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Edible films to improve quality and shelf life of fresh tortillas

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ABSTRACT

Edible films have emerged as packaging aids to replace synthetic polymers due to their biodegradable nature. The aim of this study was to develop starch-based edible films for application in packaging, to inhibit moisture transfer from the filling to the tortilla, while maintaining physicochemical and organoleptic quality characteristics, and improving shelf life. Twenty-four types of filmogenic solutions were produced by casting from corn, pea, and chestnut starch blended with agar, guar, and potassium alginate, using glycerol as a plasticizer. The films were selected due to its surface properties and water absorption capacity, which allowed selecting the films that best retained moisture. The hydration properties of the selected films allowed to verify that the corn and pea starch films show good resistance to water and microbial development. Microstructural properties showed that the pea starch films exhibit a homogeneous and smooth surface without porosity. Fourier Transform Infrared Micro-Spectroscopy allowed the characterization of the starch films at the molecular level. The mechanical properties of corn and pea starch films were evaluated in tensile strength (21.98–27.5 KPa), elongation at break (59.17–185.96%), and elastic modulus (42.35–17.09 MPa). The results suggest that pea films are more flexible than starch films due to differences in the amylose and amylopectin molecules and molecular masses of the different starches. The sensory analysis concluded that the pea starch film delayed moisture transfer from the filling to the tortilla, maintaining the texture, appearance, and organoleptic characteristics for 6 days, improving the consumption experience of these food products.

1. Introduction

Edible films have been known to protect perishable food products from spoilage and some types of loss quality. However, over the last decade there has been a growing interest in the development and use of bio-based packaging materials to replace the conventional synthetic packaging materials needed to preserve and protect foods.

The selection of the right biopolymer, for the production of packaging films, for one particulary application, is very important, to improve the quality of fresh, and processed food, and increase the shelf-life and of its packaging (Brzoska et al., 2018). Edible films, produced from biopolymers, can provide a slower respiration rate, storage, firmness retention, and controlled microbial growth, improving food product quality and increasing storage (Garcia et al., 2010).

The production of films and edible coatings is based on the dispersion of biopolymers in a solvent (water, ethanol, or organic acids), and on the addition of additives (plasticizers or binders) to obtain filmogenic solutions, which, after being prepared, undergo a drying operation to form casting films or coatings (Bourtoom, 2008).

Starch-based polymers have gained importance due to their abundant availability, ease of processing, relatively low price, and environmentally friendly profile, making them a viable alternative to petrochemical-based polymers. Such polymers can be isolated from different sources, including potato, corn, wheat, tapioca, and cassava (Horstmann et al., 2017). Starch includes two main structural components - amylose and amylopectin. Native starches generally contain 20%–30% amylose, although most pulse starches have a higher amylose content. These starches, because they have different amylose contents, have distinct functional properties. In general, starches with higher amylose content form gels that are stronger, more elastic and have

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resistance against deformation, while starches with higher amylopectin content presents higher penetrability, stickiness and adhesiveness (Li et al., 2011; Liu et al., 2006; Gutiérrez et al., 2015; Kramer, 2009). When a starch paste remains at rest, without stirring before or after cooling, the tendency is for the intermolecular forces to be established, forming a gel. The regions, in the gels, where such forces are established, increase in number during the rest period, making the network firmer and compacter to different degrees, according to the number, sizes, and distribution of the micellar regions (Mason, 2009).

The use of lipids (waxes, lacquers, fatty acids, alcohols, and acetylated glycerides) in the film formulation has been shown to provide flexibility to the polymer, and to be an excellent barrier to water transmission, affecting the transmission of some gases such as oxygen and carbon dioxide (Debeaufort and Voilley, 2009). Plasticizers used for starch-based films generally include polyols, such as glycerol, which, increases film flexibility improve mechanical properties, and decreases water permeability (Haq et al., 2014; Imran et al., 2010).

Non-starch polysaccharides, such as seaweed extracts (alginates, agar, carrageenans), or plant extracts (guar, tragacanth, and arabic) and microbial gums (xanthan) possess good film-forming properties. Alginate is a polysaccharide that forms films with good oxygen barriers, which can retard lipid oxidation in foods (Nussinovitch, 2009; Pavlath and Orts, 2009), and improve flavor, texture, and batter adhesion; however it tends to be quite brittle when dry, and a poor moisture barrier (Nieto, 2009). Agar is a galactose polymer that forms strong gels characterized by melting points far above the initial gelation temperature (Kramer, 2009; Natrajan and Sheldon, 2000). These films can be used to improve shelf-life and control pathogenic bacterial growth.

Starch-based films are gaining popularity in industrial packaging applications, being easily soluble and dispersed in water, due to excellent heat resistance, greater stability, and shelf-life. However, the studies carried out on films with different starches, and the countless combinations with other polymeric materials, the discrepancies in the form of production, the scale-up, and the specific properties for a given application, make the reproducibility, in most cases, impossible.

The objective of this study was to develop edible films, from starch-based polymers (corn, pea, or chestnut) and binary mixtures with non-starch polysaccharide sources (agar, guar, and potassium alginate), through casting technique, using glycerol as plasticizer (28.5 wt% strach based). From the different films, one (or more) formulation that could best allow to retain the moisture of the tortilla filling, prevent it from softening, keep the texture, and increase the shelf-life of these products, will be chosen. The films produced were sequentially selected considering surface characteristics, physical properties; microstructure properties and mechanical properties.

The films that exhibited the best water holding capacity, homogeneous and smooth surface without porosity, and flexible were used in the tortilla filling, and were evaluated through sensory testing.

2. Materials and methods

2.1. Materials

The following reagents were used in film preparation: corn starch, gum guar and agar (Sigma-Aldrich, Germany), potassium alginate (Proaromática, Portugal), and glycerol (AnalaR-BDH, England). The pea starch was from Yantai Shuangta Food (China), and organic water chestnut powder from Kan Phytochemicals Pvt Lda (India). The products used in the sensory test were acquired a market: wheat wraps (Koala, Europe); tuna in oil (Continent, Portugal); sweet chili sauce (Blue Dragon, Thailand), and egg (Derovo, Portugal). All other reagents used for the analysis were analytical grade.

2.2. Film preparation

Filmogenic solutions (FS) of starch-based polymers (corn, pea and chestnut) and non-starch polymers (potassium alginate, guar gum and agar) were prepared according to Costa et al. (2018) and Gutiérrez et al. (2015) as follows: (1) Polymers consisting of (2.1 g) corn, pea or chestnut starches; with or without addition of (0.6 g) glycerol (concentration of plasticizer were chosen based according to preliminary experiment from which results are not shown); (2) Blends of binary polymers consisting of (1.6 g) corn, pea or chestnut starches with (0.5 g) potassium alginate, guar or agar gum; with or without addition of (0.6 g) glycerol; (3) the polymers, selected in 2.31 and 2.3.2 sections, were performed with a diameter of 15 cm, consisting of (1.85 g) starches (corn, pea and chestnut) with (0.25 g) non-starch polymer (potassium alginate, guar or agar gum); with or without addition of (0.6 g) glycerol. The dispersed FS (100 mL) was heated in a water bath at 85 °C for 20 min under a 700 rpm stirring (Selecta Agimatic 243, Spain) until the mixture became clear or complete homogenizing of the starch. The FS were homogenized for 5 min (10.000 rpm) in an Ultra Turrax (IKA T25-S1, Germany) and gas bubbles were removed for 10 min in an ultrasonic bath (Branson 2200 B2200R-4, ultrasonic power: 240 W, USA). Films were prepared by placing 15 mL of the FS in a series of Petri dishes (5 cm diameter), or 80 mL of the FS in a series of Petri dishes (14 cm diameter), on a level surface. The FS were dried at 40 °C and 40% RH for 24 h. Five starch films with 5 cm diameter, and ten with 14 cm diameter, were made for each FS. Dry films were removed from the Petri dishs with the help of a spatula, and stored at 53 ± 1% RH and 25 ± 1 °C in desiccators for at least 7 days before any testing.

2.3. Film properties

2.3.1. Subjective evaluation

The films were classified using subjective parameters according to the methodology of Monterrey and Sobral (1999), which is based on visual observations of the films for continuity (absence of breaks and fractures after drying) homogeneity (absence of particles that are insoluble or visible to the naked eye, or areas of opacity or different colors), and handling (possibility of manipulation without risk of breakage). The films were classified according to a scale defined as 3-excellent, 2-good, 1-damaged.

2.3.2. Wettability test

The surface wettability of starch films was evaluated, the drop method which is used to estimate wetting properties of a solid surface, the water contact angle test was replaced with an internal (and subjective) method. A droplet of distilled water (100 μL) was deposited on the film surface with a micropipette. The time required for softening and breaking of the film was performed with a chronometer (Kurzzeitmesser), 20 s after initial contact of the droplet water with the sample surface. Two measurements per film were carried out.

2.3.3. Thickness and grammage measurements

Thickness and grammage measurements were carried out according to Galus and Kadzińska (2015), with modifications. Briefly, the thickness of the different cast films was measured using a digital micrometer (Powerfix Z22855, UK) and expressed in millimeters (mm). The grammage was determined by weighing a known area after conditioning in a standard 25 °C, 53% rh environment for a minimum of 24 h. Results were expressed in grams per square centimeter (g.cm-2). Each measurement was carried out in 3 film-specimens each at 5 random positions and averaging the results, while grammage measurements were performed in triplicate, and the average weight was calculated (n = 3).

2.3.4. Water activity (a_w)

The water activity of the starch films was measured using an electronic water activity meter (Rotronic-HigroPalm aw1, Switzerland) with direct reading at 25 °C, and the results were expressed in water activity (aw), and results for each film were determined by the average of three measurements (n = 3).

2.3.5. Water solubility (W_S)

The water solubility (W_S) of starch films is an indicator of the coating resistance when exposed to a medium containing water (Fakhouri et al., 2007) and was determined according to the procedure of Arham et al. (2018) with modifications. Films squares were cut (4 cm^2) put in an oven-drying for 24 h at 105 °C (Memmert UL80, Germany), and weighed. Water (80 mL) was vigorously mixed to the dried films in an Erlenmeyer, incubated in a 25 °C water bath for 24 h. The supernatant was collected in a preweighed Petri dish, and the residue was weighed after oven-drying for 24 h at 105 °C. Two measurements per film were carried out and the average weight was calculated (n = 2). The mass of the dissolved material in the original 4 cm^2 sample was calculated using the following equation:

$$WS(\%) = \frac{m_i - m_f}{m_i} \times 100$$

W_S (%) – water solubility; m_i (g) – initial mass; m_f (g) – final mass

2.3.6. Film microstructure

Film microstructure was observed with a scanning electron microscope (JEOL, JSM-7001 F, Japan) working at a voltage of 15 KeV, according to Ahmad et al. (2015). The film analyses were performed directly on the sample mounted on an aluminum sample holder, covered with a thin layer of gold obtained in vacuum evaporator with the Sputter Coating Attachment of Quorum Q150R ES. Observations were carried out on the surface of the coating film, the microphotographs were made with a camera attached to the microscope. The samples were systematically observed with a magnification of 100×, 500x, and 1000x.

2.3.7. Fourier transform infrared spectroscopy (micro-FTIR) measurements

Infrared spectroscopy of the films is used to aid in the elucidation of complex molecular structures, since it allows an association between the spectra obtained and the functional groups present in the compounds.

FTIR analysis was performed directly on the surface of the exposed films in reflectance mode and the spectra were recorded using a spectrophotometer (Spotlight 200i FTIR Microscopy System with Spectrum Two, PerkinElmer, Inc., USA), equipped with a UATR Two accessory, following the methodology of Torrenegra et al. (2018). All readings were taken directly on the films. The spectra were obtained at 4 cm^{-1} resolution and 8 scans of data accumulation. Data were recorded on a computer and FT-IR spectra were traced using FTIR Essential v3.50.185 software.

2.4. Mechanical properties

Tensile strength tests (TS), Young's modulus (YM), and elongation at break (E%) of starch-based starch films were performed in a Universal Test System texture analyzer (Instron 5566) according to ASTM D882-95 method (ASTM International, 2018). The starch films were stored for 7 days in a desiccator (23.8 °C and 56.5% r. h.) and for the tensile strength determination, the films were cut into 25-mm x120 mm strips. The strips were fixed in 2 grips separated by 120 mm. The grips were dislocated at 25 mm min^{-1} up to film rupture. The values used in the data analysis were related to the deformation force (50 N). A testing speed of 0.5 mm min^{-1} was used for Young's Modulus measurement and was run in two strips for each film sample, and ten determinations were performed, at testing conditions of 23.8 °C and 56.5% RH.

2.5. Sensory analysis

In the sensory test, the starch films, chosen in sections 2.3 and 2.4 (homogeneous and smooth surface appearance without porosity), and mechanical properties (higher TS and EB), were applied to the wheat tortillas to wrap the tuna paste filling, and the sensory properties was evaluated after 7 days. The tortillas were prepared without film (control as used in the market) and with film (150 cm^2) around the tuna paste, constituted by 60% of the total product, with tuna in oil, drained (36.7%), boiled egg (20%) coarsely chopped, and sweet chili sauce (3.3%), placed in plastic packages, sealed and chilled at 4 °C, during the established testing period. The sensory properties were carried out according Singh-Ackbarali and Maharaj (2014), using an untrained panel (16 females and 4 males), aged between 21 and 58 years, at room temperature (25 °C), and under fluorescent light. Two wrap samples, properly identified, cut into square shapes (5 cm × 5 cm), with about 45 g, were served on a disposable plate.

The number of tasters and the order of presentation of the samples followed the adapted free choice profiling method (Kemp et al., 2009) to evaluate quality attributes: appearance, color, taste (tuna), texture, and general acceptance. The parameters were measured, according McEwan and Lyon (2003), on a structured scale from 1 to 5.

In the hedonic scale, two tests were used, acceptance test and purchase intention, using the Likert scale 5 points (Kemp et al., 2009). In the acceptance test the extremes of the scale corresponded to: "did not like it very much" (1) and "liked it very much" (5); and in the purchase intention test: (1) "would probably not buy" and (5) "would buy".

Table 1
Films classification according to visual parameters.

Code	Filmogenic solution	Continuity	Homogeneity	Handling
A	Corn starch (2.1%)	3	3	3
B	Pea starch (2.1%)	3	2	1
C	Chestnut starch (2.1%)	3	2	1
D	Corn starch (1.6%) and Potassium alginate (0.5%)	1	2	2
E	Pea starch (1.6%) and Potassium alginate (0.5%)	3	3	3
F	Chestnut starch (1.6%) and Potassium alginate (0.5%)	3	3	3
G	Corn starch (1.6%) and Agar (0.5%)	3	2	2
H	Pea starch (1.6%) and Agar (0.5%)	3	3	2
I	Chestnut starch (1.6%) and Agar (0.5%)	3	2	1
J	Corn starch (1.6%) and Guar (0.5%)	3	2	2
K	Pea Starch (1.6%) and Guar (0.5%)	3	3	2
L	Chestnut starch (1.6%) and Guar (0.5%)	3	3	3
M	Corn starch (2.1%) and Glycerol (0.6%)	3	2	1
N	Pea starch (2.1%) and Glycerol (0.6%)	3	3	3
O	Chestnut starch (2,1%) and Glycerol (0,6%)	3	3	3
P	Corn starch (1.6%), Potassium alginate (0.5%) and Glycerol (0.6%)	3	3	2
Q	Pea starch (1.6%), Potassium alginate (0.5%) and Glycerol (0.6%)	3	3	3
R	Chestnut starch (1.6%), Potassium alginate (0.5%) and Glycerol (0.6%)	3	3	3
S	Corn starch (1.6%), Agar (0.5%) and Glycerol (0.6%)	3	3	3
T	Pea starch (1.6%), Agar (0.5%) and Glycerol (0.6%)	3	3	3
U	Chestnut starch (1.6%), Agar (0.5%) and Glycerol (0.6%)	3	2	1
V	Corn starch (1.6%), Guar (0.5%) and Glycerol (0.6%)	3	2	2
W	Pea starch (1.6%), Guar (0.5%) e Glycerol (0.6%)	3	2	2
X	Chestnut starch (1.6%), Guar (0.5%) e Glycerol (0.6%)	3	2	1

Table 2
-Properties of the samples of the starch films obtained.

Code	Weight (g)	Diameter (cm)	Area (cm^2)	Grammage (g. cm^{-2})	Thickness (mm)
A	0.198	4.7 ± 0.04	17.4 ± 0.30	114.5 ± 2.5	0.078 ± 0.01
B	0.315	4.3 ± 0.00	14.52 ± 0.00	217.2 ± 0.8	0.105 ± 0.00
C	0.218	5.0 ± 0.15	19.30 ± 1.16	114.8 ± 9.3	0.088 ± 0.01
D	0.195	5.4 ± 0.05	22.70 ± 0.41	86.1 ± 1.1	0.056 ± 0.01
E	0.180	4.9 ± 0.13	18.70 ± 0.93	97.0 ± 5.9	0.045 ± 0.00
F	0.177	5.3 ± 0.06	21.86 ± 0.52	81.3 ± 3.3	0.098 ± 0.01
G	0.177	4.7 ± 0.12	17.38 ± 0.90	102.8 ± 5.4	0.078 ± 0.00
H	0.259	4.0 ± 0.02	12.73 ± 0.16	204.3 ± 3.0	0.075 ± 0.00
I	0.227	4.6 ± 0.22	16.55 ± 1.53	139.7 ± 12.1	0.058 ± 0.01
J	0.209	5.1 ± 0.03	20.03 ± 0.23	104.4 ± 1.3	0.073 ± 0.01
K	0.177	4.8 ± 0.17	18.17 ± 1.31	96.8 ± 2.7	0.135 ± 0.01
L	0.164	5.1 ± 0.09	20.05 ± 0.70	81.8 ± 2.5	0.098 ± 0.01
M	0.268	4.9 ± 0.08	18.68 ± 0.58	143.8 ± 4.1	0.078 ± 0.00
N	0.301	4.5 ± 0.02	15.73 ± 0.17	191.5 ± 4.6	0.088 ± 0.00
O	0.281	5.2 ± 0.10	21.47 ± 0.85	130.7 ± 4.5	0.120 ± 0.01
P	0.284	5.1 ± 0.05	20.63 ± 0.38	137.5 ± 8.4	0.083 ± 0.01
Q	0.249	4.9 ± 0.05	19.06 ± 0.37	130.8 ± 2.6	0.068 ± 0.00
R	0.269	5.1 ± 0.05	20.23 ± 0.38	132.7 ± 13.7	0.080 ± 0.01
S	0.246	4.8 ± 0.08	17.92 ± 0.57	138.0 ± 8.7	0.085 ± 0.00
T	0.263	4.6 ± 0.05	16.81 ± 0.35	156.8 ± 6.7	0.060 ± 0.01
U	0.241	4.8 ± 0.17	17.79 ± 1.21	136.8 ± 10.1	0.083 ± 0.00
V	0.214	5.0 ± 0.10	19.27 ± 0.81	109.7 ± 14.8	0.123 ± 0.02
W	0.232	4.9 ± 0.05	18.67 ± 0.37	124.2 ± 2.1	0.101 ± 0.01
X	0.202	4.9 ± 0.02	19.05 ± 0.19	105.9 ± 1.1	0.073 ± 0.01
Min	0.202	4.03	12.73	81.3	0.045
Max	0.301	5.38	22.70	217.2	0.135
Stdv	0.005	0.35	0.262	3.8	0.003

Results are expressed as the average of four replications ± standard deviation.

2.6. Statistical analysis

The results, of the film surface and mechanical properties, were estimated to one-way analysis of variance (Anova) using the Tukey test for 95% ($p < 0.05$) significance level, and by Cell means plot using the Microsoft Excel 2010 software (Windows 10) corresponding to the average of three replicates ($n = 3$) ± standard deviation.

The results of the sensory analysis were submitted to descriptive statistical analysis and Spearman correlation tests, calculating location measurements, graphs of extremes, and quartiles using RStudio v. February 1, 1335 software (RStudio Team, 2015).

3. Results and discussion

3.1. Film surface properties

Films were initially selected based on subjective evaluation of the films' surface morphological characteristics (Table 1), namely continuity, homogeneity and film handling, as well as physical characteristics such as thickness and grammage (Table 2 and Fig. 1). The water holding capacity (Fig. 2) of the films over time was an important selection parameter. The selected starch films (Fig. 3) were characterized for physicochemical and mechanical properties (Table 3) and the film that exhibited better water capacity, a homogeneous and smooth surface without porosity, and flexible was used to the tortilla filling for the sensory tests.

3.1.1. Subjective evaluation

The results of the subjective evaluation of the starch-based films are shown in Table 1. Were found 10 starch-based films consisting of corn starch (A and S), pea (E, N, Q, and T), and chestnut starch (F, L, R, and O) that gave rise to continuous films with no fractures or breaks after drying, absence of insoluble or visible particles to the naked eye, or areas of opacity or different colors and, which were rated with the highest values for the continuity, homogeneity and handling parameters. Of these 6 formulations, they were made with the addition of glycerol and only 4 without glycerol. The 3 films that had slight difficulty detaching from the support were rated worst in the handling parameter and were made of pea starch (H and K), and corn (P). This was followed by 4 films, which were also rated worse on the homogeneity parameter, as they had more uneven surfaces, and were composed of corn (G and V), and pea (J and W) starches. While the 6 starch-based films had brittle and less pliable areas when folded, and were rated worst in the handling parameter, containing corn (M), pea (B), and chestnut (C, I, U, and X) starches. The film with the worst characteristics was the corn starch film with potassium alginate (D). This may be a consequence of the difference in gelatinisation temperature of the starch used. When the native starch was subjected to a temperature of 95 °C, which is above its

Gelatinisation temperature, the membranes of the starch granules are broken, releasing dextrin, which is a semi soluble with adhesive properties that hinder the release of the cast films from the support (Henrique et al., 2008).

3.1.2. Thickness and grammage measurements

Table 2 presents the results for the average thickness, grammage, area, diameter, and weight of the starch films. Analysis of variance (ANOVA), Two-Factor with replication, was applied to the measurement of the different polymers to verify that there is no difference in the group (null hypothesis) by analyzing the variances. Three-factor models were applied (Table 2), and the results found significant differences ($p < 0.01$). Thus, the null hypothesis is rejected and the hypotheses regarding (i) the interaction between the measurements taken and the type of polymer, (ii) at least one of the polymer blends shows different measurements, (iii) at least one measurement is different for the different polymers (Fig. 1). From the graphs obtained from the analysis of variances, we can conclude that the average weight of the films without glycerol ranged from 81.34 to 217.19 g cm^{-2}, and with glycerol ranged from 105.91 to 191.46 g cm^{-2}. The films produced with pea starch without glycerol (217.19 g cm^{-2}), had the highest grammage than those produced without glycerol (191.46 g cm^{-2}). The addition of glycerol to starch-based materials, on average, decreases the film grammage and we can conclude that the films are more homogeneous. In this study, the filmogenic suspensions always have the same concentration, for all types of starch, the observed difference in film grammage can be explained by the difference in amylose content and molecular weight of different starches (corn, pea, and water chestnut) that may influence the different properties obtained for the films. In order, the amylose content of the starches reported in the literature was as follows: water chestnut 20% < corn about 25% < pea 30%. Was found that the molecular weight of corn starch has the highest values (≈51 MDa) and pea starch and water chestnut has an intermediate value (≈25 MDa). The pea starch has higher amylose content and lower molecular weight giving rise to stronger and less stretchable films. The presented results are in

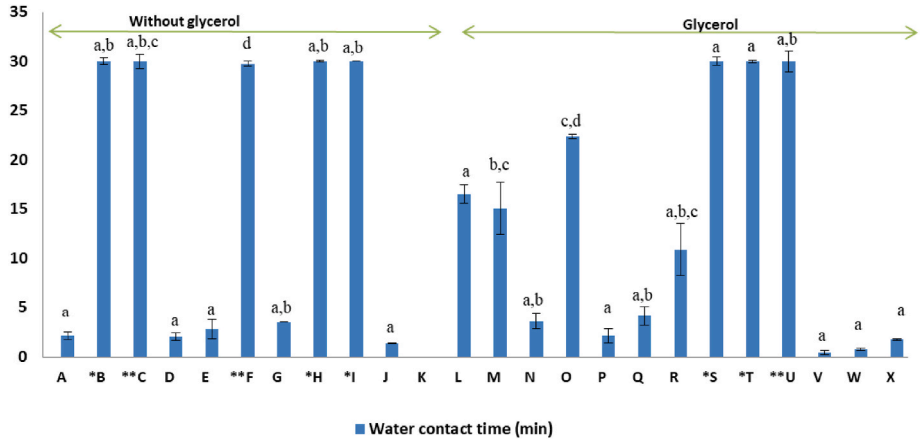

Fig. 1. Variation of water contact time for breaking the films. (*Film intact >30 min**Film damped by the water≈30 min).

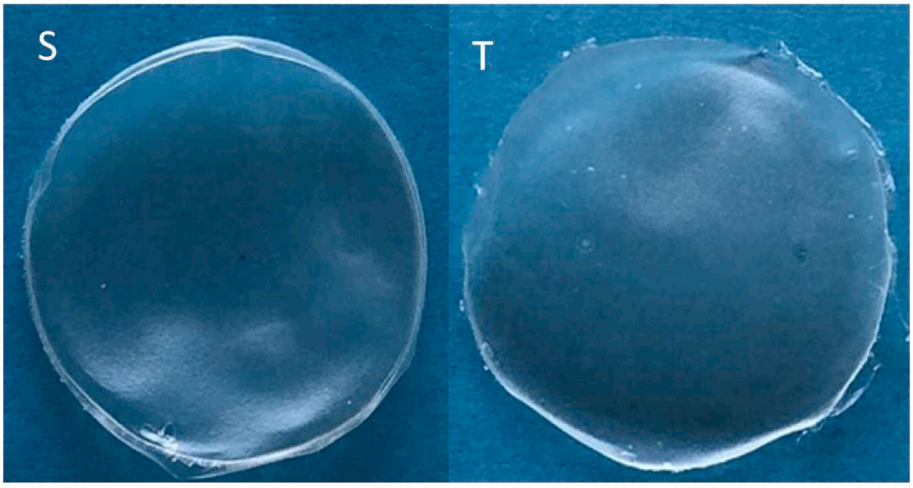

Fig. 2. The visual aspect of films obtained from corn (S) and pea (T) starch.

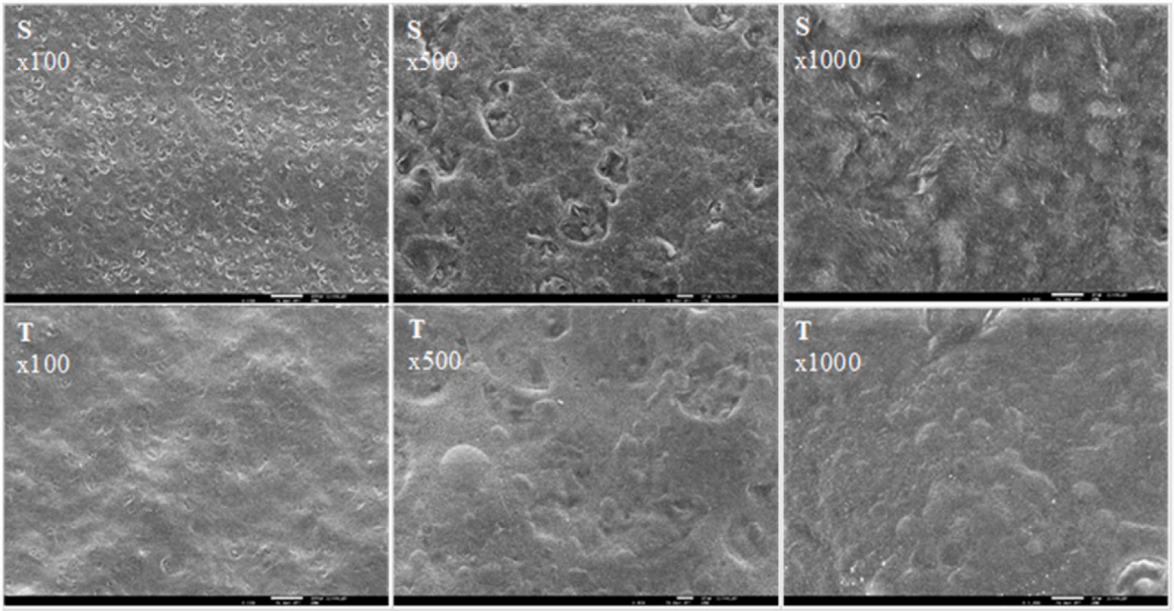

Fig. 3. SEM micrographs of corn (S) and pea (T) starch-based films (magnification ×100, x 500 and x1000).

Table 3
Thickness, grammage, area, water activity (a_w), solubility, tensile strength (TS), Young modulus (YM), elongation at break (E) of corn and pea starch films.

Parameters		Starch Films	
		Corn (S)	Pea (T)
Surface properties	**Thickness** (mm)	0.08 ± 0.01^b	0.08 ± 0.01^a
	Weigh (g)	2.26 ± 0.01^b	2.16 ± 0.12^a
	Diameter	14.14 ± 0.13^a	14.19 ± 0.23^a
	Grammage (g. cm^{-2})	143.93 ± 0.08^a	136.8 ± 8.4^a
	Area (cm^2)	157.0 ± 3.0^a	158.3 ± 4.8^a
Hydration properties	$\mathbf{a_w}$	0.56 ± 0.00^a	0.56 ± 0.00^a
	Solubility (%)	16.20 ± 0.00^a	17.40 ± 0.01^a
Mechanical properties	**TS** (kPa)	22.3 ± 4.0^a	27.5 ± 2.9^b
	YM (Mpa)	64.4 ± 64.2^a	17.1 ± 7.9^a
	E (%)	53.2 ± 26.8^a	186.0 ± 69.2^b

Values having the same letter for a parameter are not significantly different at p level <0.05.

agreement with those presented by Domene-López et al. (2019), since starches with high amylose content could presumably have lower molecular weight and a relatively more linear structure than those with a high amylopectin content. Shrinkage of the starch gel during drying is also favored by lower amylopectin content, and higher amylopectin favors more homogeneous and denser films.

The thickness of the films ranged between 0.045 and 0.135 mm (Table 2), and the standard deviation for the thickness is very small (0.005), underlining the uniformity of the films obtained. These results are in agreement with Rodríguez et al. (2006), controlling the thickness of the starch films allows to evaluate the uniformity of the polymers, repeatability of the measurement of their properties, and allows the validity of the comparisons between the films. The thickness of the starch films also affects their mechanical properties mainly the punching strength and water vapor permeability of hydrophilic films. Thicker films are more resistant to puncture and less permeable to water vapor.

3.1.3. Wettability test

The hydrophilic nature of starch films can be verified through contact with water. Assessment of surface hydrophobicity represents a key characteristic for controlling moisture transfer in polysaccharide-based films, such as testing the surface wettability of films with water. Fig. 2 shows the results for the variation of the water contact time with the film surface for all cast film samples, which ranged from 0.42 min to 30.00. The results show that films B, H, I, S resist without breaking even after 30 min, while in C, F, and U, the water passed to the support side, without breaking the film, having adhered to the support. These films do not meet the requirements and were eliminated. In the remaining films, water broke the polymer structure eliminating them. In general, all samples showed hydrophilic ability, with good wetting behavior rather than repellency characteristics. Polymeric films or blends with agar, however, were found to decrease the hydrophilic characteristics of the film and water permeability. These results are in agreement with those published by Arham et al. (2016), as agar being a cold water-insoluble polysaccharide confers waterproofing to the film. This is due to the decrease of the intermolecular attractions of the polymer chains, that increases molecular mobility, and allows water molecules to enter the film structure (Farahnaky et al., 2013; Thakur et al., 2017). Also, the addition of glycerol in biopolymer dispersions, increases the diffusion of water (water-soluble), in guar blends, and decreases water uptake, in agar blends by blocking the voids in the films (water-insoluble).

In the subjective analysis test, films A, S, E, N, Q, T, F, L, R, and O were selected due to having the highest values for the continuity, homogeneity, and handling parameters. When the films were tested for water droplet wettability on the surface, was found that films B, H, I, S, and T repelled water on the surface the longest. The S and T films were produced on a larger scale (15 cm diameter), and characterized their hydration, microstructural and mechanical properties, to select the best to be applied to the wrapper filling.

3.1.4. Water activity

In hydrophilic films the water activity (aw) and temperature to which they are subjected greatly influence the barrier properties, allowing the control of gas exchange by respiration and microbial development and can maintain the storage time of the products.

The water activity results (Table 3) for corn (S) and pea (T) starch films were similar (0.56 ± 0.005) the films are characterized as intermediate moisture products with a high preservative power because microbial development is not possible ($aw < 0.6$). This result is very positive for the stability of the film because it represents less free water available for the growth of microorganisms, and by decreasing the diffusion of water molecules, it increases the barrier properties of these materials and therefore a longer shelf-life. García et al. (2000) presented similar results (0.64) for corn starch and glycerol (1:1).

3.1.5. Water solubility

The results shown in Table 3 reveal that the difference between the solubility of corn starch film (16.2%) compared to that of pea starch (17.4%), is not very significant indicating that both have good water resistance. This reduction may be due to the greater consistency and stiffness of these films since there were not enough spaces in the polymer matrix that could be occupied by water as has been reported by Santos et al. (2003). However, higher solubility of the film may indicate a lower resistance of the film to water, leading to its dissolution and losing its protective effect on the food surface (Maizura et al., 2007). Similar results were found by Avena-Bustillos and Krochta (1993) with high amylose content of corn starch cross-linked (12%).

3.1.6. Film microstructure

The scanning electron microscopy (SEM) of the starch matrix gives relevant information about the microstructure of the films regarding their homogeneity, layer structure, pores and cracks, and surface smoothness. Depending on the nature of the components and the interactions expanded in the film-forming solutions, the hydrocolloids

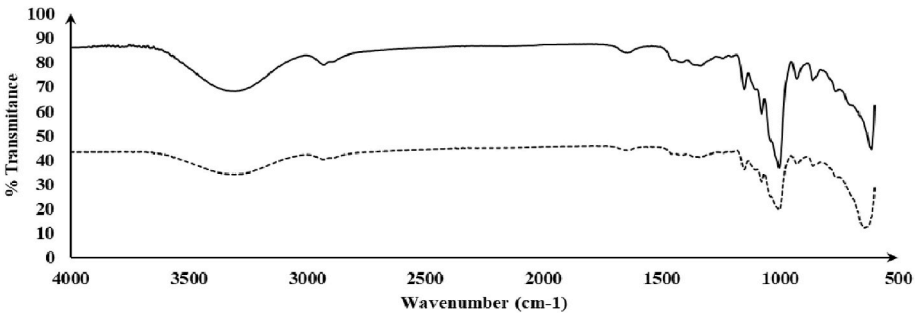

Fig. 4. FTIR spectra of corn (dashed line) and pea (full line) starch films.

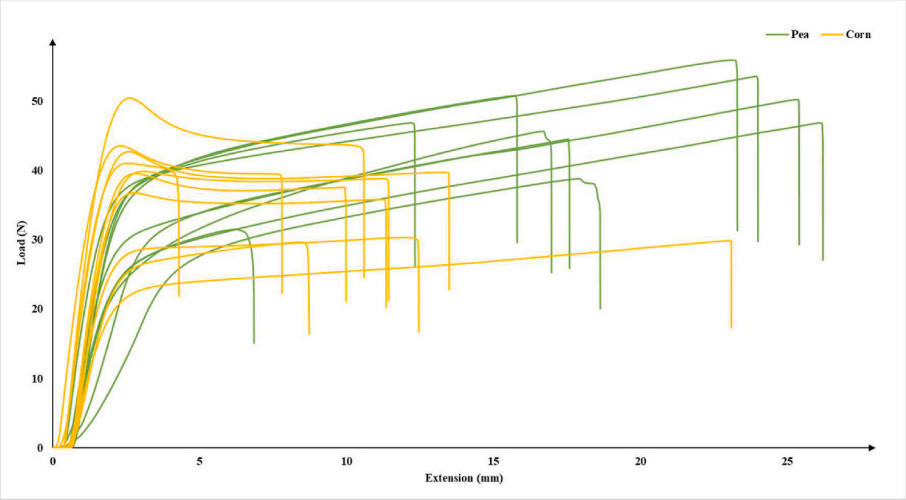

Fig. 5. Tensile test of corn (S) starch cast film.

arrange themselves in different ways in the film matrices (Domene-López et al., 2019).

Images of the microstructure of the corn and pea starch films are shown in Fig. 4. The corn starch film shows a surface with heterogeneity and porosity. Some authors (Shit and Shah, 2014) attributed these irregular microstructures to incomplete dispersion of the starch granules. Drying of starch films containing glycerol cause phase separation and migration of glycerol droplets to the top of the surface. However, the pea starch films show a homogeneous and smooth surface appearance without porosity, which can be attributed to the presence of more ordered phases and a homogeneous structural network.

Similar structures were obtained in other works (Ahmad et al., 2015).

3.1.7. Fourier-transform infrared spectroscopy (micro-FTIR) analysis

The Fourier transform infrared spectroscopy (FTIR) spectra are very effective in the study of intermolecular interaction like hydrogen bonding, allowing its observation to determine the properties of starch films. Changes in the hydrogen bond network due to changes in matrix composition alter this structure and in consequence modulate the matrix network (Liang and Ludescher, 2015). The results of the FTIR spectra of the corn and pea starch films (Fig. 4) show similar main peaks, with a band located at 3356 cm^{-1} that corresponds to vibration modes of intramolecular OH groups of the absorbed water, glycerol, and starch polymer, as also indicated by Flores-Morales et al. (2012). The band located at 2941 cm^{-1} is weak and is related to the hydrogen bond vibration. A weak shoulder was observed at about 2900 cm^{-1}, which overlaps with the band for the $–CH_2$ symmetric stretching vibration, to increase with retrogradation of normal starch (Wang et al., 2015). One explanation for the increased intensity of this shoulder is that it may be related to the amyl-lipid complex formation during retrogradation. The absorption bands at 1662, 1537, 1443, 1378, and 1265 cm^{-1} also change as retrogradation progresses, although the reasons for this are not well understood. These bands are also weak and related to CH and C═O groups and C–O vibrations (Thygesen et al., 2003). The bands at 1079 and 1000 cm^{-1} reflect the amounts of ordered and amorphous regions, respectively (Souza and Andrade, 2002). The absorption ratios of 1079/1000 and 1000/936 cm^{-1} are assumed to represent, respectively, the order in more crystalline regions and the state of organization of the double helices located inside the crystals. With retrogradation, the ratios of 1079/1000 and 1000/936 cm^{-1} increase, consistent with the greater organization of the structure.

The wavelength between 800 and 1300 cm^{-1} corresponds mainly to C–O and C–C stretching vibrations and is sensitive to changes in polymer conformation and starch hydration. The spectral band at 1000 cm^{-1} is very sensitive to water content (Wang et al., 2015). These shifts indicated that the addition of glycerol promoted the hydrogen bonding interactions between starch and glycerol. These results prove the plasticizing effect of glycerol and are expected considering the hydrophilic nature of glycerol and starch (Ambigaipalan et al., 2013).

3.2. Film mechanical properties: Tensile Strength (TS), Elongation at Break (% EAB), and Elastic Modulus (EM)

Tensile strength (TS), elongation at break (%EAB), and elastic modulus (EM) are crucial mechanical properties of active packaging

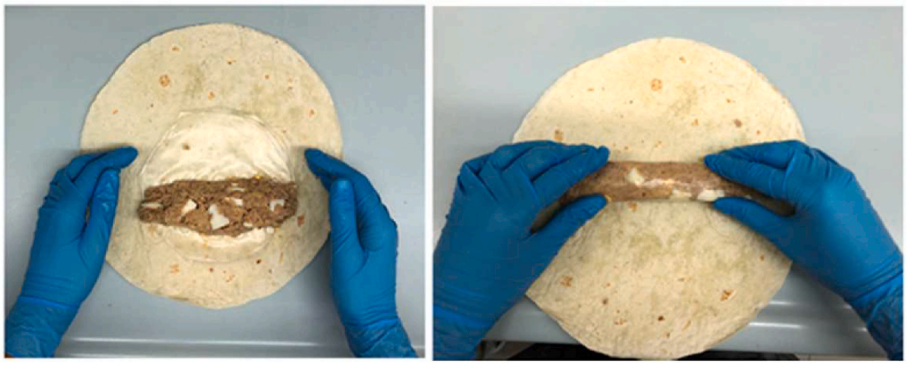

Fig. 6. Filling placement in the edible film on the tortilla top.

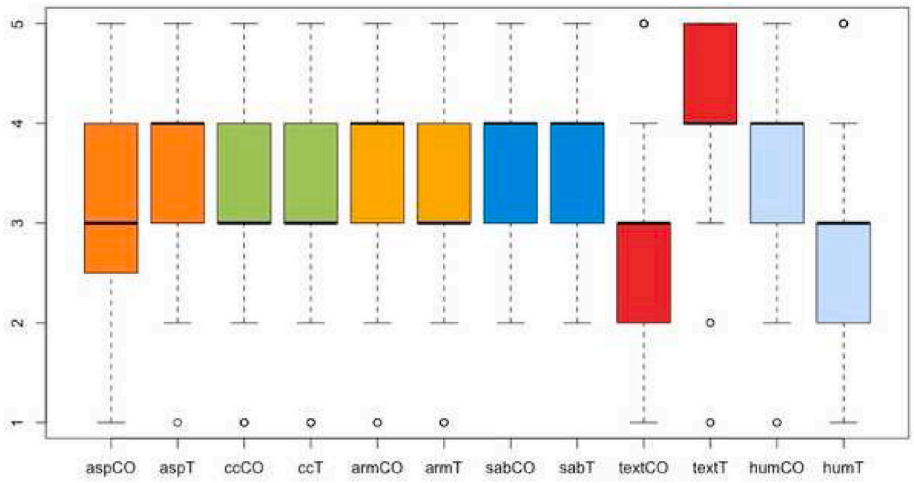

Fig. 7. General results of the sensory analysis. Legend: Co – control, T – film; asp – aspect; cc – brown color; arm – aroma; arm – aroma; sab – tuna flavor; text – tortilla texture; hum – humidity degree in mouth. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

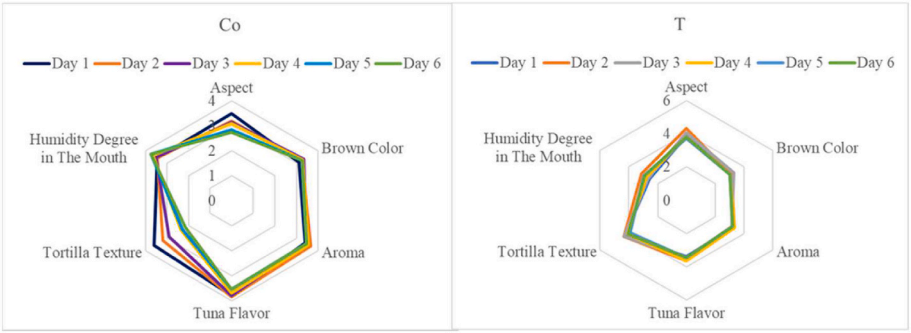

Fig. 8. Attributes of the samples over the days, Co – control, T – film.

films. TS reflects the ability of the films to withstand mechanical stress without damage during production, handling, and application (Borges and De Carvalho, 2015). EAB refers to the maximum stretch the films can withstand, while EM represents the stiffness of the films. As summarized in Fig. 5, can observe, a lack of homogeneity in the TS and EAB values for the duplicate samples of the same film, Fig. 8 shows the strips obtained after the tensile test for each sample. These results can be explained by the heterogeneity of the SF homogenization, poor distribution of the FS on the Petri box, and a non-uniform drying process.

Corn starch films have shown the more stiff characteristics (Table 3): lower tensile strength at break (TS) (21.98 ± 41.12 kPa), low elongation at break (EB) (59.17 ± 22.45%), and higher elastic modulus (EM) (42.35 ± 17.18 MPa). Comparatively, pea films have shown similar TS (27.5 ± 2.94 kPa), higher EB (185.96 ± 69.23%), and lower EM (17,09 ± 7.93 MPa), which indicate, that the pea starch polymer has higher toughness and flexibility, that may be associated with higher puncture and tearing resistance.

This can be explained by the formation of stronger hydrogen bridge bonds between the pea starch polymer chains with the agar gum polymer chains than with the corn starch polymer chains, making the structure of the pea film more compact and resistant and with a greater ability to retain water. The results obtained for corn and pea starches are difficult to compare with the literature, because of the large range of variables involved (different compositions, molecular weight, film preparation methods, and conditioning environments).

3.3. Sensory analysis

In Fig. 6, observe the application of the selected pea starch film (T).

Through the graphic of the general results of the sensory analysis (Fig. 7), it is observable that control (Co) and the pea starch film wrap (film (T)) did not influence the parameters color (brown) and flavor (tuna), with identical values. Concerning aroma (tuna), small differences were observed, since more than 50% of the panelists evaluated both samples between 3 and 4, however revealing a higher trend in control (Co). The film influenced the appearance, texture, and humidity parameters. Regarding the aspect, 69.5% of the panel evaluated control (Co), between 3 and 4, while the film (T) was evaluated with 73.3%, between 4 and 5. The moisture degree in the mouth was higher in control (Co), because 80.2% of the panel evaluated it, between 3 and 4, while T, 74% evaluated it between 2 and 3. The texture of the tortilla was the parameter that showed the most significant differences, since 81.7% of the panel evaluated film (T) between 4 and 5, while the control (Co), only 75.6% evaluated between 1 and 3.

Fig. 8 shows the attributes evolution (appearance, texture, color, aroma, flavor, and humidity) of each sample over time, where a high assessment in the humidity degree and flavor of tuna in control (Co), can be observed.

These results are in agreement with those reported regarding the wetting of the tortilla in the control (Co), denoting deterioration of texture, while the film (T) shows a high evaluation regarding the texture and appearance of the tortilla.

Thus, it can be conclude that the film delays the moisture transfer

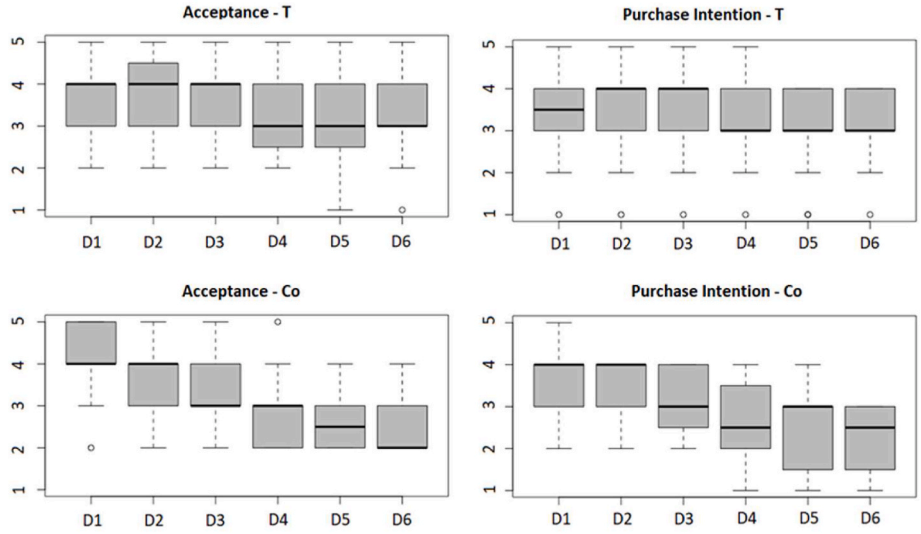

Fig. 9. Sensory analysis: acceptance test and purchase intention, over time. Legend: **Acceptance test**: 1 - dislike extremely; 2 – dislike moderately; 3 - neither like nor dislike; 4 - like moderately; 5 - like extremely. **Purchase intention**: 1 - definitely would not buy; 2 - probably wouldn't buy it; 3 – Maybe yes/maybe not buy; 4 - probably buy; 5 - buy.

from the filling to the surrounding tortilla, allowing to improve the texture and increase the useful shelf-life of these products (O'Connor et al., 2017).

It can also be observed that control (Co) had a more intense flavor (tuna) and aroma than film (T), showing that the film influenced the flavor (tuna) and aroma of the products used in the recipe. Similar effects have been observed by Boonsong et al. (2009), who reported a block in the aromas transfer of volatile compounds, in films produced from cassava starch.

In the acceptance test and purchase intention of each sample over the days, there was a decreasing behavior for both samples (Fig. 9). In the acceptance test on the last day (D6), 90% of the panelists evaluated control (Co), between 2 and 3, while the film (T), presents 75% of the values between 3 and 4. In the purchase intention test, on the last day (D6), 75% of the tasters evaluated control (Co), between 2 and 3, while in T, 85% evaluated between 3 and 4. These results denote the tortilla firmness loss and the appearance spoilage of the control (Co), over time, as the film does not delay the moisture transfer from the filling to the tortilla. Even so, it was observed that the results were always superior for the film.

In the sensory questionnaire, panelists rated overall preference for the wrap with film, because it positively influenced the appearance, texture, humidity degree in the mouth, and the aroma. It also allowed a delay in the transfer of moisture from the filling to the surrounding tortilla, avoiding soggy/moistening and improving the consumption experience of this product.

4. Conclusion

In this study, the edible pea starch film (1.6%) with agar (0.5%) and glycerol (0.6%) prepared by casting, produced a smooth surface visible to the naked eye with a uniform granular network structure of a unique film formation shape, thus exhibiting good water solubility, low microbial growth, good water resistance and mechanical properties. Furthermore, the addition of glycerol was found to improve the properties, physical and mechanical of the film. These results indicated that these films positively influence the texture, flavor, and aroma of the filling of wraps and can be used as edible materials to wrap food filling in tortillas and extend the shelf-life. Treatments also showed positive, in parallel, through the sensory analysis. These films have potential application value in these foods but need further investigation to define other applications for the food industry. Some challenges remain, such as the need to improve and standardize coating procedures, according to industry requirements, aiming to reduce costs and increase shelf-life, to meet consumer demands, without altering sensory characteristics of wrap products.

CRediT authorship contribution statement

Conception and design of study, Carmo Serrano, Cláudia Viegas, João Bordado; acquisition of data: Rafael Santos, R. Galhano dos Santos, J.A.D. Condeço, Ana Marques; analysis and/or interpretation of data: Carmo Serrano, Rafael Santos, Cláudia Viegas, M. Margarida Sapata; Drafting the manuscript: Carmo Serrano, Rafael Santos, M. Margarida Sapata; revising the manuscript critically for important intellectual content: João Bordado, Carmo Serrano, Cláudia Viegas, M. Margarida Sapata; Approval of the version of the manuscript to be published: Carmo Serrano, Rafael Santos, Cláudia Viegas, M. Margarida Sapata, R. Galhano dos Santos, J.A.D. Condeço, Ana C. Marques e J. C. Bordado.

Declaration of competing interest

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

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