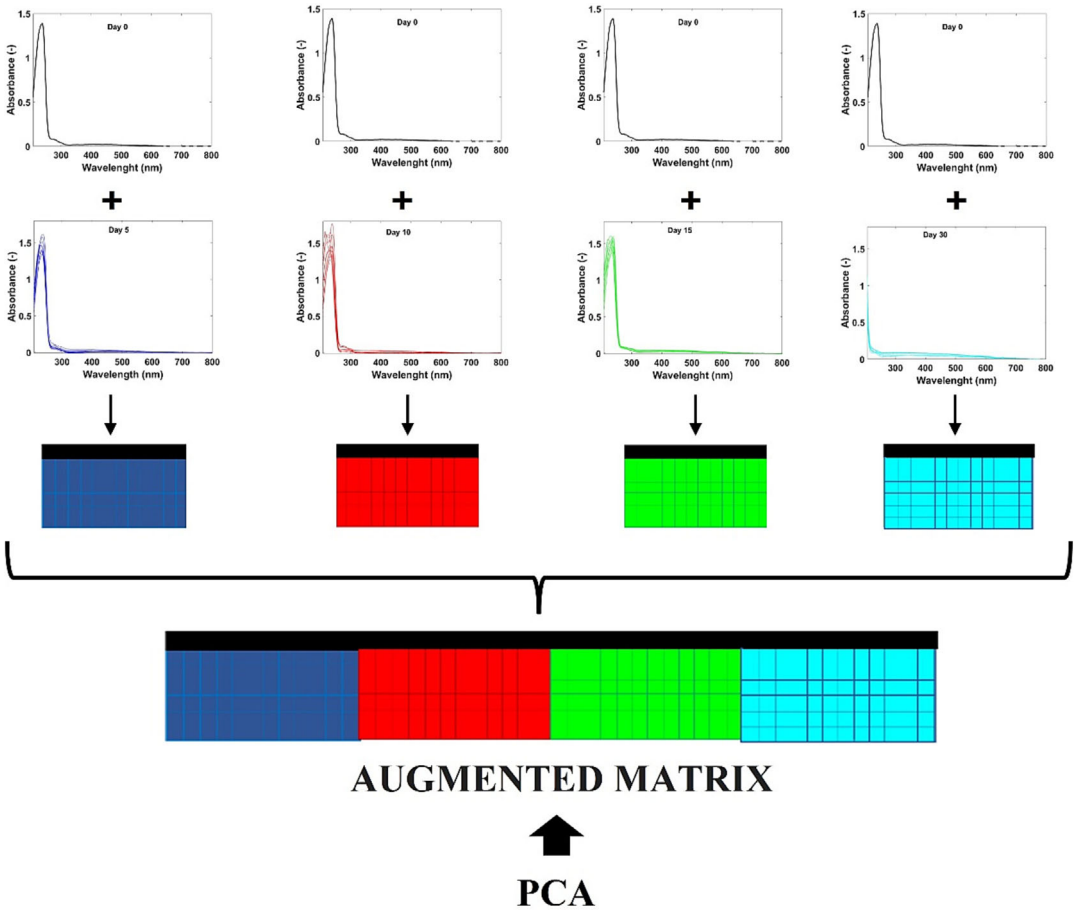

FIGURE 2 UV–Vis spectra of Brazil nut oil extracted from samples stored at different temperature conditions (10, 25 and 50°C) and packaging types (CW, pinhão aqueous extract film; WSC, water soluble curcumin film and CF, control film) grouped by storage time. Black spectrum on Day 0; blue spectrum on Day 5; red spectrum on Day 10; green spectrum on Day 15; and cyan spectrum on Day 30.

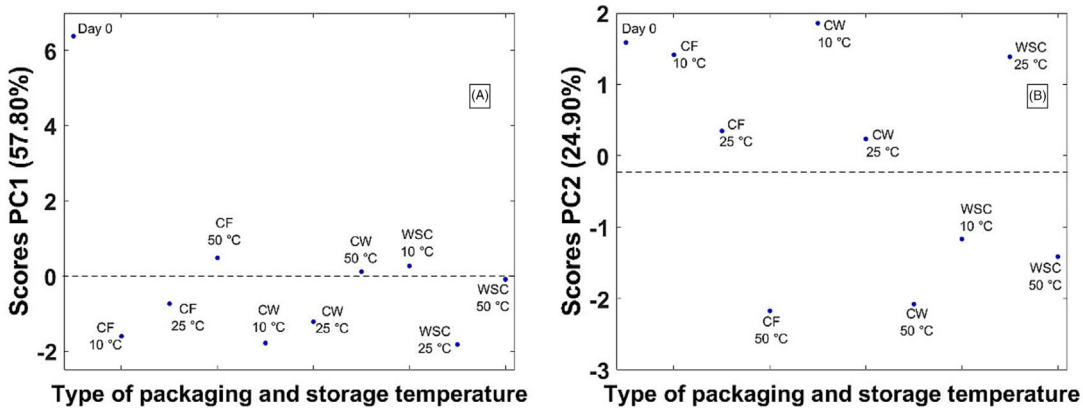

FIGURE 3 Storage time evolution for scores values from PC1 (A) and PC2 (B) for each packaging type (CW, pinhão aqueous extract film; WSC, water soluble curcumin film and CF, control film) at the storage temperatures of 10, 25 and 50°C.

Figure 2 shows how the augmented matrix was obtained, which is defined as the concatenation between two or more matrices of bilinear data from different systems, which share some or all of their compounds in a third direction, representing the qualitative or quantitative difference between samples. Thus, simultaneously analysing different mixtures of the same compounds under different conditions (in this case, the analyse day and packaging system) is a bright, reliable way to extract information about the systems' individualities.[17,34]

The PCA applied to the augmented matrices allowed the obtention of important information from the scores and loadings graphs.

And yet, based on this approach, it was possible to assess which portion of each block had the greatest influence on the dispersion presented in the scores. Two principal components (PCs) were able to retain more than 82% of the explained variance in the fused matrix (hypermatrix; 57.8% on PC1 and 24.9% on PC2), providing relevant information to explain the behaviour of Brazil nut oil during its storage in different temperature conditions (10, 25 and 50°C) and packaging materials. The PCA evaluation and data concatenation strategy were also used in the evaluation of Brazilian monovarietal olive oil in two different packaging systems.[16]

Analysing the scores presented in Figure 3, it is possible to verify that, in different packaging systems, Brazil nut oil was affected differently by storage conditions. It is evident that the samples along PC1 are very distant from Day 0 (oil sample extracted from Brazil nuts not submitted to storage), indicating that this PC is able to differentiate the non-packaged sample from the packaged ones. However, along PC2, it is possible to observe that there was a similar behaviour between the samples packaged in the CF (package without antioxidants) and CW (package added with pinhão extract) both stored at 10°C, the sample packaged in WSC (package added with water-soluble curcumin) submitted to storage at 25°C, and the sample from Day 0.

PC1 loadings (Figure 4) revealed that with up to 10 days of storage the extracted oils show similar behaviour. However, after 15 days, the primary oxidation (α,β ethylene 310–330 nm; tetraene 310–320 nm) and secondary (390–550 nm)[35] products begun to exert great influence on the distinction between the oils extracted after packaging and storage with the oil extracted without packaging at zero time.

Regarding the PC2 loadings (Figure 5), similar loading values were determined for wavelengths above 280 nm, where the correlation coefficient between those loadings on those regions was 0.9. This suggests that the oxidation products found after 30 days of storage and packaged in CF (control packaging) and CW (pinhão extract) packagings when stored at 10°C, as well as the samples of Brazil nuts packaged in WSC at 25°C were similar. In practical terms, this may be important because Brazil nuts are often commercialized at room temperature but are stored in the fridge during consumption.

The absorption in the UV–Vis region in the range of 200 to 230 nm can be attributed mainly to primary oxidation products that result from factors such as auto-oxidation. The main components of primary oxidation products are dienes, which have maximum absorption between 220 and 230 nm.[36,37] In addition to the absorption of this compound, the absorption of tocopherol from 220 to 300 nm is also reported.[35] According to O'Brien, Farr and Wan,[38] it is possible to find in small concentrations in Brazil nut oil molecules of tocopherols, diglycerides, phospholipids and carotenoids. Chunhieng et al.[39] evaluated the presence of phospholipids, tocopherols and sterols in Brazil nut oil. The authors found a total tocopherol content of 0.06 mg/g of the full nut, being β-tocopherol (88.3%) the main isomer identified. Other authors also identified and quantified tocopherols in Brazil nuts oil, such as Funasaki et al.[40] who found a total of 234.26 $\mu g/g_{oil}$ and the γ isomer (159.78 $\mu g/g_{oil}$) was the major fraction among the isomers.

Therefore, PCA results suggested that the crushed nuts can be packed in films of TPS/PBAT without antioxidants and stored at 10°C, since the packaging with antioxidants at this temperature presented the same behaviour. At this temperature range, a higher

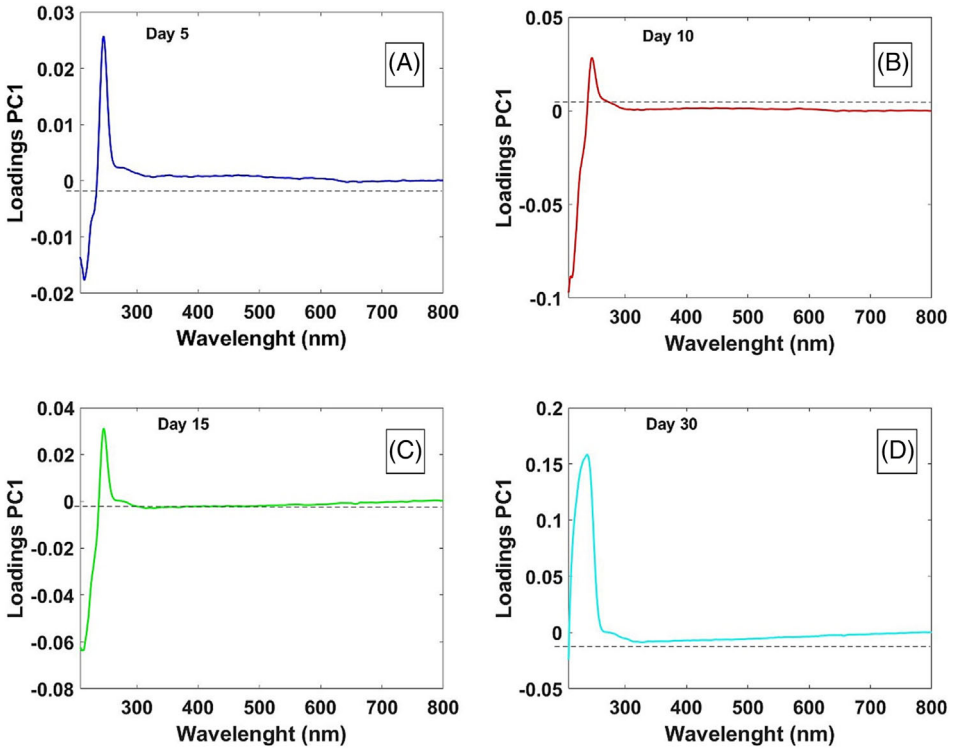

FIGURE 4 PC1 loadings for (A) Day 5, (B) Day 10, (C) Day 15 and (D) Day 30.

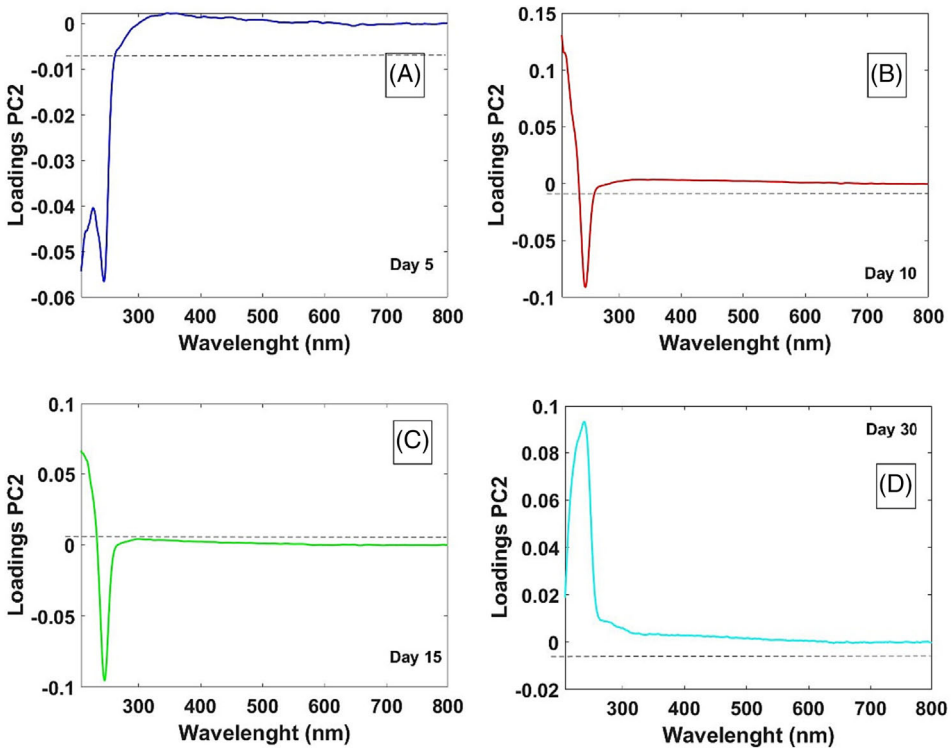

FIGURE 5 PC2 loadings for (A) Day 5, (B) Day 10, (C) Day 15 and (D) Day 30.

activity of lipase enzymes is reported in the literature for sunflower seeds storage.[41] These lipases, which are produced by fungi present on the surface of the nuts,[42,43] are responsible for enzymatic oxidation or hydrolytic rancidity,[44] another radical-based reaction that can occur on nuts.[1] Zanotto et al.[43] have isolated from Brazil nuts phytopathogenic fungi that showed hydrolytic activity. Thus, it is possible that under this storage condition (10°C) none of the packaging materials could inhibit lipase action.

However, at 25°C, nuts packaged in films containing water-soluble curcumin as an antioxidant compound were the most preserved from oxidation. Then, until 5 days, all package materials at all storage temperatures could preserve samples from the primary oxidation products, while differences started to be observed after Days 10 and 15 concerning primary oxidation compounds. Regarding tocopherols, until 15 days, the samples on package materials CF (control film) and CW (pinhão aqueous extract film) stored at 10°C, as well as samples of Brazil nuts packaged in WSC (water soluble curcumin film) stored at 25°C are similar to the content on Day 0. Day 30 showed differences in the primary oxidation products and tocopherol characteristics.

If high temperatures are sustained, the hydroperoxide is decomposed into different secondary products, such as aldehydes, ketones, alcohols, hydrocarbons, furans and epoxides, depending on the type of fatty acid that is being oxidized. Some of these secondary products are potentially toxic to humans, and they are responsible for the characteristic rancid flavour present in the oxidized fats and oils. This reaction is considered the primary factor limiting the shelf-life of nuts, which results in both economic losses and health hazards.[32]

4 | CONCLUSIONS

TPS/PBAT packaging films were added with the *A. angustifolia* seeds extract and water-soluble curcumin to improve the oxidative stability of packed foodstuff. Brazil nuts were chosen as a food model due to the high concentration of polyunsaturated fatty acids. Oxidative stability was evaluated for 30 days at different temperature conditions. PCA coupled with augmented matrix showed that the nuts packed in TPS/PBAT films without adding natural antioxidants and containing the pinhão extract could be stored at a temperature of 10°C without any deterioration of the oil for 15 days. The best preservation result was found for Brazil nuts packed in active films that contain water-soluble curcumin as a natural antioxidant. This packaging allowed Brazil nuts to be stored near ambient (25°C) for 15 days. After this storage time, independent of the package used as well as the storage temperature, the oxidation reactions can be more significant and compromise the sensory and nutritional quality of Brazil nuts. Further studies can be designed, including packaging material stability when submitted to these storage temperatures (e.g., without the nuts) and Brazil nuts yeast and mould evaluation.

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CONFLICT OF INTEREST STATEMENT

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.

DATA AVAILABILITY STATEMENT

The authors declare that all data supporting the findings of this study are available within the article.

ORCID

Vanessa de Carvalho Rodrigues https://orcid.org/0000-0002-8709-9896

Fernanda Vitória Leimann https://orcid.org/0000-0001-6230-9597

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