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Increase of walnuts' shelf life using a walnut flour protein-based edible coating

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ABSTRACT

The present study aimed to improve walnut quality during storage. An edible coating was developed from defatted walnut flour and applied to the surface of walnut kernels, which were subsequently stored at 40 °C for 84 days along with walnuts coated with methylcellulose and uncoated kernels. On day 84, the walnuts coated with the walnut flour coating presented the lowest oxidized (13.33) and cardboard (34.73) flavors, the highest walnut flavor (72.27), the highest carotenoid (2.01 mg/kg) and γ-tocopherol contents (306.78 mg/kg) and the least deterioration of oleic/linoleic fatty acids ratio. Furthermore, the kernels covered in this coating displayed higher overall consumers' acceptance than those covered with methylcellulose (5.31). The methylcellulose and walnut flour coatings also prevented against polyunsaturated fatty acids' deterioration, while walnuts coated with methylcellulose displayed a better behavior for peroxide value. Both coatings protected walnuts against the deterioration processes. Walnut flour coating could be used as a natural alternative to prolong the shelf life of walnut kernels, without the introduction of allergens nor synthetic compounds in this food product.

1. Introduction

Walnuts contain high amounts of lipids and essential fatty acids. In this context, with the increased worldwide walnut production and the demand for new specialty oils, walnuts represent an attractive source of edible oil. Walnut oil is produced on a small scale in many countries and is used by consumers as a healthy alternative that enriches the flavor of food (Martínez, Penci, Ixtaina, Ribotta, & Maestri, 2013). Walnuts can also be considered an important source of protein, which represents 12–15% of their dry weight and displays good digestibility and essential amino acid balance (Sze-Tao & Sathe, 2000). Therefore, the residue obtained from the walnut oil industry, which is a protein-rich walnut flour (Rabadán, Pardo, Gómez, & Álvarez-Ortí, 2018), can be harnessed for different purposes, like the development of an edible coating.

Edible films and coatings have an important role in protecting the quality of food products by decreasing their deterioration. An edible film is defined as a thin layer of edible material, that can be placed on or between food components and, provides a barrier to moisture, oxygen and solute movement (Bourtoom, 2008). The main attributes involved in characterizing films are: optical properties, water-solubility, water sorption/desorption, thickness, porosity, barrier properties (vapor and gaseous permeabilities) and mechanical behavior, which affect the consumer acceptability, film stability and improvement of the shelf life of food products ability (Huber & Embuscado, 2009; Murrieta-Martínez et al., 2019). Meanwhile, an edible coating is a thin layer of biodegradable packaging material, formed as a coating on a wide range of foods, with important roles in the quality, safety, transportation, and storage. These types of coatings can be prepared from materials with film-forming ability, such as proteins, polysaccharides, alginate, and other hydrocolloids (Bourtoom, 2008). Edible coatings endow different functions in food products, among them, the prevention of moisture loss and mechanic damage, decreased oxygen diffusion, and the facilitation of the transport of additives (agents with antioxidant and antimicrobial activities). Accordingly, the quality of different types of foods and raw materials can be preserved by applying an edible coating (Grosso, Asensio, Nepote, & Grosso, 2018; Larrauri et al., 2016; Martín et al., 2019; Riveros, Martin, Aguirre, & Grosso, 2018).

Due to their high contents of oil (68–72%) and polyunsaturated fatty acids (PUFAs, 90%), walnuts are highly susceptibility to oxidation, which limits their shelf life. This process results in the loss of essential fatty acids, the emergence of rancid and off-flavors, development of

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toxic compounds, and adverse color changes, thereby decreasing the product sensory, chemical, nutritional and physical quality and leading to consumer rejection (Grosso et al., 2018). Food quality is defined as the combination of attributes or characteristics of a product that have significance in determining the degree of acceptability of consumers. Most sensory researchers focus on consumer satisfaction as a measure of quality although, there is an historic tradition of using expert judges to be the arbiters of product quality. One of the main factors that affect consumer perception of food quality are storage variables. Thereby, storage stability studies are undertaken to ensure prolonged shelf-life of several food products, by using standard discriminative and descriptive sensory procedures (Lawless & Heymann, 2010). Hence, the addition of an edible coating on walnut kernels could help to preserve the storage sensory and chemical quality of walnut kernels.

The food industry is continually striving to protect and satisfy the needs of food allergic consumers (López-Calleja, de la Cruz, González, García, & Martín, 2015). Many edible coatings are made with allergic ingredients (milk whey, wheat, soy, and peanut proteins). Cellulose derivatives do not bear allergens in their composition but are semi-synthetic compounds (Peelman et al., 2013) extremely resistant to hydrolysis that cannot be digested by humans. The presence of allergens and synthetic or semisynthetic compounds in edible coatings should be specified in the food's label and would affect the number of consumers willing to purchase these products. Moreover, the application of a coating with protein as the main component could be exploited to enrich different food products. Therefore, the application of a coating on walnut kernels prepared from the same source (walnut kernels) could help to increase their shelf life, without the incorporation of allergens and synthetic compounds.

Hence, the objectives of the present study were to obtain a protein concentrate from walnut flour, develop an edible coating from this flour, and, finally, to evaluate its protective effect on walnut kernels.

2. Materials and methods

2.1. Materials

Unshelled butterfly walnuts (*Juglans regia* L., Chandler variety) from Nogales SRL (La Rioja province, Argentina) were obtained for this study (2017 crop) and preserved in high barrier bags at −20 °C. Methylcellulose (MC) was used for the preparation of a control edible coating, due to its efficient performance previously evidenced on the preservation of walnuts (Grosso et al., 2018).

2.2. Methods

2.2.1. Walnut flour (WF) preparation

WF was obtained based on the procedure conducted by Riveros et al. (2018). First, the walnut kernels were grounded using a grinder (Moulinex, model AD5663C9, Colombia). Afterwards, the milled kernels were defatted through solvent extraction, using n-hexane in a Soxhlet apparatus (10 h). The product obtained was dried at 60 °C in an air circulation oven (Garmont, Alta Gracia, Argentina) for 24 h. This defatted flour was then subjected to a soluble carbohydrate's extraction using an ethanol/water (70:30) solution in a Soxhlet apparatus (8 h). Once again, the product was subjected to another oven drying step (60 °C; 24 h). Finally, the remaining cake was milled and sifted through a 60-mesh sieve to standardize the granulometry of the final defatted and non-sugar walnut flour (WF).

2.2.2. Proximate composition

Moisture (method 925.40), protein by Kjeldahl (total nitrogen x 5.3) (Sze-Tao & Sathe, 2000; AOAC, 1990 method 955.04), ash (method 923.03) and lipid contents (method 948.22) of walnuts and WF were determined according to AOAC methods (AOAC, 1990). Total carbohydrates were estimated by difference using the following formula: carbohydrate content = 100% - (% protein + % lipid + % ashes) (Gayol, Soliani, Quiroga, Nepote, & Grosso, 2009). Crude fiber (AOCS Standard Procedure Ba 6a-05, 2017) and neutral detergent fiber (NDF) (AOCS Official Method 2002.04) were measured using an Ankom 220 fiber analyzer.

2.2.3. Edible coatings preparation

WF was used for the preparation of an edible coating (WFC) following the method conducted by Riveros et al. (2018) and Martín et al. (2019), with slight modifications. With this purpose, the WF was dispersed in distillated water (6.0 g/100 mL) under stirring conditions at 70 °C for 60 min. Then, the pH of the solution was adjusted to 9 using 0.1 N sodium hydroxide. Finally, glycerol (10 g/100 g flour) was added to the solution as a plasticizer under stirring conditions to ensure a homogenous distribution. The coating forming solution was cooled at room temperature and was centrifuged (1186 G for 6 min) to obtain the WFC.

The MC coating solution (MCC) was prepared accordingly to the procedure conducted by Grosso et al. (2018).

2.2.4. Walnuts coating procedure and storage stability study

The coating procedure was performed by spraying the coating solution in a rotative stainless steel pan containing the walnuts with external air circulation (Erweka GmbH, Heusenstamm, Alemania). 0.5 kg of walnuts were placed in the coating pan and the rotation speed was set to 200 rpm (Martín et al., 2019). The coating was applied at a concentration of 2,5% (v/w). Meanwhile, MCC coating was applied on the samples by the immersion procedure as conducted by Grosso et al. (2018).

The following treatments were obtained: WC = walnut control sample (kernels without coating), WWFC = walnuts coated with WFC and WMCC = walnuts coated with MCC. The samples were placed in plastic containers situated randomly on shelves and stored for 84 days in an air-circulation oven at accelerated oxidative conditions (40 °C). Three pouches of each treatment were retrieved from storage every 21 days to perform sensory and chemical analyses.

2.2.5. Sensory analysis

Eight trained panelists participated in the descriptive analysis of walnut samples, which had at least 4 years of experience evaluating nut products (walnuts, almonds, peanuts, etc.) and showed good levels of olfactory and gustatory perception. These panelists were selected according to the criteria followed by Grosso, Asensio, Grosso, and Nepote (2017) and had to pass a screening test. The selected ones were later trained and calibrated. The descriptive analysis consisted in a hybrid method between the Quantitative Descriptive Analysis (Tragon Corp., Redwood City, Calif., U.S.A.) and the Spectrum TM Analysis Methods (Sensory Spectrum, Inc., Chatham, N.J., U.S.A.). A 150 mm unstructured line scale was used. A paper sheet with the list of attributes and their respecting definitions were developed during the training sessions, along with the reference and warm-up intensity ratings (Table 1). To evaluate oxidized, cardboard, walnut flavor, sweetness, sourness, bitterness, saltiness and astringent attributes, crushed walnuts were placed in plastic cups coded with random numbers. The panelists were instructed to consume the whole content of the cups during evaluation. For the evaluation of color intensity, glossiness, roughness, crunchiness and hardness, entire butterfly walnuts were provided to the judges. Before beginning the evaluation, panelists retested all references and warm-up sample. Every evaluation day was divided in two sessions. A completely randomized block design was used for testing samples.

2.2.6. Consumer tests

For consumer analysis, panelists (n = 110) were recruited according to the criteria followed by Grosso et al. (2017). Fresh walnut samples (WC, WMCC and WWFC without storage) were presented in random

Table 1
Sensory descriptive analysis attributes. Definitions of sensory attributes, standard references with their corresponding intensity, and warm-up intensity ratings used for the descriptive sensory evaluation of fresh (day 0) and stored walnuts (40 °C, 84 days) without coatings (WC), with walnut flour-based coating (WWFC) and methylcellulose-based coating (WMCC).

Attribute[a]	Definition	Reference	Reference intensity[b]	Warm up intensity[b]
Appearance				
Color Intensity (brown)	The intensity or the strength of brown color from light to dark brown.	Almonds[c]	80	51
		Coffee[d]	110	
Roughness	The appearance associated with uneven surface.	Almonds[c]	30	88
		Cornflakes[e]	100	
Glossiness	The appearance associated with the amount of light reflected by the product surface.	Beans[f]	30	29
Aromatics				
Walnut flavor	The aromatic associated with walnut			80
Oxidized	The aromatic associated to rancid fats and oils.	Toasts with oxidized walnut oil[g]	40	0
Cardboard	The aromatic associated with wet cardboard.	Moist cardboard	30	15
Tastes				
Sweetness	Taste on the tongue associated with sucrose solutions.	20 g kg −1 sucrose solution	20	12
		50 g kg −1 sucrose solution	50	
		100 g kg −1 sucrose solution	100	
Saltiness	Taste on the tongue associated with sodium chloride solutions.	2 g kg −1 NaCl solution	25	5
		3,5 g kg -1NaCl solution	50	
		5 g kg -1NaCl solution	85	
Bitterness	Taste on the tongue associated with bitter solutions such as caffeine.	0.5 g kg −1 caffeine solution	20	23
		0.8 g kg −1 caffeine solution	50	
		1.5 g kg −1 caffeine solution	100	
Sourness	Taste on the tongue associated with acid agents such as citric acid solutions.	0.5 g kg −1 citric acid solution	20	9
		0.8 g kg −1 citric acid solution	50	
		1.5 g kg −1 citric acid solution	100	
Feeling Factors				
Astringent	The puckering or drying sensation on the mouth or tongue surface.	4 g of tea h in 250 ml of water at 90 °C	59	50
Texture				
Crunchiness	Force needed and amount of sound generated from chewing a sample with molar teeth.	Cornflakes[e]	120	56
Hardness	Force needed to compress a food between molar teeth.	Almonds[c]	65	36

[h]Green Hills Classic tea.
[a] Attributes listed in order as perceived by panelists.
[b] Intensity ratings are based on 150-mm unstructured line scale.
[c] Almonds, Grandiet, Córdoba, Argentina.
[d] Coffee, Nescafé ® Clásico, Nestlé Argentina S.A. Buenos. Aires, Argentina.
[e] Cornflakes, “3 Arroyos”, Buenos Aires, Argentina.
[f] Beans, “Egran”, Córdoba, Argentina.
[g] Toasts with oxidized walnut oil: White bread “Fargo”, each piece cut in 9 equal parts that were moistened with 2 ml of oxidized walnut oil.

order to the panelists to perform the test. The samples were placed inside plastic cups on a plastic tray along with a glass of water and a paper ballot sheet to evaluate color, flavor and overall acceptance. Panelists were instructed to consume the whole content of each cup and to rinse their mouths with water between samples to minimize any residual effect. A 9-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely was used to evaluate consumer acceptance.

2.2.7. Chemical analysis

For the analysis of chemical lipid oxidation indicators, the walnut oil was extracted by pressing the kernels (HE-DU hydraulic press, Hermes I. Dupraz S.R.L., Córdoba, Argentina). P-anisidine value (AV) was measured following the IUPAC (1987) standard methods and carotenoid content (CC) was analyzed at 470 nm following the procedure described by Grosso et al. (2018) using an UV–Vis spectrophotometer (Spectrum SP-2100 UV, Zhejiang, China). Peroxide value (PV) was evaluated according to the AOAC method (AOAC, 1990).

Tocopherol quantification was performed by HPLC using a Zorbax RX-SIL column (5 µm particle size, 4.6 × 250 mm, Agilent Technologies, Palo Alto, CA, USA) as described by Martín, Nepote, and Grosso (2016). The mobile phase was an isopropanol solution in hexane 0.5% v/v with a flow rate of 1.5 mL/min. The detection wavelength was 298 nm. The tocopherol content and quantification were done by external standard curves of α-, β-, γ- and δ-tocopherols (Sigma-Aldrich, St Louis, MO, USA).

To evaluate changes in the fatty acid composition, the fatty acid methyl esters were analyzed on a Perkin Elmer Clarus 600 CG-MS (Waltham, Massachusetts, USA). A TG-WAXMS capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness; Thermo Scientific™, Waltham, Massachusetts, USA) was used. Separation, identification and quantification of the fatty acid methyl esters were performed according to Martín et al. (2016).

2.2.8. Statistical analysis

The experiment was carried out in three replicates. The statistical analysis was performed using InfoStat software, 2017 version. The results obtained from the chemical and sensory indicators were analyzed via a two-way analysis of variance (factors: ‘treatment’ and ‘time’) on a completely randomized design. The data were expressed as means ± standard deviations. The level of significance for all analysis was set up at $p < 0.05$ and the difference between means was solved via least significant difference (LSD) test. Also, correlations between sensory and chemical variables were determined through a correlation analysis (Pearson's coefficients).

Table 2
Proximate composition (g/100 g of dry weight) of walnut kernels and walnut flour.

Components	g/100 g of dry weight	
	Walnut kernels[a]	WF[b]
Moisture	4.44 ± 0.37a	8.88 ± 0.30b
Fat	64.06 ± 0.25b	0.35 ± 0.13a
Protein	16.00 ± 0.02a	49.36 ± 2.09b
Ash	1.74 ± 0.06a	4.46 ± 0.11b
Total carbohydrate	18.19 ± 0.24a	45.83 ± 1.92b
Neutral detergent fiber (NDF)	17.38 ± 0.23a	43.79 ± 0.43b
Crude fiber	7.19 ± 0.09a	18.95 ± 0.44b

[a] Means ± standard deviations (n = 3; ANOVA and LSD, α = 0.05). Different letters in each column indicate significant differences between the samples (α = 0.05).
[b] Defatted and without soluble carbohydrates walnut flour.

3. Results and discussion

3.1. Proximate composition

The results regarding walnut kernels' composition (g/100 g of dry weight) (Table 2) were consistent with the literature data, exemplified by Sze-Tao and Sathe (2000). The lipid, carbohydrate, and protein contents obtained in WF (g/100 g of dry weight) were similar to a walnut flour obtained in another study, in which a different procedure of lipid extraction was used (Mao & Hua, 2012). Nevertheless, other work found lower protein and greater carbohydrate contents in other types of walnut flour (Rabadán et al., 2018). These discrepancies could be attributed to the extraction of soluble carbohydrates performed in the current study.

The protein content in the samples increased from 16% in fresh walnuts to 49% in WF, in contrast to the lipid concentration, which decreased in the WF to less than 1%. The NDF, which is constituted by the hemicellulose, cellulose, and lignin fractions, represented 95% of the insoluble carbohydrates in the edible coatings. Noshirvani et al. (2017) mentioned that the water permeability of a chitosan–carboxymethyl cellulose coating was dramatically decreased by the incorporation of a small quantity of oleic acid, aiding to protect against moisture loss and staling of sliced bread. Hence, such high NDF and low lipid contents indicate that WF could be a suitable ingredient for the preparation of an edible coating.

The prepared WF is formed by more than 90% of molecules that are commonly used for edible coating preparation (protein and cellulose), which could improve the WFC properties. Considering the composition of WF, walnuts could be protected and preserved using WFC. Walnuts are food products with low moisture content (less than 5%) and water activity. Whenever food is intended for ingestion, it must be microbiologically safe. Since low water activity and humidity conditions promote microbiological stability in food and coating membranes, the decreased moisture conditions in the coated nuts could act preventing microbiological development (Puscaselu, Gutt, & Amariei, 2019). In addition, relative humidity (RH) affects the mechanical properties and gas permeability (oxygen) of edible films. Cho and Rhee (2002) reported that films with lower glycerol contents were more sensitive to RH variation while, Hong and Krochta (2006) reported that oxygen permeability increased at a higher RH in protein-based edible coatings. In the current research, WFC is prepared using a rich proteinic material (WF) using glycerol as plasticizer. For that reason, a similar behavior as described by the previous authors is expectable in WFC.

3.2. Sensory evaluation of fresh walnut samples: descriptive analysis and consumer acceptance test

Both sensory analyses made on fresh kernels were performed to identify and quantify the sensory attributes affected by the addition of the coatings (before storage, i.e., day 0) and compare the consumers' perception among the products. For sensory descriptive analysis, no significant differences were detected among the treatments, except for the color intensity rating. WWFC presented the highest color intensity (61.28) in comparison to WC (49.33), indicating that the protein-rich walnut flour-based coating (WFC) conferred a darker color to the kernels. Coatings that contain carnauba wax also impart a dark hue (yellow) to walnuts, affecting their consumer acceptability (Mehyar, Al-Ismail, Han, & Chee, 2012). Meanwhile, MCC displayed the opposite behavior, promoting a lighter color intensity. Likewise, Grosso et al.

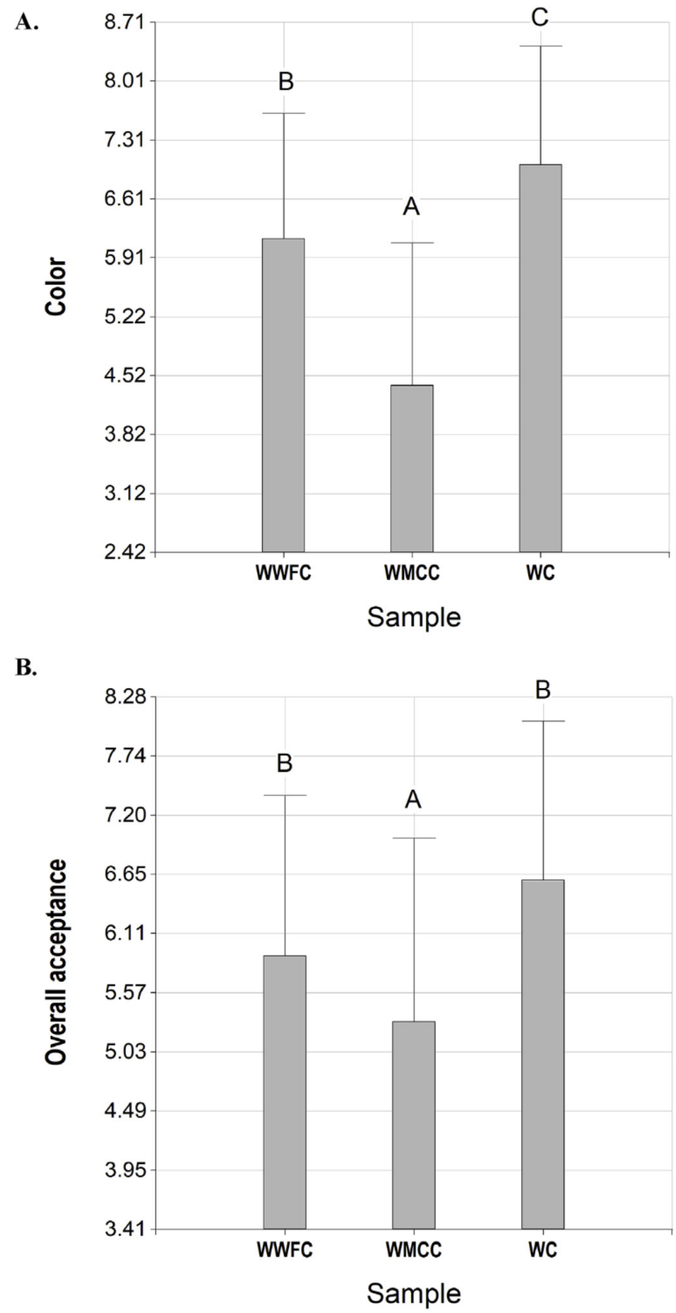

Fig. 1. Consumers' acceptance evaluation. Means (n = 80) and standard deviations of (A) color and (B) general consumers' acceptance (9-point hedonic scale) considering fresh walnut samples without the addition of an edible coating (WC) and, with the addition of a walnut protein-based coating (WWFC) and a methyl-cellulose coating (WMCC). Different letters indicate significant differences at α = 0.05 (LSD Fisher).

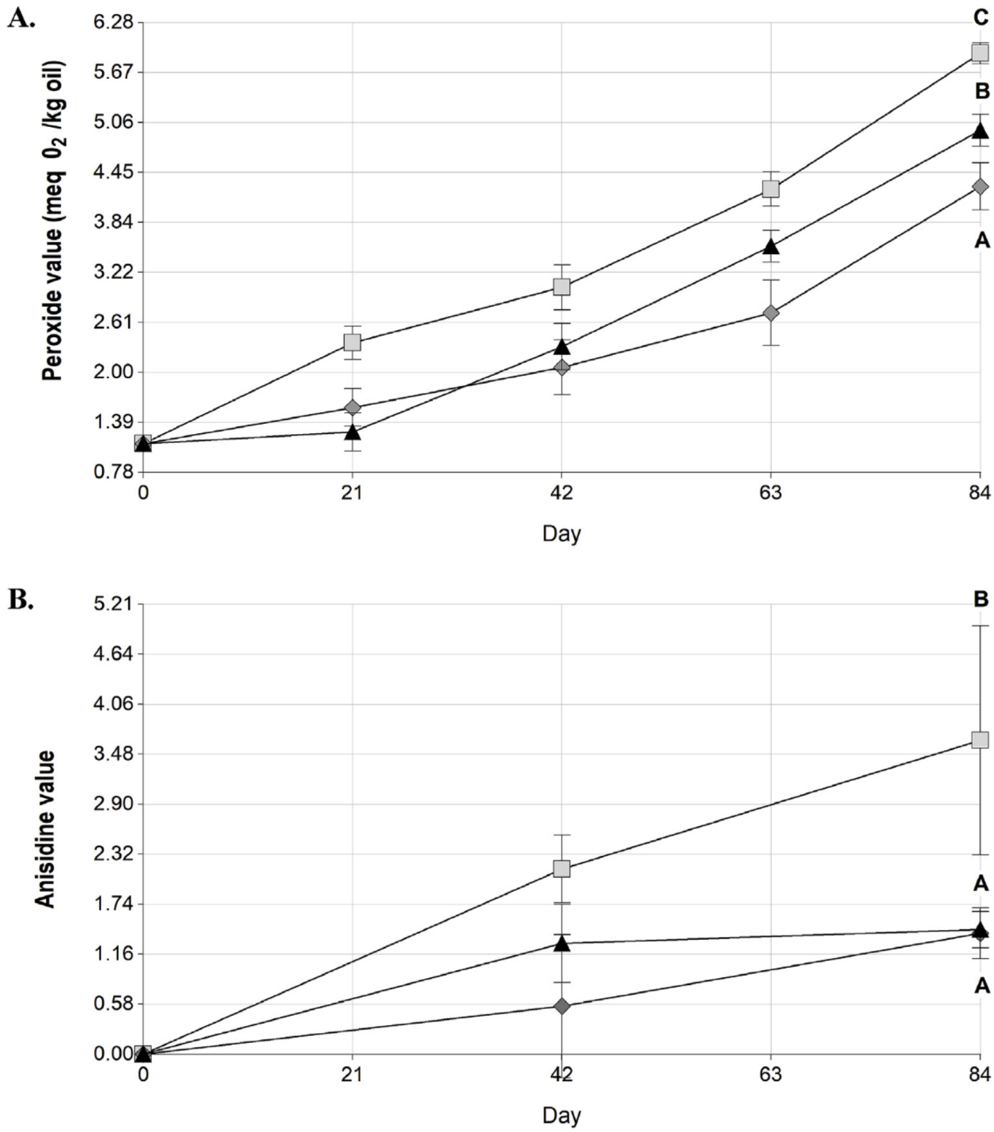

Fig. 2. Peroxide (meq O_2/kg oil) and anisidine value for walnuts coated with different coatings, stored during 84 days at 40 °C. (A) Peroxide; (B) Anisidine. (—□— WWFC, walnut protein-based edible coating; —◆— WMCC methyl-cellulose edible coating; —▲— WC, without coating). Different letters indicate significant differences between the treatments at $\alpha = 0.05$ (LSD Fisher).

(2017) already observed that the application of an MCC coating on walnuts decreased the color intensity rating of the kernels.

Concerning the consumer acceptance test, measured on a 9-point hedonic scale, WMCC displayed the lowest scores for color acceptance (4.40 = *dislike slightly*) (Fig. 1A), whereas, higher values were assigned to WWFC (6.14 = *like slightly*) and WC 7.02 = *like moderately*). These results show that the addition of the MCC coating negatively affected the consumers' perception, in agreement with the findings of Grosso et al. (2017). In contrast, the WFC coating did not provoke a negative reaction by the consumers.

For flavor acceptance, WC and WWFC presented greater scores (6.43 and 6.05, respectively) than WMCC (5.02). Hence, unlike the MCC coating, which exhibited a negative effect on the flavor perception of walnuts, WWFC did not affect the consumers' opinion of the walnut flavor.

For overall acceptance, WWFC (5.91) and WC exhibited greater scores than WMCC, which presented the lowest score (5.31) (Fig. 1B). The relatively low color and flavor acceptances of the walnuts coated with MCC could explain the poor overall acceptance of this product.

3.3. Storage study

3.3.1. Changes in the chemical parameters

Carotenoids are natural antioxidants present in plant materials which deteriorate along with lipid oxidation. On day 0, the samples presented a mean carotenoid concentration of 2.56 mg/kg oil. On the last day of storage (day 84), the carotenoid quantities were most abundant in WWFC (2.01 mg/kg oil), followed by WMCC (1.82 mg/kg oil) and, lastly, WC (1.49 mg/kg oil), indicating that WFC helped to prevent the deterioration of these compounds.

There was an increase in the PV of the samples during storage (Fig. 2A). On day 21, WC displayed the highest value, followed by WWFC and WMCC. This trend continued so that on day 84, WMCC presented the lowest value (4.27 meqO_2/kg oil), WC displayed the highest value (5.90 meqO_2/kg oil), and WWFC exhibited an intermediate PV (4.96 meqO_2/kg oil). Thus, although both coatings displayed a protective effect against lipid oxidation, MCC was more efficient at delaying primary lipid oxidation. Previous research has also shown that edible coatings based on cellulose derivatives presented better protection against PV development in walnuts when compared with those based on protein (Grosso et al., 2018). This behavior could be attributed to the properties of the polysaccharide used for coating preparation, highlighting the barrier effect afforded by MCC (Hassan,

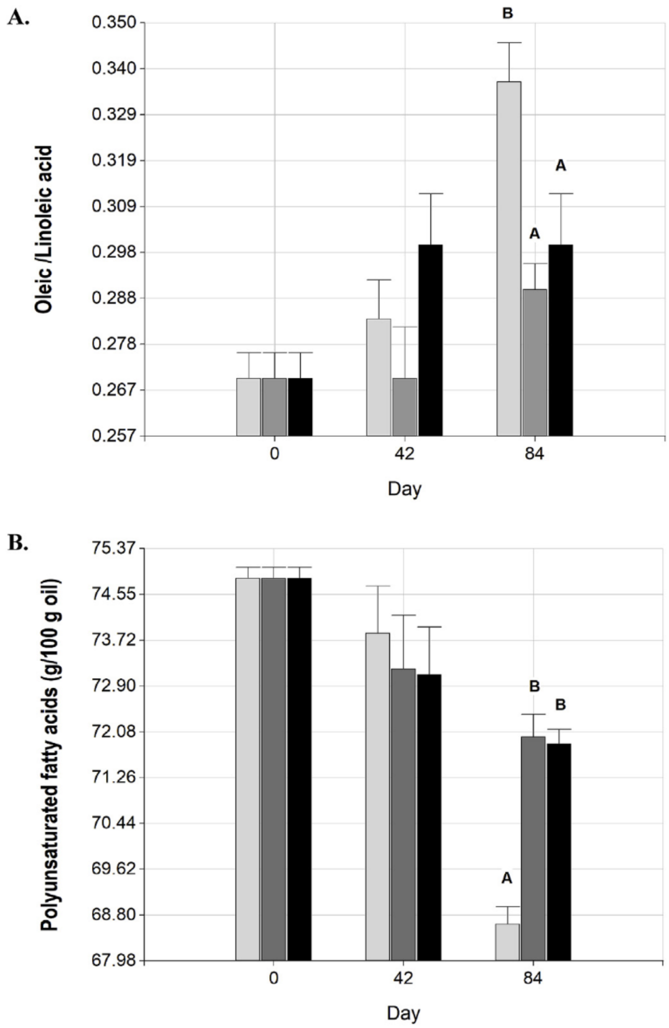

Fig. 3. Changes in the fatty acid composition (g/100 g oil) for walnuts coated with different coatings stored during 84 days at 40 °C. (A) Oleic/linoleic acid ratio; (B) Polyunsaturated fatty acids. Abbreviations: ☐ WWFC, walnut protein-based edible coating; ■ WMCC methyl-cellulose edible coating; ■ WC, without coating. Different letters indicate significant differences between the treatments at α = 0.05 (LSD Fisher).

Chatha, Hussain, Zia, & Akhtar, 2018).

Significant differences in the AV emerged between treatments during storage (Fig. 2B). On day 84, WC displayed the most pronounced value (3.64), evidencing the protective effect of the coatings (WFC and MCC) against deterioration of the walnuts, by reducing the formation of secondary oxidation compounds. These coatings could be acting as oxygen permeability barriers. Similarly, Gayol et al. (2009) proved that prickly pear syrup coatings could prevent the increase of the AV of almond samples.

Changes in the fatty acid composition of the walnut samples were detected throughout storage (Fig. 3). Concerning the oleic/linoleic ratio (O/L), an increase was detected in all samples. Nevertheless, only WC displayed a significantly higher value on day 84 in comparison to the coated walnuts (WMCC and WWFC). A similar tendency could be appreciated in the saturated/unsaturated fatty acids ratio (S/U). Concerning the PUFAs concentration, a higher decrease was observed in WC (from 74.83 to 68.63 g/100 g oil) (Fig. 3B) than in the other samples. In turn, WC showed the highest increase in the proportion of monounsaturated fatty acids (MUFAs) during the same period (from 16.64 to 19.27 g/100 g oil). The behavior of WC is a result of the oxidation of linoleic acid and linolenic acid (PUFAs), due to the weakness of their double bonds. Therefore, the protective effect displayed by the coatings (MCC and WFC) could be because of the reduction in the gas permeability. Likewise, Martín et al. (2016) showed that reducing gas permeability resulted in a lower degradation of PUFAs in peanuts.

The tocopherol composition was typical of walnuts, with γ-, δ-, α-, and β-tocopherol contents of 355.93, 27.27, 4.93, and 0.95 mg/kg oil, respectively, totaling 389.08 mg/kg oil, before storage (day 0). During storage, some tocopherols completely disappeared from the walnuts' composition (α-tocopherol and β-tocopherol), and that of others diminished. γ-Tocopherol (Fig. 4) decreased least in WWFC (306.78 mg/kg oil) relative to WC (302.42 mg/kg oil) and WMCC (300.40 mg/kg oil). δ-Tocopherol displayed the same pattern. Tocopherols are molecules that easily deteriorate and oxidize in the presence of high temperature and oxygen. Silva, Martinez, Casini, and Grosso (2010) adjudicated the loss of tocopherols in stored peanut samples to the lipid oxidation processes since these molecules are natural lipid-soluble compounds. Among the samples examined in the current study, the barrier provided by WFC better protected the walnut tocopherols throughout storage.

3.4. Descriptive sensory changes during storage

The intensity ratings of some sensory attributes changed as storage advanced (color, as well as oxidized, cardboard, and walnut flavors). The color intensity increased for all samples throughout storage (Fig. 5A). On the last day of storage, WC exhibited the highest value (70.40), followed by WWFC (66.47) and WMCC (62.33). According to the results, WC presented the greatest increase (21.07 points), followed by WMCC (17.28 points). Some studies have concluded that the browning reaction occurring during storage could be the product of enzymatic oxidation of the phenolic compounds present in the walnut skins (Manzocco, Mastrocola, Nicoli, & Marangoni, 2001). For instance, Chatrabnous, Yazdani, Tavallali, and Vahdati (2018) detected a greater browning in walnut samples that developed higher values for lipid oxidation indicators (e.g., PV, hexanal), and Christopoulos and Tsantili (2012) found the same trend in walnuts exposed to increased temperature and oxygen during storage. In the present study, WWFC showed the lowest extent of browning development, which could be attributed to the reduced gas exchange, preventing the oxidation of walnut phenolic compounds.

For oxidized and cardboard flavors, both sensory attributes related to rancidity (Grosso & Resurreccion, 2002; Riveros, Mestrallet, Quiroga, Nepote, & Grosso, 2013), the intensity ratings increased for all samples during storage. On the last day of storage, the highest ratings for oxidized (Fig. 5B) and cardboard flavors (Fig. 5C) were displayed by WC (63.80 and 59.73), followed by WMCC (29.53 and 36.73) and then WWFC (13.33 and 34.33), with the lowest values. Previously, protein coatings afforded protection against the development of oxidized flavor when applied on peanut samples (Martín et al., 2019), and walnuts covered with edible coatings prepared from MCC and whey protein slowed the development of oxidized and cardboard flavors (Grosso et al., 2017). Among the samples studied in the current work, WFC displayed a better behavior, preventing the increase of these off-flavors associated with quality deterioration.

No significant differences in walnut flavor were found between the samples (78.70) on day 0. The intensity ratings of this attribute decreased as storage progressed, for all samples (Fig. 5D). On the last day of storage, WWFC exhibited the highest intensity rating (73.27). Hence, once again, WFC provided the greatest preservation of this characteristic attribute. Previous research on the evolution of the characteristic walnut flavor (Colarič; Štampar, Hudina, & Solar, 2006) or nutty flavor (Jensen & Sørensen, 2001) revealed a strong association between the increase of rancid flavor and the decrease of nutty flavor, suggesting that the lipid oxidation was the primary parameter affecting the sensory quality of the product (Jensen & Sørensen, 2001).

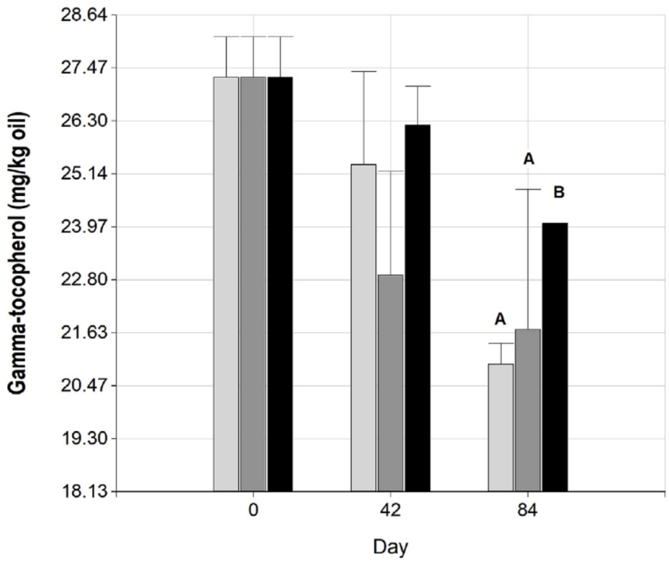

Fig. 4. Gamma-tocopherol content (mg/kg oil) for walnuts coated with different coatings stored during 84 days at 40 °C (□ WWFC, walnut protein-based edible coating; ■ WMCC, methyl-cellulose edible coating; ■ WC, without coating). Different letters indicate significant differences between the treatments at α = 0.05 (LSD Fisher).

3.5. Correlation analysis (Pearson's coefficients)

Strong correlations were found between some of the variables. For instance, color intensity was positively correlated with PV ($r = 0.50$) and AV ($r = 0.53$), and negatively associated with the PUFAs concentration ($r = -0.53$) ($p < 0.0001$). Such observations highlight that the intense browning reaction, mainly in WC, was associated with higher scores in chemical lipid oxidation indicators and degradation of the main fatty acids in the walnut composition. Meanwhile, oxidized flavor showed negative correlations with walnut flavor ($r = -0.95$), the concentration of PUFAs ($r = -0.70$), δ-tocopherol concentration ($r = -0.68$), and CC ($r = -0.65$), and was positively related to cardboard flavor ($r = 0.95$), AV ($r = 0.86$), and PV (0.65). The control sample (WC) presented the highest intensity for oxidized flavor throughout storage, as well as the highest increase in the chemical lipid oxidation indicators and the highest degradation of carotenoids and tocopherols, while WWFC displayed the lowest increase through storage.

Walnut flavor was positively correlated with γ- and δ-tocopherol ($r = 0.50$ and 0.61, respectively), CC ($r = 0.63$) and the PUFAs content ($r = 0.65$), indicating that WWFC, which presented the highest value for this attribute during storage, allowed a better preservation of the natural antioxidants and fatty acids in walnuts. AV was strongly correlated with other chemical indicators, like O/L ($r = 0.64$), PUFAs ($r = -0.70$), and γ-tocopherol ($r = -0.60$). PUFAs were strongly correlated with δ- ($r = 0.71$) and γ-tocopherol ($r = 0.79$), showing that these compounds diminish together.

4. Conclusion

Through the defatting and extraction of soluble carbohydrates, a high proteic value walnut flour is obtained, suitable for the preparation of an edible coating. When applied on walnuts, the walnut flour-based coating exerts protection against lipid deterioration, displays a marked effect on the preservation of walnut sensory properties and prevents the development of sensory attributes related to lipid deterioration. Furthermore, walnuts covered with WFC exhibit a similar consumers acceptance when compared to the control sample and is preferred over the walnuts coated with MCC. Meanwhile, MCC presents similar or lower protection against most of the chemical indicators of lipid oxidation. To conclude, both coatings help to preserve the walnut kernels from lipid deterioration in different ways, prolonging their shelf life. Furthermore, the walnut flour-based edible coating is advantageous because walnut kernels covered with this protective coating rich in

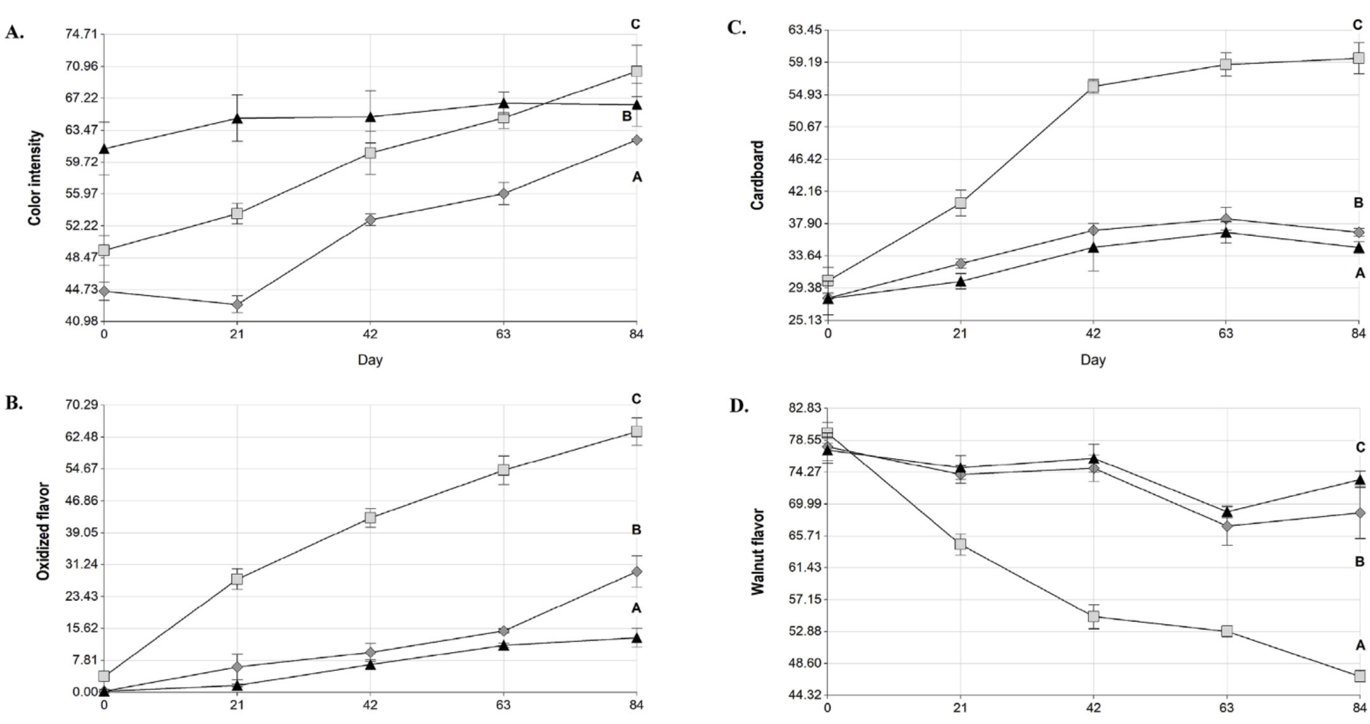

Fig. 5. Sensory attributes for walnuts coated with different coatings stored during 84 days at 40 °C. (A) Color intensity; (B) Oxidized flavor; (C) Cardboard; (D) Walnut flavor. Abbreviations: WWFC, walnut protein-based edible coating; WMCC methyl-cellulose edible coating; WC, without coating. Different letters indicate significant differences between the treatments at α = 0.05 (LSD Fisher).

protein avoids cross-contamination with allergens. However, the influence of the relative humidity condition of walnut's storage place should be considered because it could adversely affect the stability of this edible coating.

The application of the WFC on walnuts by the food industry, is a feasible process from an economical and practical point of view. Concerning the economic point of view, walnut oil-cake, which is a residue from walnut oil industry, is the main ingredient used for preparing walnut flour. This residual material has a low cost and could be harnessed by the same oil industry or other industry to obtain this flour.

Considering the practical feasibility, the preparation of the walnut flour and WFC requires simple industrial processes and machineries already used by the food industry, such as, solid/liquid extraction systems. With respect to the application of the walnut coating, the recommended procedure is spraying liquid inside of a rotative steel pan, which, is very simple and currently used by the food industry for many products.

For all these reasons, we believe that it is possible to transfer this knowledge for practical application in the food industry, which will contribute to solve the main issue in walnut preservation by decreasing lipid oxidation in a natural and environmentally friendly way.

Declaration of competing interest

The authors report no conflicts of interest.

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