CORN STARCH-GELATIN BASED FILMS ADDED WITH GUABIROBA PULP FOR APPLICATION IN FOOD PACKAGING

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ABSTRACT: The guabiroba pulp (*Campomanesia xanthocarpa*) has natural antioxidant compounds, such as phenolic compounds and ascorbic acid. The objective of this work was to produce an active biodegradable film based on blends of gelatin and corn starch and activated with guabiroba pulp, for application as package for extra-virgin olive oil, as a sachet. For that, the effect of blends composition was first evaluated in passive films, and then, a formulation was chosen to produce the active film with guabiroba pulp. The effect of the incorporation of guabiroba pulp on the barrier, mechanical and optical properties of these films was studied, and the antioxidant effect in the storage of extra virgin olive oil was verified in a specific test. Blends with gelatin (GEL) (5 and 10%) and native corn starch (NCS) (2 and 4%) were prepared, in the ratios (2:1, 1:1 and 1:2). The blend film with 5% GEL and 2% NCS in the 1:1 ratio, was selected for the addition of GP (10 and 20%). The addition of GP caused a reduction in tensile strength (TS) and increased elongation values. The film with 10% GP was selected for the preparation of the sachet, due to its lower value of water vapor permeability (WVP) (6.75 g mm m^{-2} d^{-1} kPa^{-1}). At the end of the storage period (15 days), the values of acidity index (AI) and peroxide index (PI) of the extra-virgin olive oil did not reach the maximum limit allowed by the current legislation. Therefore, it can be concluded that the results obtained may be useful for future studies and applications using active and biodegradable packaging in the storage of olive oil.

KEY WORDS: Biodegradable packaging; shelf -life; Olive Oil; Oxidation

HIGHLIGHTS

- The addicion of guabiroba pulp increase the thickeness and elongation of the films.
- Films have shown significant differences in mechanical and barrier properties.
- Acidity from olive oil did not reach the limit allowed by in Brazil's legislation in 14 days.
- Peroxide index from olive oil, in 14 days, are within the limit allowed by in Brazil's legislation.

1 INTRODUCTION

Packaging has the primary function of wrapping products, facilitating transportation and protecting the integrity of the product contained therein (Medeiros, Horodyski & Passador 2017). In addition, it assists in the conservation of food against external factors, such as humidity, tem- perature and light, which can lead to a change in quality and cause its degradation (Carocho, Morales & Ferreira, 2015), such as lipid oxidation, which can occur in products such as olive oil leading to decreased shelf life. Thus, there are numerous packaging options on the market, the most used being flexible plastic films, which ensure the desired protection for many products; however, when improperly disposed they cause serious environmental problems generating a large amount of waste (Koushal et al., 2014).

Today, many industries, companies and organizations already recognize the need to minimize the amount of materials that are difficult to break down, especially synthetic plastics, by undertaking research efforts to find viable alternatives for their substitution. Therefore, one of the solutions found is the development of biodegradable packaging and films, which use polymers from environmentally friendly renewable sources (Razavi, Amini & Zahedi, 2015)

Active packaging is defined as a type of packaging that acts on the storage conditions, extending the shelf life and improving safety or sensory properties, while maintaining product quality (Martucci , Gende, Neira, & Ruseckaite, 2015). Among the substances that promote such effects we highlight the antioxidant agents. The use of antioxidant packaging in food systems may reduce the oxidation reactions, being an advantage mainly in the food industries (Júnior et al., 2015).

The active packaging that use antioxidant agents usually employ synthetic antioxidants; however, their use has been questioned, since several papers affirm that these antioxidants promote toxic and carcinogenic effects to individuals (Sila et al., 2014). With this, the search for natural antioxidants, extracted from vegetables, which are able to retard the oxidation of food and replace the synthetic additives, has gained interest among researchers (Caleja, Barros, Antonio, Oliveira & Ferreira, 2017).

The guabiroba (*Campomanesia xanthocarpa*) is a food plant belonging to the *Myrtaceae* family, being native to Brazil (Viecili et al., 2014). Among the fruits that contain natural antioxidant compounds, guabiroba is an important source of phenolic compounds and ascorbic acid (Lima, Castro, Sabino, Lima & Torres 2016). Thus, the use of such additive in

the elaboration of a film can be interesting, because besides being incorporated in biodegradable matrices, it also has antioxidant action, which can be an alternative to decrease the lipid oxidation of products.

In this context and taking into account the substitution of conventional antioxidants for guabiroba pulp, a natural antioxidant. The objective of this work was to produce an active biodegradable film based on blends of gelatin and corn starch and activated with guabiroba pulp, for application as package for extra-virgin olive oil, as a sachet. For that, the effect of blends composition was first evaluated in passive films, and then, a formulation was chosen to produce the active film with guabiroba pulp. The effect of the incorporation of guabiroba pulp on the barrier, mechanical and optical properties of these films was studied, and the antioxidant effect in the storage of extra virgin olive oil was verified in a specific test

2 MATERIALS AND METHODS

2.1 Materials for the production of films

The materials used to make the films were: gelatin type A (NP comércio de produtos alimentícios LTDA, São Paulo, Brazil), native corn starch (AMILOGILL®2100) supplied by Cargill (São Paulo, Brazil), distilled water, guabiroba pulp, obtained in Laranjeiras do Sul - PR, extra virgin olive oil (Basso Fedele & Figli LTDA, Italy) and acrylic plates.

2.2. Methods

2.2.1. PULPING AND CHARACTERIZATION OF GUABIROBA PULP

The rinsed and sanitized guabirobas were pulped using a previously sanitized pulping machine (with a 0.6-mm sieve). The pulp mass obtained was stored in plastic polyethylene packages with up to 200 g and frozen in a horizontal freezer (-18ºC), until the time of use. The pulp was characterized for moisture, ash-residue by incineration, crude fiber, protein and phenolic compounds (Nollet, 2004; Kwiatkowski, França, Oliveira, Rosa & Clemente, 2010).

2.2.1 PREPARATION OF BLEND FILMS

First, 4 different filmogenic solutions were prepared, two of gelatin and two of corn starch (Tanada-Palmu et al., 2002). The film- forming gelatin-based (GEL) solution was ob tained by hydrating 5 and 10 g of gelatin in 100 mL water for 1 h at 25 ± 3 °C. Afterwards, the temperature of this solution was raised to 55 °C in a thermostatic bath for 10 min. After

solubilization, glycerol was added under mag- netic stirring, and the natural pH of the solu tion was maintained (Bertan, 2008).

The film-forming native corn starch solution (NCS) (2 and 4%), glycerol (10% relative to the dry mass of the starch) and distilled water, under manual stirring and heating at 80°C for 15 min (Bertan, 2008).

Apos a elaboraçao das solucoes filmogenicas, as mesmas foram misturadas em diferentes proporções para obtenção das distintas formulações sendo: A: films made of GEL/NCS/GLY (5 and 2%), Formulaçao B: GEL/NCS/GLY (5 and 4%), Formulaçao C GEL/NCS/GLY (10 and 2%), and formulaçao D: GEL/NCS/GLY (10 and 4%) were made by mixing the film-forming solutions of GEL (Item 2.2.1) and NCS (Item 2.2.2), in the magnetic stirrer for 60 sec, in various ratios (1:1; 1:2 and 2:1 v v-1) (Tanada-Palmu, Fakhouri & Grosso, 2002).

The volume of each ratio of the film-forming solution (20 ml) was poured onto acrylic plates (14 cm x 14 cm) and dried at room temperature (25°C) for approximately 24 hrs. After drying, they were removed from the plates with a metal spatula and stored in desiccators with saturated solution of magnesium nitrate P.A., at 25 ± 3°C and 52 ± 3% RH, for 48 hrs, before the characterization analyzes (Bertan, 2008).

2.2.2 PREPARATIN OF ACTIVE BLEND FILMS

In the film-forming solution GEL/NCS/GLY (5 and 2%) at the 1:1 ratio, previously sieved guabiroba pulp (GP) was added at concentrations of 10 and 20% (m v^{-1}). The solution was homogenized with magnetic stirrer for 120 seconds and then 20mL of the solution was poured onto acrylic plates and dried at room temperature (25°C) for approximately 24 hrs.

2.3 CHARACTERIZATION OF THE BLEND FILMS

2.3.1 VISUAL AND TACTILE ANALYSIS

The films were characterized for the visual aspect through a visual and tactile analysis (Bertan, 2008).

2.3.2 THICKNESS, WATER VAPOR PERMEABILITY AND WATER SOLUBILITY.

Film thickness was measured using a Mitutoyo micrometer (model MDC-25M, Japan). The final value represented the average of 10 random measurements taken at different parts of the film, including the center, edges and middle parts.

The water vapor permeability was determined gravimetrically according to a modified to the standard method E96/96M-16 of the American Society for Testing and Material (ASTM, 2016). Films were placed in permeation cells (0.06 m internal diameter × 0.035 m depth) were sealed on the top of permeation cells (0.06 m internal diameter × 0.035 m depth) containing calcium chloride (relative humidity close to 0.01% at 25 ◦C). The cells were placed in desiccators containing a saturated NaCl solution (25 ◦C, 75% RH). The weight gain of the cells was monitored every 24 h during 7 days. The experiments were conducted in triplicate.

The water solubility was obtained by the percentage of dry material of the film solubilized in water (25 ◦C), (Gontard et al., 1994). All the analyzes were conducted in triplicate.

2.3.3 MECHANICAL PROPERTIES

The tensile strength (TS) and elongation at break (ELO) were determined in a TA-XT2 Texture Analyzer (Stable Micro Systems, Hamilton, USA), according to the ASTM Standard Method D882-12 (ASTM D882-12, 2012), applying modifications according to Tanada-Palmu, Fakhouri & Grosso, (2002). A temperatura do ensaio foi de 25 ◦C.

2.4 PACKAGING PREPARATION

The development and application were based on the study by Reis et al., (2015), with modifications. Two films were used to evaluate the stability of the extra virgin olive oil, being: films made of 5% GEL, 2% NCS, 10% GP added with glycerol and the control formulation, without GP, both at the 1:1 ratio.

Packaging in the shape of rectangular bags (sachets), 6.5 cm x 3 cm in dimension (19.5 cm^2) (Figure 1), defined according to the dimensions of commercial sachets, were made using a foot pedal sealer with timer temperature control. Olive oil aliquots of 6 mL were stored in the sachets and stored in a desiccator (63 ± 3% RH) and in the drying oven under accelerated oxidation conditions, with daytime and nighttime simulation at 30 ± 3°C. (Figure 1), with the purpose of verifying the stability of the product under such conditions (Reis et al., 2015).

Figure 1 - Sachets for the packaging of olive oil, in individual portions, produced from: a) GEL (5%), NCS (2%) and GLY and b) GEL (5%), NCS (2%), GP (10%) and GLY.

2.5 STABILITY MONITORING OF EXTRA VIRGIN OLIVE OIL

After obtaining the sachets, they were stored in a desiccator, with sodium nitrate, at 63 ± 3% RH and in the drying oven at 30 ± 3°C (Reis et al., 2015), with daytime and night time simulation. Packaged extra-virgin olive oils were characterized for (i) acidity index (AI), (ii) peroxide index (PI) and (iii) phenolic compounds content (PC). All the analyzes were performed on days 0, 7 and 15.

2.5.1 Acidity Index (AI) and Peroxide Index (PI)

The acidity index and the peroxide index of the packaged product was determined according to the AOCS methodology (2001).

2.5.2 Phenolic compounds content (PC)

Determination of the phenolic compounds was carried out based on the method of Follin-Ciocauteau, according to Kwiatkowski, França, Oliveira, Rosa & Clemente, 2010.

3 STATISTICAL ANALYSIS

The statistical analyses of variance (ANOVA) were performed using ASSISTAT software version 7.7 Beta (Silva & Azevedo, 2009). Significant differences between means were identified through the Tukey test ($p < 0.05$).

4 RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF THE GUABIROBA PULP

The guabiroba pulp has shown values: moisture 85.4% ± 0.2, ashes 0.64% ± 0.01, carbohydrates 13.29% ± 0.1, protein 0.82% ± 0.24 and phenolic compounds 2660 mg GA $100g^{-1}$± 0.10. Os valores encontrados para umidade e cinzas foram ligeiramente superiores aos valores encontrados por Alves e al, 2013, sendo estes 80 e 0,43%, respectivamente. Os autores encontraram valores para compostos fenólicos em torno de 1222,59 mg GA $100g^{-1}$± 0.10 para a polpa e 1797,56 mg GA $100g^{-1}$± 0.10 para o resíduo da fruta.

4.2 CHARACTERIZATION OF THE BLEND FILMS

4.2.1 VISUAL AND TACTILE ANALYSIS

The films made in all concentrations and proportions had good aspect, easy to handle and were visibly homogeneous, with no presence of insoluble particles or brittle areas. In general, the blend films that contained a higher GEL ratio (2:1) were less flexible and slightly opaque, and those with higher NCS ratio (1:2) had greater opacity, flexibility, and were more fragile. With the increase in GEL concentration (from 5 to 10%) the films were more rigid, and with a slightly yellowish color to the naked eye, which occurred due to the increase in protein concentration. Similar aspect was obtained by Bertan (2008).

4.2.2 THICKNESS, WATER VAPOR PERMEABILITY AND SOLUBILITY IN WATER.

The thickness of the films ranged from 0.0251 mm to 0.0691 mm, being lower for the formulation A film, ratio 1:2, and higher for the formulation B film, ratio 2:1 (Table 1). The difference occurred due to the different proportions used in the preparation of blend films, which are dependent on the composition inherent to each solution. It was observed that by varying the content of NCS (from 2 to 4%), in blend films of GEL (5%), at the 2:1 and 1:2 ratios, there was an increase in film thickness. The same behavior also occurred at the 2:1, 1:1 and 1:2 ratios in films with GEL (10%), varying the NCS proportion from 2 to 4%. The increase in GEL concentration from 5 to 10% caused an increase in thickness in all the proportions studied, both with NCS 2% and 4%. This difference is related to the increase in the amount of dry matter. Hence, when there is an increase in protein concentration in the films, there is an increase in solids in the polymer matrix formed after drying, resulting in an increase in the thickness values (Brandelero, Grossmann & Yamashita, 2013).

The water vapor permeability (WVP) ranged from 3.88 to 7.60 g mm m-2 d-1 kPa-1. He lowest values were obtained in formulation A films, at the 1:1 ratio, and higher for the formulation D films, at 2:1 ratio (Table 1). The formulation A films were not statistically different from each other in relation to all the proportions studied. An increase in NCS concentration from 2 to 4% in films made with 5% GEL (formulations A and B) caused an increase in WVP of the films at the 2:1 and 1:1 ratios. This behavior was probably led by the increase in thickness, as well as the increase in the hydrophilicity of the matrix.

According to Fakhouri, Martelli, Bertan, Yamashita, Mei & Queiroz (2012) in the development of cassava starch with gelatin films, when the protein is incorporated into the matrix of the film-forming solution of the starch, it stays between the amylopectin and amylose chains reducing their interactions, increasing the free volume, resulting in greater diffusion of the water.

Table 1-Thickness, water vapor permeability and solubility of the films made of gelatin (GEL), native corn starch (NCS), plasticized with glycerol (GLY).

Formulation*	Ratio (GEL: NCS)**	Thickness (mm)***	WVP (g mm m^{-2} d^{-1} kPa^{-1})***	SOL (%)***
A	2:1	0.0365 ± 0.0022^{fg}	4.22 ± 0.10^{fg}	43.30 ± 0.52^{bc}
	1:1	0.0335 ± 0.0027^{g}	3.88 ± 0.35^{g}	19.78 ± 0.20^{h}
	1:2	0.0251 ± 0.0039^{h}	4.17 ± 0.62^{fg}	33.97 ± 0.22^{e}
B	2:1	0.0414 ± 0.0042^{e}	5.66 ± 0.37^{bcd}	44.35 ± 0.33^{b}
	1:1	0.0370 ± 0.0023^{fg}	4.85 ± 0.30^{def}	20.60 ± 0.53^{gh}
	1:2	0.0377 ± 0.0019^{ef}	4.65 ± 0.19^{efg}	26.03 ± 0.38^{f}
C	2:1	0.0627 ± 0.0031^{b}	6.33 ± 0.28^{b}	48.32 ± 0.13^{a}
	1:1	0.0477 ± 0.0029^{d}	5.40 ± 0.19^{cde}	42.36 ± 0.53^{c}
	1:2	0.0402 ± 0.0037^{ef}	5.87 ± 0.16^{bc}	37.24 ± 0.95^{d}
D	2:1	0.0691 ± 0.0037^{a}	7.60 ± 0.11^{a}	37.43 ± 0.60^{d}
	1:1	0.0562 ± 0.0027^{c}	6.21 ± 0.27^{bc}	21.59 ± 0.22^{g}
	1:2	0.0495 ± 0.0042^{d}	6.14 ± 0.12^{bc}	14.16 ± 0.39^{i}

*Formulation A: GEL (5%)/NCS (2%)/GLY, Formulation B: GEL (5%)/NCS (4%)/GLY, Formulation C: GEL (10%)/NCS (2%)/GLY, Formulation D: GEL (10%)/NCS (4%)/GLY, where GEL=gelatin; NCS=native corn starch; GLY=glycerol. **The ratios are based on solutions made of GEL (5 and 10%) and NCS (2 and 4%). ***Mean and standard deviation. Values that have the same letter, in the same column, are not significantly different ($p>0.05$) by the Tukey Test with 95% confidence.

The increase of the GEL concentration (from 5 to 10%) in the blend films with 2 and 4% NCS, caused an increase in WVP, in all proportions. According to Bertan, Tanada-Palmu, Siani & Grosso (2005), this is due to the hydrophilic character of the gelatin, which induces interaction with water, causing an increase in the WVP value of the films.

In studies of native cassava starch-based films, plasticized with invert sugar and sucrose, for the incorporation of antimicrobial additives, Kechichian et al., (2010) obtained values ranging from 1.47 to 5.67 g mm m^{-2} d^{-1} kPa^{-1}, close to those found in this study.

The values for water solubility (SOL), ranged from 14.16 to 48.32%, with the lowest value obtained with the 1:2 ratio of formulation D films, and the highest value obtained with the 2:1 ratio of formulation C blend films (Table 1). The increase in the concentration of NCS (from 2 to 4%) in 5% GEL films decreased the SOL value in the 1:2 ratio from 33.97% to 26.03%. Similar behavior was obtained at all the ratios (2:1, 1:1 and 1:2) of the 10% GEL (Formulations C and D) films when there was an increase in NCS content. This behavior probably occurred due to the formation of a more cohesive matrix, which made solubilization difficult.

The increase of the GEL concentration (5 to 10%) caused an increase in the SOL of the films made with 2% NCS (formulation C) in all the proportions studied. Conversely, for the formulation D films, a reduction in SOL was observed for films in the 2:1 ratio (44.35 to 37.43) and 1:2 ratio (26.03 to 14.16%). The SOL in water of the films is directly related to their components, i.e., with the hydrophilicity/hydrophobicity, interaction between the components and consequently their structure.

Similar results were obtained by AL-Hassan & Norziah (2017), in the development of films of gelatin and starch, also obtained SOL values close to those found in this work, ranging from 16.33 to 46.56%.

4.2.3 MECHANICAL PROPERTIES

The tensile strength (TS) of the blend films varied from 10.95 to 79.04 MPa, being lower for formulation C, at the ratio of 1:2 and higher for the films prepared with formulation A, at the 2:1 ratio.

In Table 2, it is observed that when the NCS concentration is increased from 2 to 4% for the films made with 5% GEL (formulations A and B), ratio 2:1, there was a decrease in TS of the films from 79.04 to 48.53 MPa. The same increase in the NCS concentration of the blend films at the ratio of 1:2, caused an increase in the TS of the films, regardless of the concentration of GEL (5 or 10%) used. This was probably due to the higher concentration of solids present in the matrix of the film, making it more cohesive.

Table 2-Mechanical properties (tensile strength and elongation) of the films made of gelatin (GEL), native corn starch (NCS), plasticized with glycerol (GLY).

Formulation*	Ratio (GEL: NCS)**	TS (MPa)***	ELO (%)***
A	2:1	79.04 ± 11.26 [a]	4.17 ± 0.89 [abcd]
	1:1	23.61 ± 4.77 [fg]	1.45 ± 0.61 [d]
	1:2	21.16 ± 6.56 [g]	1.67 ± 0.59 [cd]
B	2:1	37.98 ± 5.36 [de]	2.53 ± 0.75 [bcd]
	1:1	25.15 ± 5.29 [efg]	2.36 ± 0.52 [bcd]
	1:2	48.53 ± 9.74 [cd]	4.21 ± 1.73 [abc]
C	2:1	37.34 ± 4.16 [def]	2.63 ± 0.92 [bcd]
	1:1	61.95 ± 14.69 [bc]	5.01 ± 1.62 [ab]
	1:2	10.95 ± 4.62 [g]	1.60 ± 0.79 [cd]
D	2:1	69.23 ± 0.89 [ab]	4.89 ± 2.56 [ab]
	1:1	69.14 ± 4.71 [ab]	4.90 ± 1.65 [ab]
	1:2	64.86 ± 3.45 [ab]	6.19 ± 2.12 [a]

*Formulation A: GEL (5%)/NCS (2%)/GLY, Formulation B: GEL (5%)/NCS (4%)/GLY, Formulation C: GEL (10%)/NCS (2%)/GLY, Formulation D: GEL (10%)/NCS (4%)/GLY, where GEL=gelatin; NCS=native corn starch; GLY=glycerol. **The ratios are based on solutions made of GEL (5 and 10%) and NCS (2 and 4%). ***Mean and standard deviation. Values that have the same letter, in the same column, are not significantly different ($p>0.05$) by the Tukey Test with 95% confidence.

On the other hand, increasing the GEL content (5 to 10%) leads to an increase in TS values for films made with NCS (2%), in the ratios of 1:1 (23.61 to 61.95 MPa) and for films made with NCS (4%) in all the proportions studied, i.e., TS improved with the increase of GEL concentration. Therefore, as with SOL, the TS values also increased with the addition of GEL (dry matter) content in blend films. The effect of the increase in TS with increased GEL concentration on the NCS blend films was also observed by Bertan (2008).

The percentage of elongation (ELO) of the blend films ranged from 1.45 to 6.19%, being lower in the blend film of formulation A, at the 1:1 ratio, and higher in the blend film of formulation D, at the 1:2 ratio. The increase in starch concentration (2 to 4%) caused an increase in the ELO value at the ratio of 1:2, both for 5% GEL films (1.67 and 4.21%), as well as for films made with 10% GEL (1.60 to 6.19%). The increase in the concentration of GEL in the film-forming solution from 5 to 10% caused an increase in the ELO percentage for films made with 2% NCS in the ratios of 1:1 (1.45 to 5.01%) and for films made with 4% NCS in the ratios of 2:1 (2.53 to 4.89%), 1:1 (2.36 to 4.90%) and 1:2 (4.21 to 6.19%). These differences observed when the concentration of the components used is increased, i.e., GEL (5 to 10%) and NCS (2 to 4%), as well as the variation of the ratio (2:1, 1:1 and 1:2), is directly related to the interactions that these components perform, consequently influencing the obtained properties.

Bertan (2008) has made native or modified waxy corn starch-based and GEL-based films, and obtained ELO values between 2.01 and 4.96%, below those found in this study.

Comparing these results with those of low density polyethylene films (LDPE), the TS values obtained for the blend films in this work are similar to the TS of the LDPE (6.9 – 24 MPa); however, the elongation is much lower than that reported for this synthetic polymer (225-600%) (Twede & Goddard, 2010).

4.3 SELECTION OF BLEND FILM

Based on the results obtained, the film made with 5% GEL and 2% NCS at the 1:1 ratio was selected for the preparation step of active composite films. That formulation was chosen because it presents (i) low WVP (3.88 ± 0.35 g mm m^{-2} d^{-1} kPa^{-1}), (ii) good handling, i.e., the film did not break when subjected to the manual effort produced by the hands, and (iii) low SOL (19.78%)

4.4 CHARACTERIZATION OF THE ACTIVE BLEND FILMS, ADDED WITH GUABIROBA PULP (GP)

4.4.1 VISUAL AND TACTILE ANALYSIS

The incorporation of GP (10 and 20%) into the blend films made them sticky and less resistant, especially in the one that contained the largest amount of GP. In addition, it was observed that the addition of GP caused the blend films to show an orange color, with this color being more intense in the active blend film with addition of 20% GP.

The incorporation of 10 and 20% GP in the polymer matrix of the blend films showed, on only one side, a granular texture, resulting from insoluble GP fibers that did not solubilize in the film-forming solution, since the GP used in the study had 13.3 g/100 g of crude fiber. Similar aspect was obtained by Reis et al., (2015) when making films based on manioc starch incorporated with mango pulp and yerba mate extract.

4.4.2 THICKNESS, WATER VAPOR PERMEABILITY (WVP) AND SOLUBILITY (SOL)

The thickness values of the active films ranged from 0.0895 mm to 0.1243 mm, with the lowest thickness for the control blend film and the highest for the active blend film added with the highest concentration of GP (Table 3). One possible explanation may be related to the components of the GP, such as fibers (13.3 g $100g^{1}$), carbohydrates and proteins (0.82g $100g^{-1}$), which are high molecular mass polymers, in which it increased the solids in the solution, thus contributing to the increase in thickness. Similar behavior was reported by

Souza et al. (2011) who developed films based on cassava starch (4%), with mango pulp (0 to 20%) and acerola pulp (0 to 20%), added with sucrose (1.4%) and invert sugar (0.7%).

The WVP values of active blend films ranged from 6.75 to 12.95 g mm m-2 d-1 kPa-1, while the value for the control film was 3.88 g mm m^{-2} d^{-1} kPa^{-1}, showing significant differences ($p < 0.05$) between the formulations (Table 3). The increment of GP, in both concentrations (10 and 20%), resulted in an increase in WVP values when compared to the control film. According to Avérous, Fringant and Moro (2001), this result can be explained by the presence of hydrophilic materials in the polymeric matrix of the films, coming from both starch and fruit pulp fibers.

According Pereira et al. (2012), when the guabiroba fruit was characterized, they found that it contains 9.75 g 100g-1 of total dietary fiber, with the fraction of soluble fibers being 0.28 g.100 g-1 and insoluble fibers 9.47 g.100 g-1, showing that more insoluble fibers are found in the fruit, which may have influenced the increased thickness and WVP of active blend films.

Tulamandi et al., (2016), by incorporating papaya puree, gelatin and defatted soy protein in the preparation of starch-based films, plasticized with glycerol, obtained WVP measurements close to those found in this study, with values ranging from 5.55 to 8.45 g mm m^{-2} d^{-1} kPa^{-1}.

Nota-se que o maior valor obtido de solubilidade foi de 36,92% oriundo do filme ativo, com 20% de polpa, enquanto que o menor valor obtido foi a do filme controle, cujo valor foi de 19,78%, apresentando diferença estatística ($p < 0,05$) significativa entre as formulações. Foi observado que, assim como os valores de espessura e PVA aumentaram, com a incorporação de PG na matriz dos filmes compostos, ocorreu o mesmo para SOL. Desta forma, a adição da polpa de fruta propiciou aumento na espessura, PVA e SOL dos filmes em virtude da presença de alguns compostos naturais presentes em frutas, tais como a glicose, sacarose, maltose, e celulose, que podem afetar significativamente a matriz filmogenica, pois os filmes à base de amido, são altamente hidrofílicos. Assim com a presença dos componentes naturais das frutas, a sua hidrofilicidade deve ter sido aumentada, absorvendo ainda mais água (VEIGA-SANTOS; DITCHFIELD; TADINI, 2011), acarretando em um aumento de SOL e PVA.

Table 3- Thickness, vapor permeability, solubility, tensile strength and percentage of elongation of the blend films made with guabiroba pulp.

Formulation*	**Thickness (mm)**	**WVP (g mm m^{-2} d^{-1}**	**SOL (%)****	**TS (MPa)****	**ELO (%)****

			kPa^{-1})**		
B	0.0335± 0.0027 [c]	3.88± 0.35 [c]	19.78± 0.20 [c]	23.61 ± 4.77 [a]	1.45 ± 0.61 [c]
B1	0.0895± 0.0076 [b]	6.75± 0.38 [b]	28.84± 0.93 [b]	8.67 ± 0.67 [b]	11.04 ± 1.09 [b]
B2	0.1243± 0.0107 [a]	12.95± 0.61 [a]	36.92± 0.47 [a]	4.24 ± 0.16 [c]	19.67 ± 2.39 [a]

*Formulation B: GEL (5%)/NCS (2%)/GLY, Formulation B1: GEL (5%)/NCS (2%)/GP (10%)/GLY, Formulation B2: GEL (5%)/NCS (2%)/GP (20%)/GLY, where GEL=gelatin; NCS=native corn starch; GLY=glycerol; GP = guabiroba pulp. ** WVP= water vapor permeability; SOL= water solubility; TS=tensile strength; ELO=elongation **Mean and standard deviation. Values that have the same letter, in the same column, are not significantly different (p>0.05) by the Tukey Test with 95% confidence.

4.4.4 MECHANICAL PROPERTIES

The tensile strength (TS) of the active blend films ranged from 4.24 to 8.67 MPa (Table 3). The addition of GP, in both concentrations (10 and 20%), in the blend film caused a reduction in TS, being more evident at the maximum concentration used. This was probably due to GP having insoluble fibers, lipids and carbohydrates, which affect the polymeric matrix of the films, decreasing binding forces, and consequently leading to a decrease in TS.

Pereira et al (2012) evaluated the Brazilian biodiversity through the physical-chemical characterization of three species, among them, guabiroba (*Campomanesia xanthocarpa* O. Berg), which presented lipid values of 3.7g / 100g and carbohydrates of 15.67g / 100g. Thus, the lipids present in the fruit may have led to the TS values of the active compound films.

The ELO of the active blend films ranged from 11.04 (Formulation B1) to 19.67% (Formulation B2), with significant differences between formulations at $p < 0.05$. It can be observed that both the addition and increase of GP concentration caused an increase in ELO. One possible explanation would be the natural presence of carbohydrates (glucose and fructose) and lipids in the guabiroba pulp, which may have influenced the ELO values, since these constituents have the function of plasticizing agent, contributing to the flexibility of the films. According to Matta Junior (2011), when the film structure becomes less rigid, the strength/tension properties are reduced and the elongation increases.

Reis et al., (2015), when formulating films based on manioc starch, mango pulp, yerba mate extract, plasticized with GLY, obtained ELO values ranging from 55.15 to 69.36%, above the values found in this work. The author observed that the formulations with higher concentrations of mango pulp presented higher ELO. Santana et al., (2013) studying the incorporation of different concentrations of anatto as antioxidant additive in chitosan-based

films, plasticized with GLY, obtained values above those found in this study, ranging from 23.20 to 23.98%.

4.5 SELECTION OF THE ACTIVE BLEND FILM

Based on the results obtained, the active blend film with 10% GP was selected to be used in the preparation of sachets and in the packaging of extra virgin olive oil. This film was chosen due to its low WVP value (6.75 g mm m^{-2} d^{-1} kPa^{-1}). Although the selected film appears to present suitable properties for making the sachets, it also exhibited fragility upon contact with the product, along the storage period, only making it possible to perform stability analyzes of the extra virgin olive oil for 15 days.

4.6 STABILITY MONITORING OF THE EXTRA VIRGIN OLIVE OIL

4.6.1 Acidity Index (AI), peroxide index (PI) and and Phenolic Compounds (PC)

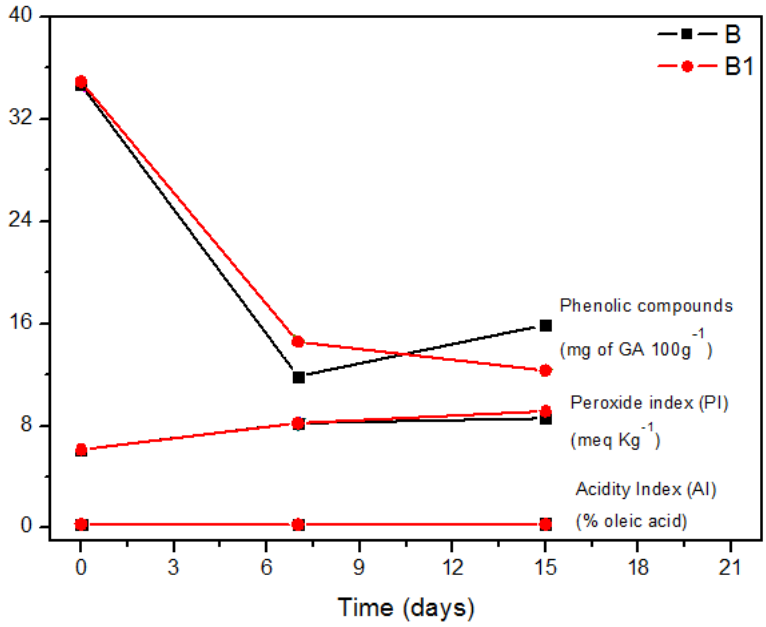
The results shown (Figure 2) for the AI were not significantly different in relation to the storage time, regardless of the film used for the packaging. Stoll, Silva, Costa, Flôres, & Rios, 2017, studying the efficacy of the use of a biodegradable cassava starch film with addition of anthocyanins on the quality of the extra virgin olive oil over 12 days of storage, found results close to those presented in this study. In this study, the author observed that in both packaging conditions (biodegradable film with anthocyanin and polypropylene film) and control, the quality of the extra virgin olive oil was within the limit established by the Codex Alimentarius (2001) (<0.8%) until the end of the storage period.

Regarding the results obtained for the PI, after 7 days it is observed that the values increased from 6.14 to 8.21 meq kg-1, for both treatments (control and with 10% GP), with significant difference ($p < 0.05$) observed between the formulations, from time 0 with the other timepoints (7 and 15 days). This increase in PI after 7 days of storage may have occurred due to the presence of oxygen that remained inside the packaging (headspace) during the packaging process, causing oxidation of lipids in the first stages of product storage (Stoll, Silva, Costa, Flôres, & Rios, 2017). No statistical difference ($p < 0.05$) was observed in the PI value from day 7 to day 15 for both treatments, as well as between treatments throughout the study period.

It can be observed that after 15 days of storage the samples were within the standards required by the Brazilian legislation in force, where the maximum acidity index allowed is

0.8% of oleic acid and the maximum peroxide index amount allowed for extra virgin olive oil is 20 meq kg-1 (ANVISA, 2005; MAPA, 2012)

Figure 2 – Values obtained for Acidity index, peroxide index and Phenolic Compounds (PC) in the extra virgin olive oil stored in control and GP films.



*Formulation B: GEL (5%)/NCS (2%)/GLY, Formulation B1: GEL (5%)/NCS (2%)/GP (10%)/GLY, where GEL=gelatin; NCS=native corn starch; GLY=glycerol. ** The ratios are based on solutions made of GEL (5), NCS (2) e GP(10). ***Mean and standard deviation. Means with equal lowercase letters in the same column do not differ at the level of p> 0.05.

Similar results to those herein were found by Carpiné et al., (2015), who stored olive oil in biodegradable films of isolated soybean protein, coconut oil and natural surfactants and evaluated the stability of the olive oil, packed in the composite film for 28 days. The authors found that the PI in the films increased with the storage time, ranging from 7.59 to 13.197 meq kg^{-1}, in the formulation with higher ratio of coconut oil and 7.59 to 12.56 meq kg^{-1}, in composite films with lower ratio of coconut oil.

The effect of incorporating 10% GP into the sachets (Figure 2), during the 15-day storage period indicated that there were significant losses of PCs present in the extra virgin olive oil during storage. It is possible to observe significant difference from timepoint 0, to the other timepoints (7 and 15 days), for both films. PC losses in the samples are probably related to the photodegradation of PCs during the storage period under accelerated oxidation because they are sensitive to light as well as to elevated temperatures.

When comparing the PC values present in the product stored in the control and active packages, no significant difference was observed between the treatments. This behavior probably occurred because the PCs present in the extra virgin olive oil were degraded during storage in the sachet, when exposed to light. According to Stefanoudaki Williams, & Harwood, 2010, when it comes to the degradation and loss of stability of olive oil, light is one of the main factors that must be considered. Additionally, Piscopo & Poiana, 2012, report that the light can initiate chemical and biochemical reactions in the presence of oxygen and consequently causes oxidation of lipids and other compounds.

This study can be compared with that by Santana, Machado, Larroza & Druzian, 2013, who evaluated the effect of incorporating 0.25, 0.5 and 1.0% annatto in biodegradable chitosan-based packages and found that during a 45-day period there were significant losses of phenolic compounds in the packaged product (palm oil), given that for packages with 1% anatto at timepoint 0, the CP content was 199.91 mg g^{-1} and decreased to 89.41 mg g^{-1} after 45 days. Machado et al. 2012, evaluated the antioxidant action of cassava starch films containing nanocellulose as mechanical reinforcement, and yerba mate extract as antioxidant additive, when packaging palm oil. A decrease was reported in the values of phenolic compounds of the films, from 102.70 to 60.72 mg g^{-1}, after 40 days of storage.

5 CONCLUSION

Foi possivel elaborar saches a base de amido de milho e gelatina, com a adição de polpa de guabiroba, para acondicionar óleo de oliva extra virgem. A formulação do sache foi baseada na caracterização dos filmes elaborados uma vez que the addition of GP (10 and 20%), to the selected blend film, caused a reduction in TS and increase in moisture content, thickness, WVP, SOL and ELO quando comparados ao filme sem adiçao de polpa.

The active blend film with 10% GP was selected for the preparation of the sachet, which in turn was used in the packaging of extra virgin olive oil, due to its lower WVP (6.75 g mm m^{-2} d^{-1} kPa^{-1}). The sachets made with blend films added with 10% GP were more fragile than the sachets without addition of GP.

At the end of the packaging period of the extra virgin olive oil, the AI and PI values for the olive oil packaged in both the GP and control films, did not reach the maximum limit allowed by the current Brazilian legislation, in the period studied. The values obtained from the PCs showed that from the timepoint 0 days to the timepoint 7 days, the PC of the extra virgin olive oil decreased, but on the timepoint 15 days, such values did not change

significantly. The addition of guabiroba pulp to the films did not have an additional effect on the oxidative stability of the extra virgin olive oil.

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