COMPARATIVE STUDY ON PROPERTIES OF EDIBLE FILMS BASED ON
PINHÃO (*Araucaria angustifolia*) STARCH AND FLOUR

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Abstract

The aim of this study was to develop and compare the properties of edible films based on pinhão starch and pinhão flour. Seven formulations were developed by casting methodology: 5% pinhão starch with 0, 1, 1.5, and 2% glycerol, and 5% pinhão flour with 1, 1.5, and 2% glycerol. The films were evaluated and compared to each other for thickness, morphological analysis by Scanning Electronic Microscopy (SEM), glass transition temperature (T_g), apparent porosity ($\emptyset$), water vapor permeability (WVP), tensile properties, color, opacity, and Fourier transform infrared analysis (FTIR). In

addition, the raw materials were analyzed for moisture content, water activity (A_w), apparent porosity and pasting profile. Pinhão starch and flour did not show differences in moisture content and pinhão starch presented lower A_w , $\emptyset$, but higher peak viscosity than pinhão flour. Films made with pinhão starch exhibited lower thickness, $\emptyset$, WVP and opacity than pinhão flour films, but higher Young's modulus, tensile strength and elongation at break. In addition, films with higher amounts of glycerol exhibited higher WVP and elongation at break, with lower tensile strength and Young's modulus. The pinhão flour films structure presented irregularities and were rougher than pinhão starch films. Differences in glycerol content did not affect T_g values of these films. Flour films were yellowish and also had a higher opacity value. The FTIR spectra of pinhão starch and pinhão flour films showed similar IR absorbance patterns, with no structural change in the presence or absence of glycerol.

Keywords: pinhão; starch film; flour film; characterization; tensile properties; water vapor permeability.

1. Introduction

Plastics derived from petroleum are the most common film packaging materials in the food industry. Plastic degradation takes a long time and can cause many environmental problems by not being biodegradable (Sorrentino, Gorrasi, & Vittoria, 2007; Xu, Kim, Hanna, & Nag, 2005). The interest in developing edible films and coatings as an alternative to plastic materials has increased because of their capability to improve global food quality and extend food shelf-life. Many studies show evidence of

the beneficial effects of using such films on fresh and processed foods (Chillo, et al., 2008; Flores, Famá, Rojas, Goyanes, & Gerschenson, 2007). Biodegradable packaging made from entirely renewable natural polymers can decrease environmental pollution, as they degrade after their disposal, creating new markets for agricultural products (Gontard & Guilbert, 1994; Xu, et al., 2005). In addition, some biodegradable films may also be edible and be consumed with the food they are wrapping.

Biodegradable and edible films can be used to limit moisture, aroma, and lipid migration between food components, to restrict absorption of oxygen, to improve mechanical handling properties, or to offer an alternative to commercial packaging materials (Bourtoom & Chinnan, 2008). Starch-based films from different sources and with different properties have been widely studied (Al-Hassan & Norziah, 2012; Chen & Lai, 2008; Chillo, et al., 2008; Fakhoury, et al., 2012; Famá, Flores, Gerschenson, & Goyanes, 2006; Garcia, Pinotti, & Zaritzky, 2006; Kechichian, Ditchfield, Veiga-Santos, & Tadini, 2010; Mali, Grossmann, García, Martino, & Zaritzky, 2004, 2005; Mali, Grossmann, García, Martino, & Zaritzky, 2006; Maran, Sivakumar, Sridhar, & Prince Immanuel, 2013; Souza, et al., 2012; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009) and have indicated that these carbohydrates are promising materials in this regard. Starches show physical properties similar to plastic materials, are readily available and have good ability to form odorless, colorless, transparent, non-toxic and biologically degradable films (Cano, Jiménez, Cháfer, González, & Chiralt, 2014; Famá, Rojas, Goyanes, & Gerschenson, 2005; Flores, et al., 2007; Soliva-Fortuny, Rojas-Graü, & Martín-Belloso, 2012). Starch-based and composite edible films can enhance food quality, safety and stability as they can control mass transfer between components within

a product, as well as between product and the environment (García, Pinotti, Martino, & Zaritzky, 2009).

Edible films can also be developed from a mixture of components, as proteins, lipids and carbohydrates. Sometimes, these components present incompatibilities between one another. One approach to avoid incompatibility between the polymers from different sources is to use unrefined raw materials, such as flours. Then, the films will have the natural mixtures of carbohydrates, proteins, lipids and fibers in a totally compatible way (Maniglia, Domingos, de Paula, & Tapia-Blácido, 2014). In recent years, some studies have reported the use of flour as a raw material suitable for preparing films (Andrade-Mahecha, Tapia-Blácido, & Menegalli, 2012; Colla, do Amaral Sobral, & Menegalli, 2006; Dias, Müller, Larotonda, & Laurindo, 2010; Mariniello, et al., 2003; Rayas, Hernandez, & Ng, 1997; Salas-Valero, Tapia-Blácido, & Menegalli, 2015; Tapia-Blácido, Sobral, & Menegalli, 2005). The use of natural blends of proteins, polysaccharides, and lipids directly obtained from agricultural sources takes advantage of each component in the original system and appears to be a new opportunity for composite materials in the area of edible films (Tapia-Blácido, et al., 2005).

Since the earliest times, cultivation of plants containing high quantities of starch has been of great importance to humanity. Cereal grains, legume seeds, tubers, and certain fruits contain from 30% to 85% starch on a dry basis. In the native state, starch exists as insoluble granules with characteristic shape and some crystallinity. Starches contain between 18% and 30% amylose, except for high amylose starches and waxy corn types that are practically all amylopectin. Each raw starch has its own characteristic viscosity/temperature profile, as a result of its particular granular composition and

structure (Kramer, 2009) and these difference influence the final characteristics of starch-based films.

Pinhão is a regional seed of South America widely consumed in the local cuisine. The seeds have starch as the main component, at approximately 36% on wet basis (w.b.) (nearly 72% on dry basis) (Cordenunsi, et al., 2004). Raw pinhão flour has a greater amount of starch, at about 64%, and it contains between 3 and 5% proteins, 2 and 6% lipids, around 6% dietary fiber and 2.5% ash (Capella, Penteadó, & Balbi, 2009; Cladera-Olivera, Marczak, Noreña, & Pettermann, 2011). These characteristics show that pinhão flour and starch are good sources of complex carbohydrates and potential film forming ingredients for edible films. In addition, this regional seed in new sustainable products can support Brazil's conservation efforts that encourage consumption of this crop. New uses for pinhão can encourage agricultural extension for small farmers and extend the use of pinhão year round, rather than only during the harvesting season, increasing its market (Figueiredo-Filho, Orellana, Nascimento, Dias, & Inoue, 2011; Santos, Corso, Martins, & Bittencourt, 2002).

Based on the constant necessity of food industry for new and innovative products, pinhão (*Araucaria angustifolia*) seeds were proposed for the first time in this study as a natural source of starch and flour to develop edible films containing only pinhão starch or flour and glycerol. There are few studies applying pinhão starch as base for biofilms (Luchese, Frick, Patzer, Spada, & Tessaro, 2015; Spada, da Silva, & Tessaro, 2014), but, these studies also incorporated xanthan gum into the films. To the best of our knowledge, the development of pinhão flour-based films was not tested yet, nor was the use of pinhão starch in films not including xanthan gum. The use of pinhão flour has the advantage of

using the whole seed, decrease the process steps and waste. The aim of this study was to develop edible films based on pinhão starch and pinhão flour plasticized with glycerol, evaluate film physical properties, and compare starch-based and flour-based films with different glycerol contents.

2. Methodology

2.1. Materials

Pinhão seeds harvested in Southeastern Brazil in 2014 were used to develop edible films. One kilogram of pinhão seeds was used to obtain two fractions: starch and flour. The starch was obtained according to Daudt et al. (2014) and the flour was obtained from raw seeds. For the flour, the seeds were peeled, and dried in an oven with air circulation (SL 102/100, Solab, Brazil), at 50 °C for 24 h. After drying, the seeds were pre-milled in a cutting-grinding head (IKA[®] MF 10.1 basic, Staufen, Germany) to produce a coarse flour with around 2 mm particle size and finely milled to pass through a 0.25 mm mesh with a Cyclone sample mill (Udy Corp., Fort Collins, CO, USA). Vegetable 100% pure glycerol was used as plasticizer (Starwest Botanicals, Sacramento, CA, USA) in film preparation.

2.2. Methods

2.2.1. Physical properties of pinhão starch and flour

Physical properties of pinhão starch and flour were evaluated to understand the mechanisms involved during film formation and the final product properties.

Moisture content of pinhão starch and flour samples was determined according to AOAC method 934.06 (AOAC, 1997).

Water activity of pinhão starch and flour samples was measured using an AquaLab 4TE Water Activity Meter (Decagon, Pullman, WA, USA). It was previously calibrated using saturated salt solutions, according to the calibration procedure for this instrument. Water activity was measured at room temperature (25 ± 2 °C).

To determine true density of powders and films, a gas pycnometer (Micrometrics AccuPyc II 1340, Norcross, GA, USA) was employed to measure the displaced volume from samples. The corresponding mass of the dried sample was previously measured. The true density was calculated by dividing mass by volume of samples in five replicates.

Bulk density was measured according to the Method 616 from the United States Pharmacopeia for powders (USP, 2008) with some modifications. Five replicates made using 1.6×10^{-4} m³ cylindrical container filled using a funnel, with its discharge opening located 25 mm above the top edge of the container. Since the powder in the funnel bridged, it was stirred with a thin glass bar to provide a continuous flow through the funnel discharge. The powder in the container was leveled by rolling a round, stainless steel bar across the container in two perpendicular directions. Then, the container was weighed. The bulk density was calculated by dividing the mass of the sample by the volume of the container. The apparent porosity ($\emptyset$) was calculated from the true density (ρ_t) and bulk density (ρ_b) according to Eq. 1 (Datta, 2007).

$$\emptyset = 1 - \frac{\rho_b}{\rho_t} \quad (1)$$

The samples pasting properties was determined with a Rapid Visco Analyzer (RVA-30, Newport Scientific, Sidney, Australia). The pasting properties involve the

granular swelling and exudation of the molecular components. During the analysis, the pasting properties are measured as pasting onset temperature, peak temperature, peak viscosity, and final viscosity. Pinhão starch and flour samples (3.5 g of starch or flour corrected using the moisture content of the sample, ± 0.01 g) were mixed into slurries with distilled water (25.0 g as a function of the amount of adjusted sample, ± 0.1 g) in aluminum cans. The measurement started at 50 °C, which was held for 1 min, raised to 95 °C in 4 min, held for 3 min, cooled to 50 °C in 4 min, and finally held for 1 min. Stirring speed was 960 rpm for 10 s (to ensure initial dispersion) and 160 rpm for the remainder of the test period. The high speed of rotation was conducted to ensure the complete mixing of the starch and flour samples with the water. The results are presented in a graph form, showing the viscosity of samples as a function of time and temperature used. The values of pasting onset temperature, peak temperature, peak viscosity (the maximum viscosity during the heating), and final viscosity were extracted from the graph and were also reported.

Experiments were carried out in triplicate and an arithmetic average was taken for data processing.

2.2.2. Film preparation by casting

Edible films using pinhão starch and flour were prepared using the following procedure. The pinhão starch and flour films were obtained by mixing pinhão starch or flour with glycerol and distilled water. All films contained 5% of pinhão starch or pinhão flour and 0, 1, 1.5 and 2% of glycerol in starch films (named S5, SG5:1, SG5:1.5 and SG5:2, respectively), and 1, 1.5 and 2% in flour films (named FG5:1, FG5:1.5 and

FG5:2, respectively). The glycerol contents used represent 20, 30 and 40% of starch or flour (w/w). Pinhão flour films without glycerol cracked and were not able to be formed. The solutions were heated in a water bath stirring at 120 rpm (Model 12504, Cole-Parmer Instrument Co., Chicago, IL, USA) for 30 min at 80 °C. After the solutions were prepared, the flasks were allowed to rest for 10 min to degas in a vacuum pump (2032B DryFast Ultra®, Welch Technologies, Monroe, USA) in order to prevent micro bubble formation and subsequent pores or holes within and through the films. The solutions were then poured on glass plates (30x30 cm) covered with Mylar polyester film (DuPont, Hopewell, VA, USA) for film preparation by casting. The mixes of starch were cast at a wet thickness of 1.14 mm (starch films) and 0.89 mm (flour films) onto the plates by spreading the solutions with casting bars and the plates were placed on a leveled surface at room temperature and allowed to dry for 24 h. After drying, the films were removed from the Mylar sheets and conditioned in plastic bags at room conditions: 25 ± 1 °C and $28 \pm 2\%$ RH.

2.2.3. Film thickness

Film thickness was measured with a digital micrometer (Mitutoyo Manufacturing, Tokyo, Japan) at 5 random positions on the film samples for further tests. The films were at $28 \pm 2\%$ RH.

2.2.4. Apparent porosity (ϕ)

Apparent porosity was measured according to Eq. 1 (Datta, 2007), considering bulk density (ρ_b) and true density (ρ_t). As the films represent a different system of

powders, the bulk density (ρ_b) was measured according to de Moura et al. (2011). The films bulk density (ρ_b) was obtained from at least twenty measurements of 20 mm diameter film discs pre-equilibrated at 0% RH (calcium sulphate, W.A. Hammond Drierite, Xenia, OH) for 24 h. Each film was weighed to the nearest 0.0001 g using an analytical balance, and the thickness was measured in four random places plus the center with a digital micrometer (Mitutoyo Manufacturing) to the nearest 0.001 mm. Then, the ρ_b was calculated dividing the mass by the volume.

The true density (ρ_t) was determined using a gas pycnometer, model AccPyc II 1340 Series Pycnometer (Micromeritics Instrument Co., Norcross, GA). Samples were cut into pieces, dried for 24 h at 0% RH with calcium sulfate (W.A. Hammond Drierite, Xenia, OH), and compressed into a 1 mL cylindrical container for density measurements. The samples were analyzed in triplicates by the measurement of the volume of samples by helium displacement.

2.2.5. Morphological Analysis by Scanning Electron Microscopy (SEM)

Film cross sections were prepared by dropping a film into liquid nitrogen followed by fracturing with a pre chilled razor held in a vice grip locking pliers. The freshly fractured film pieces were retrieved from the liquid nitrogen bath and placed into a Petri dish containing a piece of filter paper and placed in a desiccator (0% RH) to dry at room temperature. Fractured film pieces were then mounted fractured surface up on a half-stub attached to a full stub using carbon adhesive tabs (Ted Pella Inc., Pleasanton, CA). Once the samples were secured to the SEM stubs, they were coated with gold-palladium in a Denton Desk II sputter coating unit (Denton Vacuum U.S.A, Moorestown,

NJ). All samples were then viewed and photographed in a Hitachi S-4700 field emission scanning electron microscope (Hitachi, Japan) at 2 kV.

2.2.6. Glass transition temperature (T_g)

The glass transition temperature of the films was determined by DSC thermograms. The samples were analyzed using DSC 8000 (Perkin Elmer Inc, Waltham MA, USA). The samples (6–10 mg) were weighed in aluminum pans and hermetically sealed and an empty pan was used as the reference. Scans were carried out over the temperature range of 20-100 °C, and heating rate of 10 °C min⁻¹ under nitrogen at 20 mL/min. The films were at 28 ± 2% RH before the analysis.

2.2.7. Water Vapor Permeability (WVP) of Films

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh, Avena-Bustillos, & Krochta, 1993) was used to determine WVP. Cabinet temperature of 25 ± 1 °C was maintained in a controlled temperature cabinet. Fan speeds were set to achieve air velocities of 152 m/min to ensure 0% RH over films throughout the cabinets. Cabinets were pre-equilibrated to 0% RH using anhydrous calcium sulphate (W.A. Hammond Drierite, Xenia, OH). Circular test cups made from polymethylmethacrylate (Plexiglas™) were used. A film at 28 ± 2% RH was sealed to the cup base with a ring containing a 20.3 cm² opening using 4 screws symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant. Distilled water (6 mL) was placed in the bottom of the test cups to expose the film to a high % RH inside the test cups. Average stagnant air gap height between the water

surface and the film were considered to estimate % RH at films underside. Test cups holding films were then weighed to obtain the initial weight and put into the 0% RH desiccator cabinets. Each cup was weighed 8 times at least at 2 h intervals. Eight replicates of each film were tested. % RH at the film underside and top were estimated and WVPs calculated using the WVP Correction Method (McHugh, et al., 1993).

The WVP of the films was calculated by multiplying the steady state water vapor transmission rate by the average film thickness determined as described above and dividing by the water vapor partial pressure difference across the films (Eq. (2)):

$$WVP = \frac{(WVTR) \cdot (thickness)}{(p_{A1} - p_{A2})} \quad (2)$$

where *WVTR* is the water vapor transmission rate and p_{A1} is the water vapor partial pressure inside the film in cup and p_{A2} is the water vapor partial pressure outside the cup.

2.2.8. Tensile properties

Films used for tensile tests were cut to have a rectangular dimension in accordance to ASTM D882-12, using films with midsection 13 mm wide, 100 mm long, flaring to 25 mm by 35 mm square sections on each end. These films were conditioned in a cabinet by exposing them to MgNO₃ saturated solutions ($A_w = 0.529 \pm 0.002$ at 25 °C) around 50% RH at 25 °C for 48 h before measurements. An Instron Model 55R 4502 Universal Testing Machine (Instron, Canton, MA, U.S.A.) with a 100 N load cell was used to determine the maximum tensile strength (TS), maximum percentage elongation at break (%E), and Young's modulus (YM). The test speed was 25 mm/min and the distance between the grips was 100 mm. Tensile properties were calculated from the plot of stress (tensile force/initial cross sectional area) versus strain (extension as a fraction of

the original length) (Pérez-Gago & Krochta, 2001). The Young's modulus was obtained by the ratio of stress to strain at the initial linear portion of the curve. The elongation to break was calculated by dividing the extension at rupture of the films by the initial length of the specimen (100 mm) and multiplying by 100.

2.2.9. Film color and opacity

A Model CM-3500d Minolta Spectrophotometer (Minolta, Inc., Tokyo, Japan) was operated to determine the color parameters (CIE L^* , a^* , and b^* coordinates and opacity) of pinhão starch and flour based edible films at $28 \pm 2\%$ RH. The equipment was set using a D65 illuminant with an opening of 8 mm and a 10° standard observer angle. The instrument was calibrated before measurements with the white and black tiles provided by the manufacturer. The color values were expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness). Three films from each treatment were evaluated and five readings were made in each replicate.

The opacity (Y) was calculated as the ratio between the opacity of each sample on the black standard (Y_b) and opacity of each sample on the white standard (Y_w). The parameters Y_b and Y_w were determined in three films with black and white standards behind the films, respectively, in three random positions and expressed in percentage (Eq.(3)):

$$Y(\%) = \frac{Y_b}{Y_w} 100 \quad (3)$$

2.2.10. Attenuated total reflectance-Fourier transform infrared analysis (ATR-FTIR)

Fourier transform infrared (FTIR) spectroscopy was used to identify the chemical structure of the pinhão starch and pinhão flour films and possible interactions between their components. The conditioned pinhão starch-alone, starch-plasticized and flour-plasticized films at $28 \pm 2\%$ RH were analyzed on a FTIR instrument (Nicolet iS10 FT-IR Spectrometer, Thermo Scientific, USA). The spectra were obtained in the range between 500 and 4000 cm^{-1} wavenumber using 16 scans at 1 cm^{-1} resolution. Before film analysis, an open beam background spectrum of a clean crystal was recorded. Data analysis of each film was performed with Thermo Scientific™ OMNIC™ (OMNIC™ Series Software, Madison, WI, USA) program.

2.2.11. Statistical analysis

Data were analyzed by 2- sample t-Student tests, and one-way ANOVA with Tukey's multiple comparison tests at 95% confidence level using Minitab version 14.12.0 statistical software (Minitab Inc., State College, PA, USA).

3. Results and Discussion

3.1. Physical properties of raw material

Physical properties of raw material used in edible films development, pinhão starch and flour, were evaluated and the results are showed in Table 1 and Figure 1.

Regarding the moisture of raw material evaluated, both samples presented low moisture with no statistic difference. Generally, flours and starches have moisture content around 12% (Adedokun & Itiola, 2010; Tomassetti, Campanella, Delfini, & Aureli, 1987), and this parameter depends on source, storage conditions, climate and others.

There were reported values of around 9% for native tapioca and corn starches (Adedokun, et al., 2010; Atichokudomchai, Shobsngob, Chinachoti, & Varavinit, 2001), around 10% for plantain and corn starch (Pérez-Sira, 1997), and between 11 and 17% for potato starch (Moorthy, 2002). In general, low moisture content, as found in pinhão starch and pinhão flour analyzed in this work, is preferable for storage.

The water activity (A_w) is another important parameter to estimate storage time under different environmental conditions. In this work, A_w is higher in pinhão flour than pinhão starch. This difference between samples does not affect the quality of them, since there is no microorganism proliferation in A_w lower than 0.6 (Barbosa-Cánovas, Fontana Jr., Schmidt, & Labuza, 2007).

It can be seen that the true and bulk densities of pinhão starch were significantly higher than pinhão flour. On the other hand, the apparent porosity of pinhão starch was significantly lower than pinhão flour. This happened because the difference between true and bulk densities is higher for pinhão flour. Bian et al. (2015) studied two wheat flour varieties and found higher values of bulk density, 0.658 and 0.717 g/cm³. Marousis and Saravacos (1990) studied starches with low and high amylose at different moisture contents. For fully dried samples (just like the samples used in the present work) corn starches with high and low amylose content presented true densities around 1.45 g/cm³, bulk densities around 0.75 g/cm³ and porosity values around 0.5. For maize native starch there were reported values of 1.48 g/cm³ for true density, 0.52 g/cm³ for bulk density and apparent porosity of 0.65 (Musa, Gambo, & Bhatia, 2011).

As observed in Table 1, pinhão starch presented a significantly higher peak viscosity and final viscosity than pinhão flour. The pasting profiles of both are presented

350 in Figure 1 and the curves shape of both are very similar. The samples pasting onset
351 temperature and peak temperatures did not present significant difference, since the
352 granules start to swell around 66 °C, after the start of gelatinization, and achieve the
353 maximum viscosity around 86 °C. In general, starches that present high viscosities
354 contain small amounts of amylose, i.e., the higher the paste viscosity, the lower the
355 amylose content (Juhász & Salgó, 2008). Amylose is also closely associated with the
356 ability to form films and coatings due to its predominantly linear nature. A film or
357 coating can be made from any type of starch that contains amylose (Kramer, 2009). Lee
358 et al. (2007) demonstrated that native waxy corn starch presented peak viscosity higher
359 than native corn starch. Pinhão starch has around 22% amylose content (Conforti &
360 Lupano, 2007), lower than the value for native corn starch which is around 28% (Belitz
361 & Grosh, 1986). The difference in viscosity between the two samples may not be
362 associated with the amylose content, because they belong to the same species. It is
363 hypothesized that this difference is due to the different composition of the starch versus
364 the flour. Pinhão flour has approximately 1.7% dietary fiber, 5.4% protein, 2.5% total fat
365 and 80.6% carbohydrate, most of which is starch (Leite, de Jong, Noreña, & Brandelli,
366 2008). Pinhão starch is 94% pure starch (dry basis) (Daudt, et al., 2014). Yu, Ma,
367 Menager, and Sun (2012) studied the pasting properties of rice starch and flour and also
368 found that the flours presented lower viscosity. In the same way of the present work, they
369 studied same species in different presentations: starch and flour. These differences
370 suggested that the proteins and lipids of rice flour influenced the pasting properties and it
371 may be also the cause of the differences in pinhão starch and flour viscosity. This was

maybe caused by the reaction of amylose and the other components present in pinhão flour.

3.2. Physical properties of pinhão starch and pinhão flour based films

Following raw material evaluations, pinhão starch and pinhão flour were used to develop edible films. Table 2 shows the physical characteristics of pinhão starch-based and pinhão flour-based edible films.

In general, film thickness varied according to the total solids of the films (from 0.039 mm in the starch film without glycerol to 0.057 mm in the pinhão flour film with 1.5% of glycerol). Films with the higher thicknesses were made with pinhão flour. All the films were considered homogeneous, since the standard deviations are low. The thickness differences did not influence other film properties, like water vapor permeability and tensile properties.

The true density of the films did not vary significantly between different film types. However, bulk density was significantly lower in the flour films and, because of that, these films showed a higher apparent porosity ($\emptyset$). These results are in agreement with those found for pinhão starch and flour raw materials. As flour presented higher porosity, flour films also presented higher porosity than starch films.

The scanning electron microscopy of the cross sections of the pinhão starch and flour films can be seen in Figure 2. The Figure 2 presents the cross section images of S5, SG5:1, FG5:1, SG5:2 and FG5:2. It can be noticed that S5 film (made only with pinhão starch) presented a smother surface and a lower thickness, as can be also seen in Table 2. Comparing pinhão starch films with pinhão flour films, it is possible to affirm that the

flour film cross-section is rougher and show pores and granules, and that the increase of glycerol appears to make the films more pliable. These irregularities and pores are in agreement with previous authors that studied flour films of different sources (Andrade-Mahecha, et al., 2012; Colla, et al., 2006). Pelissari, Andrade-Mahecha, Sobral, and Menegalli (2013) also founded similar structures when comparing plantain banana starch and flour films, such as a smother structure for starch film and more irregularities for flour film. The irregularities on the structure of the pinhão flour films may be related to the presence of other components besides starch in the polymer matrix, as protein, lipids and fiber. Although the concentration of these components contents are low (Leite, et al., 2008), they can provide interactions with starch, and between each other, that influence in the final film structure. In addition, the higher roughness of the flour film may explain the higher apparent porosity, opacity and WVP obtained for this film (Table 2).

The glass transition temperature (T_g) of the films did not show significant differences between each other, and did not influence other properties (Table 2). According to Chang, Cheah, and Seow (2000), plasticization weakens the intermolecular forces between polymer chains and, consequently, diminishes the overall cohesion, reducing T_g . Then, the lower T_g is related with higher plasticizer amount, higher WVP, higher elongation, and lower tensile strength. In the present work, the values of T_g was up to 70 °C. The films have sufficiently high T_g values and this implies that these materials still are in the glassy state at room temperature (~25 °C), the temperature used in all the characterization.

The water vapor permeability (WVP) of films may be influenced by the porosity. Pinhão flour films presented higher porosity and films with flour presented higher WVP

when compared to starch films with the same glycerol amount. WVP of the flour versus starch films may also have been influenced by the compositional differences between these two raw materials. Pinhão flour contains dietary fiber, protein and lipids. Lipophilic compounds, as lipids, may decrease water absorption and WVP (García, Martino, & Zaritzky, 2000). However, in the present work, the pinhão flour films presented higher WVP than pinhão starch films. Similar behavior was observed by Dias, et al. (2010) that studied rice starch and flour films. They reported that the rice flour films presented WVP much higher than rice starch films, both plasticized with the same glycerol content, and that this can be explained by the more irregular structure of flour based films, as can also be observed in pinhão flour (Figure 2). According to Martin-Polo, Mauguin, and Voilley (1992), an increase in the uniformity of film microstructure implies a decrease in WVP.

The glycerol amount also influenced WVP, as observed in Table 2. Higher glycerol contents resulted in higher WVP values as has been observed by many other researchers (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007; Mali, Sakanaka, Yamashita, & Grossmann, 2005; Muscat, Adhikari, Adhikari, & Chaudhary, 2012). The pinhão flour presented higher A_w than pinhão starch (Table 1). On the other hand, the monolayer moisture content calculated by BET and GAB value are higher for pinhão starch (between 0.08 and 0.10 kg water kg⁻¹ dry solids at 20 °C) (Thys, Noreña, Marczak, Aires, & Cladera-Olivera, 2010) than for pinhão flour (0.0604 kg water kg⁻¹ dry solids at 20 °C) (Cladera-Olivera, et al., 2011). The sorption water isotherms of starch and flour films plasticized have similar behavior with a sigmoid curve (Bertuzzi, et al., 2007; Dias, et al., 2010; Pelissari, et al., 2013). The interaction between glycerol and starch or flour influenced on A_w and hydrophilicity of the film, as the flour films presented higher WVP

comparing the same glycerol amount used. In general, low A_w decreases WVP and high A_w increases it. Glycerol plasticized films equilibrate at high moisture contents (Muscat, et al., 2012). The increase in the glycerol content enhances the WVP values because of the hydrophilic character of glycerol, which favors the adsorption and desorption of water molecules (Mali, Karam, Ramos, & Grossmann, 2004).

Pinhão starch films demonstrated to be stronger than pinhão flour films at specific glycerol content, with significantly higher Young's modulus and tensile strength values. Both of these characteristics decreased with increasing concentrations of glycerol. The elongation, on the other hand, increased significantly in films as glycerol concentration increased. Water generally decreases the tensile strength of films by weakening intermolecular forces (Kramer, 2009) and, when films have more glycerol, they become more hygroscopic and, consequently, decrease in tensile strength. The hydrophilicity makes the films equilibrate at high moisture when they are exposed to high relative humidity (Muscat, et al., 2012). This behavior makes the decreasing in tensile strength and the increasing in A_w and WVP, as mentioned before. On the other hand, films with higher amounts of glycerol presented higher elongation. Although the higher glycerol content makes the film weaker, it also makes them more pliable, with higher elongation at break. Comparing both types of films at the same amounts of glycerol, the starch films presented significantly higher elongation values than the flour films for the films with 1.5 and 2% of glycerol.

Tensile properties are influenced by the type and quantity of raw material used to make films, as well as the plasticizers used. The rougher pinhão flour films structure (Figure 2) may also contribute to weaker tensile strength. Salas-Valero et al. (2015)

developed canihua flour films from two different varieties. In their study, both varieties presented tensile strength around 6 MPa, but different elongations (around 15% and 24%) and Young's modulus (around 380 MPa and 460 MPa). Dias et al. (2010) in their study with rice starch films found values of 10.9 MPa, 2.8% and 532 MPa for tensile strength, elongation and Young's modulus, respectively in films with 20% glycerol (equivalent to 1% glycerol in present work). For films with 30% glycerol (equivalent to 1.5% glycerol in the present work), they found values of 1.6 MPa, 59.8% and 21.3 MPa for the same properties. These authors also analyzed rice flour films and noticed that there were no statistical differences between rice starch and rice flour films and that only the amount of glycerol influenced their characteristics. They reported that increasing the glycerol concentration decreased the tensile strength and Young's modulus and increased the elongation. A similar behavior was observed in this study, with some differences. Comparing the pinhão starch and flour films with the same amount of glycerol it can be noticed that the pinhão starch film presented higher tensile strength and Young's modulus than flour film with 1% of glycerol, and higher elongation than pinhão flour films with 1.5 and 2% of glycerol (Table 2). Pelissari et al. (2013) developed films with banana starch and flour and reported differences in tensile properties between flour and starch. The banana starch films presented higher tensile strength and Young's modulus (as SG5:1 compared with FG5:1), while banana flour films presented higher elongation at break.

The plasticizer effect of glycerol influenced mostly tensile properties and WVP. The low molecular size of glycerol (92 g/mol) allows it to occupy intermolecular spaces between polymer chains, reducing secondary forces among them. In addition, these

molecules change the three-dimensional molecular organization of polymers, reducing the energy required for molecular motion and the formation of hydrogen bonding between the chains (Vieira, da Silva, dos Santos, & Beppu, 2011). For this reason, films with low glycerol content presented lower WVP, resulting in strong molecule interactions, and a dense and more compact network and structure. The high plasticizer concentration increases the structural modification of the polymer molecular interaction to a looser network. This behavior promotes the mobility and flexibility of polymer network chains and the film matrices become less dense with higher WVP values (Sanyang, Sapuan, Jawaaid, Ishak, & Sahari, 2015).

In the same way, the glycerol induces greater tensile strength reduction as it facilitates easy interaction between glycerol–polymer molecular chains. The plasticizer diminishes the strong intra-molecular attraction between the polymer chains and promotes the formation of hydrogen bonds between plasticizers and polymer molecules; as a consequence, there is a reduction of the tensile strength of plasticized films due to the weakening of the hydrogen bonds between polymer chains (Muscat, et al., 2012; Sanyang, et al., 2015). On the other hand, the film elongation increases as glycerol content increases. This happened because plasticizers decrease the intermolecular bonds between the pinhão starch and flour components of the matrix, substituting these bonds by hydrogen bonds formed between plasticizer and polymer molecules.

Regarding color of edible films, the largest difference is in the parameter b^* . In flour films this value is positive and greater than in starch films. Pelissari et al. (2013) also found higher b^* values for banana flour films than banana starch films. This higher value indicates that these films (banana flour film and the pinhão flour films) are slightly

510 yellowish when compared with starch films. In addition, flour films were more opaque
511 than starch films. Even though the opacity was greater in pinhão flour films, it was still a
512 low value and all the films developed demonstrated transparency. Other authors reported
513 values of opacity over 50% in flour films made with different sources (Pelissari, et al.,
514 2013; Salas-Valero, et al., 2015).

515 FTIR spectra were recorded for the pinhão starch and flour films in order to
516 characterize and investigate possible interactions taking place in the film structures.
517 These spectra are presented in Figure 3. No differences were found between pinhão
518 starch and flour films, and all films showed similar IR absorbance patterns. The more
519 pronounced peaks were at about 3290 cm^{-1} , characteristic of stretching of the -OH
520 groups, caused by the formation of hydrogen bonds, and at about 995 cm^{-1} , characteristic
521 of alkene groups for =C-H bonding (Silverstein, Bassler, & Morrill, 1981). The intensity
522 of these peaks is higher in starch films with the greater amounts of glycerol (SG5:2) and
523 lower for starch films without glycerol (S5). There were no chemical structural changes
524 between films in the presence or absence of glycerol, indicating that there was no
525 chemical reaction between glycerol and starch or flour. This is confirmed by similar IR
526 patterns reported for other starch and flour based edible films (Maizura, Fazilah, Norziah,
527 & Karim, 2007; Pelissari, et al., 2013) as well as for different sources of native starch
528 (Kizil, Irudayaraj, & Seetharaman, 2002).

529 The value of the band at about 2930 cm^{-1} indicates the existence of CH_2 groups
530 (Santha, Sudha, Vijayakumari, Nayar, & Moorthy, 1990). According to Kizil et al. (2002)
531 the different intensities of the bands between 2800 and 3000 cm^{-1} can be attributed to
532 amylose and amylopectin ratio; the higher the intensity, the higher the amylose content.

There was no great variation in this band intensity because starch and flour are from the same source. The band around 1640 cm^{-1} corresponds to amide and it is related to the amide group of proteins, particularly to the C=O stretching (Silverstein, et al., 1981). The presence of this band in the pinhão starch films is probably due to protein traces that remained in the raw material. Other authors related a broad infrared band with a peak at 1637 cm^{-1} as the water absorbed in the amorphous region of starches (Kizil, et al., 2002; Santha, et al., 1990; Wilson, et al., 1991). As the crystallinity of starches increases, this band becomes weaker.

4. Conclusion

Pinhão seeds demonstrated to be a good source for starch and flour, which can be properly used as base for edible films. The differences between pinhão starch and flour composition and structure, as well as the glycerol content added, may determine the film's use and their properties. Pinhão starch and pinhão flour films showed similar IR absorbance patterns, with no chemical structural changes for any films in the presence or absence of glycerol. In spite of their hygroscopic nature, films made with pinhão starch presented a better water vapor barrier and lower apparent porosity than pinhão flour films. These films showed a more homogeneous and smoother structure than flour films, providing a sound structure to block the water vapor transfer more effectively than flour films. The pinhão starch films also exhibited good transparency with high tensile strength, Young's modulus and elongation at break, when compared with pinhão flour films at same specific glycerol content.

Pinhão flour films had higher water vapor permeability, apparent porosity and opacity than pinhão starch films with the same glycerol content. Pinhão flour contains other components besides starch, as proteins, lipids and fibers. These components and possible interactions between them and starch, and between each other, may influence the higher water vapor permeability, porosity, opacity and the irregular structure of pinhão flour films. Physical characteristics were mainly affected by the glycerol content. Films with higher glycerol content are more hygroscopic and, consequently, have inferior water vapor barrier, tensile strength and superior elongation. The glycerol content can be adjusted in the future to tailor film properties to food application needs. Hydrophilic films, such as those studied in the present work, may be applicable as edible coatings on vegetables and fruits, or even as packaging materials of dehydrated food that require prior hydration before consumption.

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6. References

Adedokun, M. O., & Itiola, O. A. (2010). Material properties and compaction characteristics of natural and pregelatinized forms of four starches. *Carbohydrate Polymers*, 79(4), 818-824.

- 579 Al-Hassan, A. A., & Norziah, M. H. (2012). Starch-gelatin edible films: Water vapor
580 permeability and mechanical properties as affected by plasticizers. *Food*
581 *Hydrocolloids*, 26(1), 108-117.
- 582 Andrade-Mahecha, M. M., Tapia-Blácido, D. R., & Menegalli, F. C. (2012).
583 Development and optimization of biodegradable films based on achira flour.
584 *Carbohydrate Polymers*, 88(2), 449-458.
- 585 AOAC. (1997). Official method of analysis n°. 934.06. In. Arlington, USA: Association
586 of Official Analytical Chemist.
- 587 Atichokudomchai, N., Shobsngob, S., Chinachoti, P., & Varavinit, S. (2001). A Study of
588 Some Physicochemical Properties of High-Crystalline Tapioca Starch. *Starch -*
589 *Stärke*, 53(11), 577-581.
- 590 Barbosa-Cánovas, G. V., Fontana Jr., A. J., Schmidt, S. J., & Labuza, T. P. (2007). *Water*
591 *Activity in Foods: Fundamentals and Applications*. Ames, Iowa: Blackwell
592 Publishing Professional.
- 593 Belitz, H.-D., & Grosh, W. (1986). *Food chemistry*. New York: Springer-Verlag.
- 594 Bertuzzi, M. A., Castro Vidaurre, E. F., Armada, M., & Gottifredi, J. C. (2007). Water
595 vapor permeability of edible starch based films. *Journal of Food Engineering*,
596 80(3), 972-978.
- 597 Bian, Q., Sittipod, S., Garg, A., & Ambrose, R. P. K. (2015). Bulk flow properties of hard
598 and soft wheat flours. *Journal of Cereal Science*, 63, 88-94.
- 599 Bourtoom, T., & Chinnan, M. S. (2008). Preparation and properties of rice starch–
600 chitosan blend biodegradable film. *LWT - Food Science and Technology*, 41(9),
601 1633-1641.
- 602 Cano, A., Jiménez, A., Cháfer, M., González, C., & Chiralt, A. (2014). Effect of
603 amylose:amylopectin ratio and rice bran addition on starch films properties.
604 *Carbohydrate Polymers*, 111, 543-555.
- 605 Capella, A. C. V., Penteado, P. T. P. S., & Balbi, M. E. (2009). Semente de *Araucaria*
606 *angustifolia*: aspectos morfológicos e composição química da farinha. *Boletim do*
607 *Centro de Pesquisa de Processamento de Alimentos*, 27(1), 135-142.
- 608 Chang, Y. P., Cheah, P. B., & Seow, C. C. (2000). Plasticizing—Antiplasticizing Effects
609 of Water on Physical Properties of Tapioca Starch Films in the Glassy State.
610 *Journal of Food Science*, 65(3), 445-451.
- 611 Chen, C.-H., & Lai, L.-S. (2008). Mechanical and water vapor barrier properties of
612 tapioca starch/decolorized hsian-tsao leaf gum films in the presence of plasticizer.
613 *Food Hydrocolloids*, 22(8), 1584-1595.

- Chillo, S., Flores, S., Mastromatteo, M., Conte, A., Gerschenson, L., & Del Nobile, M. A. (2008). Influence of glycerol and chitosan on tapioca starch-based edible film properties. *Journal of Food Engineering*, 88(2), 159-168.
- Cladera-Olivera, F., Marczak, L. D. F., Noreña, C. P. Z., & Pettermann, A. C. (2011). Modeling water adsorption isotherms of pinhão (*Araucaria angustifolia* seeds) flour and thermodynamic analysis of the adsorption process. *Journal of Food Process Engineering*, 34(3), 826-843.
- Colla, E., do Amaral Sobral, P. J., & Menegalli, F. C. (2006). *Amaranthus cruentus* Flour Edible Films: Influence of Stearic Acid Addition, Plasticizer Concentration, and Emulsion Stirring Speed on Water Vapor Permeability and Mechanical Properties. *Journal of Agricultural and Food Chemistry*, 54(18), 6645-6653.
- Conforti, P. A., & Lupano, C. E. (2007). Starch Characterisation of *Araucaria angustifolia* and *Araucaria araucana* Seeds. *Starch - Stärke*, 59(6), 284-289.
- Cordenunsi, B. R., Menezes, E. W., Genovese, M. I., Colli, C., Souza, A. G., & Lajolo, F. M. (2004). Chemical composition and glycemic index of brazilian pine (*Araucaria angustifolia*) seeds. *Journal of Agricultural and Food Chemistry*, 52(11), 3412-3416.
- Datta, A. K. (2007). Porous media approaches to studying simultaneous heat and mass transfer in food processes. II: Property data and representative results. *Journal of Food Engineering*, 80(1), 96-110.
- Daudt, R. M., Külkamp-Guerreiro, I. C., Cladera-Olivera, F., Thys, R. C. S., & Marczak, L. D. F. (2014). Determination of properties of pinhão starch: Analysis of its applicability as pharmaceutical excipient. *Industrial Crops and Products*, 52, 420-429.
- de Moura, M. R., Avena-Bustillos, R. J., McHugh, T. H., Wood, D. F., Otoni, C. G., & Mattoso, L. H. C. (2011). Miniaturization of cellulose fibers and effect of addition on the mechanical and barrier properties of hydroxypropyl methylcellulose films. *Journal of Food Engineering*, 104(1), 154-160.
- Dias, A. B., Müller, C. M. O., Larotonda, F. D. S., & Laurindo, J. B. (2010). Biodegradable films based on rice starch and rice flour. *Journal of Cereal Science*, 51(2), 213-219.
- Fakhoury, F. M., Martelli, S. M., Bertan, L. C., Yamashita, F., Mei, L. H. I., & Queiroz, F. P. C. (2012). Edible films made from blends of manioc starch and gelatin - Influence of different types of plasticizer and different levels of macromolecules on their properties. *LWT - Food Science and Technology*, 49(1), 149-154.
- Famá, L., Flores, S. K., Gerschenson, L., & Goyanes, S. (2006). Physical characterization of cassava starch biofilms with special reference to dynamic mechanical properties at low temperatures. *Carbohydrate Polymers*, 66(1), 8-15.

- 652 Famá, L., Rojas, A. M., Goyanes, S., & Gerschenson, L. (2005). Mechanical properties of
653 tapioca-starch edible films containing sorbates. *LWT - Food Science and*
654 *Technology*, 38(6), 631-639.
- 655 Figueiredo-Filho, A., Orellana, E., Nascimento, F., Dias, A. N., & Inoue, M. T. (2011).
656 Produção de sementes de *Araucaria angustifolia* em plantio e em floresta natural
657 no centro-sul do Estado do Paraná. *Floresta*, 41(1), 155-162.
- 658 Flores, S., Famá, L., Rojas, A. M., Goyanes, S., & Gerschenson, L. (2007). Physical
659 properties of tapioca-starch edible films: Influence of filmmaking and potassium
660 sorbate. *Food Research International*, 40(2), 257-265.
- 661 García, M. A., Martino, M. N., & Zaritzky, N. E. (2000). Lipid Addition to Improve
662 Barrier Properties of Edible Starch-based Films and Coatings. *Journal of Food*
663 *Science*, 65(6), 941-944.
- 664 García, M. A., Pinotti, A., Martino, M. N., & Zaritzky, N. E. (2009). Characterization of
665 Starch and Composite Edible Films and Coating. In M. E. Embuscado & K. C.
666 Hubber (Eds.), *Edible Films and Coatings for Food Applications*. New York:
667 Springer-Verlang.
- 668 Garcia, M. A., Pinotti, A., & Zaritzky, N. E. (2006). Physicochemical, Water Vapor
669 Barrier and Mechanical Properties of Corn Starch and Chitosan Composite Films.
670 *Starch - Stärke*, 58(9), 453-463.
- 671 Gontard, N., & Guilbert, S. (1994). Bio-packaging: technology and properties of edible
672 and/or biodegradable material of agricultural origin. In M. Mathlouthi (Ed.), *Food*
673 *Packaging and Preservation* (pp. 159-181). Dordrecht: Springer
674 Science+Business Media.
- 675 Juhász, R., & Salgó, A. (2008). Pasting Behavior of Amylose, Amylopectin and Their
676 Mixtures as Determined by RVA Curves and First Derivatives. *Starch - Stärke*,
677 60(2), 70-78.
- 678 Kechichian, V., Ditchfield, C., Veiga-Santos, P., & Tadini, C. C. (2010). Natural
679 antimicrobial ingredients incorporated in biodegradable films based on cassava
680 starch. *LWT - Food Science and Technology*, 43(7), 1088-1094.
- 681 Kizil, R., Irudayaraj, J., & Seetharaman, K. (2002). Characterization of Irradiated
682 Starches by Using FT-Raman and FTIR Spectroscopy. *Journal of Agricultural*
683 *and Food Chemistry*, 50(14), 3912-3918.
- 684 Kramer, M. E. (2009). Structure and Function of Starch-Based Edible Films and
685 Coatings. In M. E. Embuscado & K. C. Huber (Eds.), *Edible Films and Coatings*
686 *for Food Applications* (pp. 113-134). New York, NY, USA: Springer Science +
687 Business Media.

- 688 Lee, S.-J., Sandhu, K. S., & Lim, S.-T. (2007). Effect of Microwave Irradiation on
689 Crystallinity and Pasting Viscosity of Corn Starches Different in Amylose
690 Content. *Food Science and Biotechnology*, 16(5), 832-835.
- 691 Leite, D. M. C., de Jong, E. V., Noreña, C. P. Z., & Brandelli, A. (2008). Nutritional
692 evaluation of *Araucaria angustifolia* seed flour as a protein complement for
693 growing rats. *Journal of the Science of Food and Agriculture*, 88(7), 1166-1171.
- 694 Luchese, C. L., Frick, J. M., Patzer, V. L., Spada, J. C., & Tessaro, I. C. (2015). Synthesis
695 and characterization of biofilms using native and modified pinhão starch. *Food*
696 *Hydrocolloids*, 45, 203-210.
- 697 Maizura, M., Fazilah, A., Norziah, M. H., & Karim, A. A. (2007). Antibacterial Activity
698 and Mechanical Properties of Partially Hydrolyzed Sago Starch–Alginate Edible
699 Film Containing Lemongrass Oil. *Journal of Food Science*, 72(6), C324-C330.
- 700 Mali, S., Grossmann, M. V. E., García, M. A., Martino, M. N., & Zaritzky, N. E. (2004).
701 Barrier, mechanical and optical properties of plasticized yam starch films.
702 *Carbohydrate Polymers*, 56(2), 129-135.
- 703 Mali, S., Grossmann, M. V. E., García, M. A., Martino, M. N., & Zaritzky, N. E. (2005).
704 Mechanical and thermal properties of yam starch films. *Food Hydrocolloids*,
705 19(1), 157-164.
- 706 Mali, S., Grossmann, M. V. r. E., García, M. A., Martino, M. N., & Zaritzky, N. E.
707 (2006). Effects of controlled storage on thermal, mechanical and barrier properties
708 of plasticized films from different starch sources. *Journal of Food Engineering*,
709 75(4), 453-460.
- 710 Mali, S., Karam, L. B., Ramos, L. P., & Grossmann, M. V. E. (2004). Relationships
711 among the Composition and Physicochemical Properties of Starches with the
712 Characteristics of Their Films. *Journal of Agricultural and Food Chemistry*,
713 52(25), 7720-7725.
- 714 Mali, S., Sakanaka, L. S., Yamashita, F., & Grossmann, M. V. E. (2005). Water sorption
715 and mechanical properties of cassava starch films and their relation to plasticizing
716 effect. *Carbohydrate Polymers*, 60(3), 283-289.
- 717 Maniglia, B. C., Domingos, J. R., de Paula, R. L., & Tapia-Blácido, D. R. (2014).
718 Development of bioactive edible film from turmeric dye solvent extraction
719 residue. *LWT - Food Science and Technology*, 56(2), 269-277.
- 720 Maran, J. P., Sivakumar, V., Sridhar, R., & Prince Immanuel, V. (2013). Development of
721 model for mechanical properties of tapioca starch based edible films. *Industrial*
722 *Crops and Products*, 42, 159-168.
- 723 Mariniello, L., Di Pierro, P., Esposito, C., Sorrentino, A., Masi, P., & Porta, R. (2003).
724 Preparation and mechanical properties of edible pectin–soy flour films obtained in

- 725 the absence or presence of transglutaminase. *Journal of Biotechnology*, 102(2),
726 191-198.
- 727 Marousis, S. N., & Saravacos, G. D. (1990). Density and Porosity in Drying Starch
728 Materials. *Journal of Food Science*, 55(5), 1367-1372.
- 729 Martin-Polo, M., Mauguin, C., & Voilley, A. (1992). Hydrophobic films and their
730 efficiency against moisture transfer. 1. Influence of the film preparation
731 technique. *Journal of Agricultural and Food Chemistry*, 40(3), 407-412.
- 732 McHugh, T. H., Avena-Bustillos, R., & Krochta, J. M. (1993). Hydrophilic edible films:
733 modified procedure for water vapor permeability and explanation of thickness
734 effects. *Journal of Food Science*, 58, 899-903.
- 735 Moorthy, S. N. (2002). Physicochemical and Functional Properties of Tropical Tuber
736 Starches: A Review. *Starch - Stärke*, 54(12), 559-592.
- 737 Musa, H., Gambo, A., & Bhatia, P. G. (2011). Studies on some physicochemical
738 properties of native and modified starches from *Digitaria iburua* and *Zea mays*. In
739 *International Journal of Pharmacy and Pharmaceutical Sciences* (Vol. 3, pp. 28-
740 31).
- 741 Muscat, D., Adhikari, B., Adhikari, R., & Chaudhary, D. S. (2012). Comparative study of
742 film forming behaviour of low and high amylose starches using glycerol and
743 xylitol as plasticizers. *Journal of Food Engineering*, 109(2), 189-201.
- 744 Pelissari, F. M., Andrade-Mahecha, M. M., Sobral, P. J. d. A., & Menegalli, F. C. (2013).
745 Comparative study on the properties of flour and starch films of plantain bananas
746 (*Musa paradisiaca*). *Food Hydrocolloids*, 30(2), 681-690.
- 747 Pérez-Gago, M. B., & Krochta, J. M. (2001). Lipid Particle Size Effect on Water Vapor
748 Permeability and Mechanical Properties of Whey Protein/Beeswax Emulsion
749 Films. *Journal of Agricultural and Food Chemistry*, 49(2), 996-1002.
- 750 Pérez-Sira, E. (1997). Characterization of Starch Isolated from Plantain (*Musa*
751 *paradisiaca normalis*). *Starch - Stärke*, 49(2), 45-49.
- 752 Rayas, L. M., Hernandez, R. J., & Ng, P. K. W. (1997). Development and
753 Characterization of Biodegradable/Edible Wheat Protein Films. *Journal of Food*
754 *Science*, 62(1), 160-162.
- 755 Salas-Valero, L. M., Tapia-Blácido, D. R., & Menegalli, F. C. (2015). Biofilms based on
756 Canihua flour (*Chenopodium Pallidicaule*): design and characterization. *Química*
757 *Nova*, 38, 14-21.
- 758 Santha, N., Sudha, K. G., Vijayakumari, K. P., Nayar, V. U., & Moorthy, S. N. (1990).
759 Raman and infrared spectra of starch samples of sweet potato and cassava.
760 *Journal of Chemical Sciences*, 102(5), 705-712.

- 761 Santos, A. J., Corso, N. M., Martins, G., & Bittencourt, E. (2002). Aspectos produtivos e
762 comerciais do pinhão no Estado do Paraná. *Floresta*, 32(2), 163-169.
- 763 Sanyang, M., Sapuan, S., Jawaid, M., Ishak, M., & Sahari, J. (2015). Effect of Plasticizer
764 Type and Concentration on Tensile, Thermal and Barrier Properties of
765 Biodegradable Films Based on Sugar Palm (*Arenga pinnata*) Starch. *Polymers*,
766 7(6), 1106.
- 767 Silverstein, R. M., Bassler, G. C., & Morrill, T. C. (1981). *Spectrometric Identification of*
768 *Organic Compounds*. New York: John Wiley and Sons.
- 769 Soliva-Fortuny, R., Rojas-Graü, M. A., & Martín-Belloso, O. (2012). Polysaccharide
770 coatings. In R. D. H. a. J. B. Elizabeth A. Baldwin (Ed.), *Edible Coatings and*
771 *Films to Improve Food Quality* (pp. 103-136). Boca Raton: CRC Press, Taylor &
772 Francis Group.
- 773 Sorrentino, A., Gorrasi, G., & Vittoria, V. (2007). Potential perspectives of bio-
774 nanocomposites for food packaging applications. *Trends in Food Science &*
775 *Technology*, 18(2), 84-95.
- 776 Souza, A. C., Benze, R., Ferrão, E. S., Ditchfield, C., Coelho, A. C. V., & Tadini, C. C.
777 (2012). Cassava starch biodegradable films: Influence of glycerol and clay
778 nanoparticles content on tensile and barrier properties and glass transition
779 temperature. *LWT - Food Science and Technology*, 46(1), 110-117.
- 780 Spada, J. C., da Silva, E. M., & Tessaro, I. C. (2014). Production and characterization of
781 pinhão starch biofilms. In *Agrária - Revista Brasileira de Ciências Agrárias*
782 (Vol. 9, pp. 365-369).
- 783 Tapia-Blácido, D., Sobral, P. J., & Menegalli, F. C. (2005). Development and
784 characterization of biofilms based on Amaranth flour (*Amaranthus caudatus*).
785 *Journal of Food Engineering*, 67(1-2), 215-223.
- 786 Thys, R. C. S., Noreña, C. P. Z., Marczak, L. D. F., Aires, A. G., & Cladera-Olivera, F.
787 (2010). Adsorption isotherms of pinhão (*Araucaria angustifolia* seeds) starch and
788 thermodynamic analysis. *Journal of Food Engineering*, 100(3), 468-473.
- 789 Tomassetti, M., Campanella, L., Delfini, M., & Aureli, T. (1987). Determination of
790 moisture in food flours: A comparative thermogravimetric and NMR study, part
791 2. *Thermochimica Acta*, 120, 81-95.
- 792 USP, G. C. (2008). Bulk Density and Tapped Density. In *United States Pharmacopeia*
793 (pp. 616). Rockville: The United States Pharmacopeial Convention.
- 794 Vásconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N.
795 (2009). Antimicrobial activity and physical properties of chitosan-tapioca starch
796 based edible films and coatings. *Food Research International*, 42(7), 762-769.

- 797 Vieira, M. G. A., da Silva, M. A., dos Santos, L. O., & Beppu, M. M. (2011). Natural-
798 based plasticizers and biopolymer films: A review. *European Polymer Journal*,
799 47(3), 254-263.
- 800 Wilson, R. H., Goodfellow, B. J., Belton, P. S., Osborne, B. G., Oliver, G., & Russell, P.
801 L. (1991). Comparison of fourier transform mid infrared spectroscopy and near
802 infrared reflectance spectroscopy with differential scanning calorimetry for the
803 study of the staling of bread. *Journal of the Science of Food and Agriculture*,
804 54(3), 471-483.
- 805 Xu, Y. X., Kim, K. M., Hanna, M. A., & Nag, D. (2005). Chitosan–starch composite
806 film: preparation and characterization. *Industrial Crops and Products*, 21(2), 185-
807 192.
- 808 Yu, S., Ma, Y., Menager, L., & Sun, D.-W. (2012). Physicochemical Properties of Starch
809 and Flour from Different Rice Cultivars. *Food and Bioprocess Technology*, 5(2),
810 626-637.

Figure Legends:

Figure 1. Pasting profile of pinhão starch and pinhão flour.

Figure 2. SEM cross-section images of S5, SG5:1, FG5:1, SG5:2 and FG5:2 films.

Figure 3. FTIR spectra of pinhão starch and pinhão flour based edible films.

FIGURE 1

FIGURE 2

FIGURE 3

TABLES

Table 1: Characteristics of raw material (pinhão starch and flour) used as base to form films

	Pinhão starch	Pinhão flour
Moisture (g/100 g)	8.3 ± 0.4 ^{NS}	9.6 ± 0.6
Aw	0.415 ± 0.002 [*]	0.574 ± 0.002
True density (g/cm ³)	1.503 ± 0.001 [*]	1.491 ± 0.002
Bulk density (g/cm ³)	0.59 ± 0.01 [*]	0.36 ± 0.02
Apparent porosity	0.60 ± 0.01 [*]	0.76 ± 0.01
Pasting onset temperature (°C)	65.7 ± 0.5 ^{NS}	66.0 ± 0.1
Peak temperature (°C)	86 ± 2 ^{NS}	87 ± 0
Peak viscosity (cP)	5778 ± 110 [*]	2818 ± 120
Final viscosity (cP)	3564 ± 510 [*]	2572 ± 49

Aw: water activity. ^{*} indicates statistical difference by t-test (p < 0.05). NS indicates not statistical difference.

Table 2: physical characteristics of pinhão starch and flour based edible films.

	S5	SG5:1	SG5:1.5	SG5:2	FG5:1	FG5:1.5	FG5:2
Thickness (mm)	0.039 ± 0.002 ^d	0.045 ± 0.002 ^c	0.046 ± 0.003 ^c	0.051 ± 0.003 ^b	0.050 ± 0.001 ^b	0.057 ± 0.001 ^a	0.053 ± 0.002 ^b
True density (g/cm ³)	1.487 ± 0.014 ^{a,b,c}	1.490 ± 0.006 ^{a,b}	1.484 ± 0.009 ^{b,c,d}	1.483 ± 0.006 ^{b,c,d}	1.494 ± 0.010 ^a	1.480 ± 0.010 ^{c,d}	1.474 ± 0.004 ^d
Bulk density (g/cm ³)	1.12 ± 0.06 ^b	1.15 ± 0.06 ^{a,b}	1.14 ± 0.06 ^b	1.20 ± 0.06 ^a	0.84 ± 0.04 ^c	0.85 ± 0.04 ^c	0.89 ± 0.05 ^c
Apparent porosity	0.24 ± 0.04 ^b	0.23 ± 0.04 ^b	0.23 ± 0.04 ^b	0.19 ± 0.04 ^b	0.44 ± 0.02 ^a	0.42 ± 0.03 ^a	0.40 ± 0.03 ^a
<i>T_g</i> (°C)	77 ± 3 ^{NS}	75 ± 2	73 ± 2	71 ± 4	75 ± 4	73 ± 4	71 ± 3
WVP (g·mm/kPa·h·m ²)	1.05 ± 0.07 ^d	1.12 ± 0.02 ^d	1.24 ± 0.07 ^{c,d}	1.67 ± 0.04 ^b	1.35 ± 0.05 ^c	1.70 ± 0.05 ^b	1.89 ± 0.28 ^a
Young's modulus (MPa)	2586 ± 175 ^a	1025 ± 61 ^b	42 ± 3 ^d	19 ± 2 ^e	706 ± 55 ^c	48 ± 2 ^d	20 ± 1 ^e
Tensile strength (MPa)	46.54 ± 2.78 ^a	18.56 ± 1.39 ^b	2.64 ± 0.13 ^d	2.14 ± 0.23 ^d	13.08 ± 0.72 ^c	2.09 ± 0.01 ^d	1.60 ± 0.13 ^d
Elongation (%)	3.1 ± 0.1 ^c	2.8 ± 0.2 ^c	64.7 ± 5.3 ^a	57.4 ± 6.4 ^a	2.5 ± 0.3 ^c	24.7 ± 1.8 ^b	29.8 ± 0.7 ^b
<i>L</i> [*]	95.2 ± 0.6 ^a	93.1 ± 0.2 ^b	93.4 ± 0.2 ^b	93.0 ± 0.5 ^b	90.6 ± 0.2 ^c	91.2 ± 0.5 ^c	94.3 ± 0.3 ^a
<i>a</i> [*]	-0.198 ± 0.011 ^d	-0.176 ± 0.005 ^c	-0.166 ± 0.009 ^c	-0.160 ± 0.012 ^c	0.010 ± 0.000 ^a	-0.010 ± 0.000 ^b	-0.080 ± 0.010 ^b
<i>b</i> [*]	-0.220 ± 0.012 ^c	-0.182 ± 0.004 ^c	-0.230 ± 0.012 ^c	-0.210 ± 0.014 ^c	2.86 ± 0.06 ^b	2.69 ± 0.23 ^b	4.52 ± 0.08 ^a
Opacity (%)	8.9 ± 0.2 ^{b,c}	8.5 ± 0.3 ^c	8.94 ± 0.02 ^{b,c}	8.88 ± 0.08 ^{b,c}	10.30 ± 0.20 ^a	10.10 ± 0.30 ^a	9.20 ± 0.20 ^b

Average ± standard deviation. Edible films made with 5% of pinhão starch (S5), 5% of pinhão starch and 1, 1.5 or 2% of glycerol (SG5:1, SG5:1.5, and SG5:2, respectively), and 5% of pinhão flour and 1, 1.5 or 2% of glycerol (FG5:1, FG5:1.5, and FG5:2, respectively). *T_g*: glass transition temperature; WVP: water vapor permeability. Edible films with different letters in a row indicate difference by Tukey-test ($p > 0.05$). NS indicates not statistical difference in a row.