

RESEARCH ARTICLE

Potential of Pinhão husk (*Araucaria angustifolia*) as a structural reinforcement agent in the properties of edible films of Pinhão flour and gelatin

Deocleciano Cassiano De Santana Neto¹  | Thainnane Silva Paiva¹  |
Rogério Willian Silva Dos Santos¹  | Cristiane Vieira Helm²  |
Fábio Anderson Pereira Da Silva³  | Luiz Mário De Matos Jorge⁴  |
Regina Maria Matos Jorge¹ 

¹Graduate Program in Food Engineering, Federal University of Paraná, Curitiba, Brazil

²Non-Wood Technology Lab, Embrapa Florestas, Colombo, Paraná, Brazil

³Graduate Program in Agrifood Technology, Federal University of Paraíba, Bananeiras, Brazil

⁴Department of Chemical Engineering, State University of Maringá, Maringá, Brazil

Correspondence

Deocleciano Cassiano De Santana Neto and Regina Maria Matos Jorge, Graduate Program in Food Engineering, Federal University of Paraná, Av. Francisco Heráclito dos Santos, 100, 81530-900, Curitiba, PR, Brazil.
Email: rjorge@ufpr.br and deocleciano.cassiano7@gmail.com

Abstract

In this study, the use of Pinhão husk as a source of reinforcement material for development of edible films, where the Pinhão seed flour and bovine gelatin were used as matrices for the films. Mechanical properties, water vapor permeability, solubility and opacity, microstructure, and thermal degradation characterized the films produced. The films presented homogeneous and cohesive structures. Pinhão husk content positively affected film properties by increasing tensile strength (TS) and decreasing water vapor permeability (WVP), with Pinhão flour film formulations (5.0% Pinhão flour, 1.2% glycerol, and 0.4% Pinhão husk) and gelatin (5.0% gelatin, 2.0% glycerol, and 0.4% Pinhão husk) those that presented the best results (5.06 MPa for TS and 0.14 g.mm/kPa.h.m² for WVP) and (3.88 MPa for TS and 0.28 g.mm/kPa.h.m² for WVP), respectively. The thermal degradation study revealed that the films are stable at temperatures below 150°C, losing only free water and volatile compounds of low molecular weight. Pinhão husk can reinforce films, making them suitable as biodegradable and edible packaging materials for eco-friendly food products. This contributes to the circular economy, preserves biodiversity, and reduces plastic waste, offering promising sustainable packaging solutions.

KEYWORDS

biomaterials, edible film, microstructure, packaging, reinforcement material

1 | INTRODUCTION

With the COVID-19 crisis, the lifestyle of the world's population changed, mainly in consumer behavior related to the purchase of food products.¹ The rapid spread of the pandemic triggered a sharp increase in demand for packaging, especially those made of plastic petroleum-based products that are non-degradable and cause many

environmental problems associated with their disposal.^{2,3} Due to the lack of safe, biodegradable options available at a competitive cost.

In 2021, world plastic production was 390.7 million tons, with 90.2% of the production of plastics of fossil origin, while bio-based/bioattributed plastics represented only 1.5%.⁴ The high production and use of plastic by society, most of which end up being discarded

incorrectly. It is estimated that between 1.15 and 2.41 million tons of plastic particles enter the ocean annually, causing problems such as microplastics and garbage islands in the ocean, with plastic garbage corresponding to about 90% of the garbage in the environment.^{5,6} In addition, microplastics are ingested by a wide range of marine organisms, such as fish and mollusks, significantly impacting marine ecosystem safety and human health.⁶

Faced with this problem, the potential of developing biodegradable polymers, such as films, and edible and biodegradable packaging for application in foods, has been considered an alternative.^{2,3,7–10} Research on the use of biodegradable materials based on biopolymers has been carried out as one of the strategies to reduce plastic pollution of fossil origin, with biopolymers based on proteins and polysaccharides being the most common alternatives to plastics.¹¹ The films obtained from these matrices have good oxygen barrier properties due to the ability of these components to form neatly compacted structures through bonds, mainly hydrogen bonds.¹²

One of the main proteins used in film production is gelatin due to its abundance, low price, accessibility, non-toxicity, resistance to gases and oils, biodegradability, and good film-forming properties with low environmental impact.^{13–15} Several studies have been reported using gelatin as a base for edible films in recent years.^{13–19} However, gelatin-based films have high water vapor permeability rates and low moisture resistance, thus restricting their application to food products with high moisture rates.^{18,19}

As for carbohydrates, starch has been widely used for packaging production, as a substitute for conventional plastics, due to its low cost, high biodegradability, natural abundance, chemical stability, and film-forming capacity.^{20,21} *Araucaria angustifolia* (Bert.) O. Kuntze is a coniferous plant native to the southern regions of Brazil, northeastern Argentina, and eastern Paraguay.²² The seed almond corresponds to 74% of its weight²³ Pinhão flour can be considered a good source of starch, presenting between 63.7 and 81.0% of starch and between 5.0 and 6.36% of proteins, 1.0 and 2.2% of lipids, and about 4.7 to 15% of dietary fiber.^{23–26} Some studies have used Pinhão flour or starch as a base for edible films.^{8,27–30} However, starch-based films generally have limited barrier capacity to water vapor permeability and high water solubility due to their hydrophilicity, which makes their use in industrial applications difficult.^{12,31}

An alternative to improve these properties of films based on starch and gelatin is using materials that act as structure reinforcers, such as nanoparticles and functional additives, aiming to increase resistance and provide functionality.³² Among the studies incorporating nanoparticles or nanomaterials, nanofibers are more

recurrent. In contrast, studies using cellulose nanofibers/nanocrystals reported improvements in the permeability to water vapor, tensile strength, and elasticity of films.^{11,21,31,33–39} Some studies have been using nanomaterials from less explored sources, such as ramie nanofibers.⁴⁰ Other studies point to using materials with superior granulometry prepared by milling, among which we mention grape stalks,¹⁰ cassava bagasse²⁰ and Pinhão husk.^{2,8}

Based on the food industry's constant search for new and innovative products using renewable resources, valuing biodiversity, and seeking to add value to the Pinhão. It contributed to its Circular Economy by using and valuing the husk and eliminating the waste for better use. Since its husk corresponds to approximately 22% of the seed's weight, it is discarded into the environment without any reuse. This study aimed to develop new films based on Pinhão flour and gelatin with the addition of Pinhão husk powder as a structural reinforcer to a possible application as food packaging.

2 | MATERIALS AND METHODS

2.1 | Materials

Pinhão seeds, harvested and donated by Embrapa Floresta in Colombo, PR, Brazil (2020 Harvest, geographic coordinates: 25°19'18.2"S, 49°09'33.7"W), were used to develop edible films. The fresh seeds were dried in an oven at 45°C for 48 h and manually peeled. The dry almonds were ground in a knife mill (STAR FT-50, Fortinox, Brazil) and sieved with a 60 mesh (Pinhão flour). The seed husks were crushed in a knife mill and sieved to 100 mesh (Pinhão husk powder). Commercial powdered bovine gelatin (unflavored) with a moisture content of 11% and a nitrogen content greater than 15% was purchased from the company Gelnex© (Bloom: 240, 30 mesh, Itá, Santa Catarina, Brazil). Glycerol (Alphatec, Santana-SP, Analytical Grade, Brazil) was used as a plasticizer to prepare films.

2.2 | Analysis of the composition of flour and husk powder of Pinhão

The ground Pinhão flour and husk were characterized by chemical composition, evaluating moisture, ash, protein, and lipid contents using the AOAC protocols.⁴¹ The total starch and resistant starch contents of Pinhão flour were quantified using the Megazyme Assay Kit K-TSTA and K-RSTAR, respectively (Megazyme International, Wicklow, Ireland). The enzymatic method determined the total dietary fiber content using the Megazyme Assay Kit

Total Dietary Fiber (Megazyme International, Wicklow, Ireland). All measurements were taken three times.

2.3 | Experimental design

A two-level ($2^3 = 8$) full factorial design was performed for each type of film to assess three independent factors. For the films based on Pinhão flour, the independent factors were: the amount of Pinhão flour (3.0 and 5.0%), glycerol (1.2 and 2.5%), and powder concentration of Pinhão husk powder (0.2 and 0.4%). While for the gelatin-based films, the independent factors were: the amount of gelatin (3.5 and 5.0%), glycerol (2.0 and 3.0%), and the concentration of Pinhão husk powder (0.2 and 0.4%) (Table 1).

The response variables evaluated were elongation at break, tensile strength, and water vapor permeability. Experimental data were analyzed by full factorial design to fit the following first-order polynomial equation, such Equation 1:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \varepsilon \quad (1)$$

Where Y corresponds to the response variable, β_0 , β_1 , ..., β_{23} , represent the model coefficients, x_1 , x_2 , and x_3 correspond to the independent variables and ε is error.

TABLE 1 Composition of the films based on Pinhão flour and gelatin based with the addition of different levels of Pinhão husk powder.

Pinhão flour film			
Formulation	Pinhão flour (%)	Glycerol (%)	Pinhão husk (%)
PF1	3.0	1.2	0.2
PF2	5.0	1.2	0.2
PF3	3.0	2.5	0.2
PF4	5.0	2.5	0.2
PF5	3.0	1.2	0.4
PF6	5.0	1.2	0.4
PF7	3.0	2.5	0.4
PF8	5.0	2.5	0.4
Gelatin Film			
Formulation	Gelatin (%)	Glycerol (%)	Pinhão husk (%)
GF1	3.5	2.0	0.2
GF2	5.0	2.0	0.2
GF3	3.5	3.0	0.2
GF4	5.0	3.0	0.2
GF5	3.5	2.0	0.4
GF6	5.0	2.0	0.4
GF7	3.5	3.0	0.4
GF8	5.0	3.0	0.4

2.4 | Preparation of the film based on Pinhão flour and gelatin with the addition of the Pinhão husk powder

The films were prepared using the casting method, in which the filmogenic solution is poured into Petri dishes and dehydrated by evaporating the solvent under controlled conditions of time and temperature. For the elaboration of the films, preliminary tests were carried out to determine the amounts of the constituents of the films and the conditions used (data not shown). In the films with Pinhão flour and Pinhão husk powder, the amounts of Pinhão flour, glycerol, and Pinhão husk powder were varied. For the gelatin films, the amounts of gelatin, glycerol, and Pinhão husk powder were varied (Table 1).

For films based on Pinhão flour, aqueous suspensions were prepared by mixing Pinhão flour (3.0% to 5.0%) in distilled water. Glycerol (1.2% to 2.5%) was used as a plasticizer and Pinhão husk powder (0.2% to 0.4%) as a reinforcing agent, according Figure 1a. The aqueous suspension was heated and maintained under constant stirring (Fisatom, model 752A, Brazil) in order to achieve complete gelatinization of the starch (80°C/30 min), and then cooled to room temperature. Each aqueous suspension (30 mL) was then poured into sterile Petri dishes (15 cm in diameter) and dried in an oven with forced air

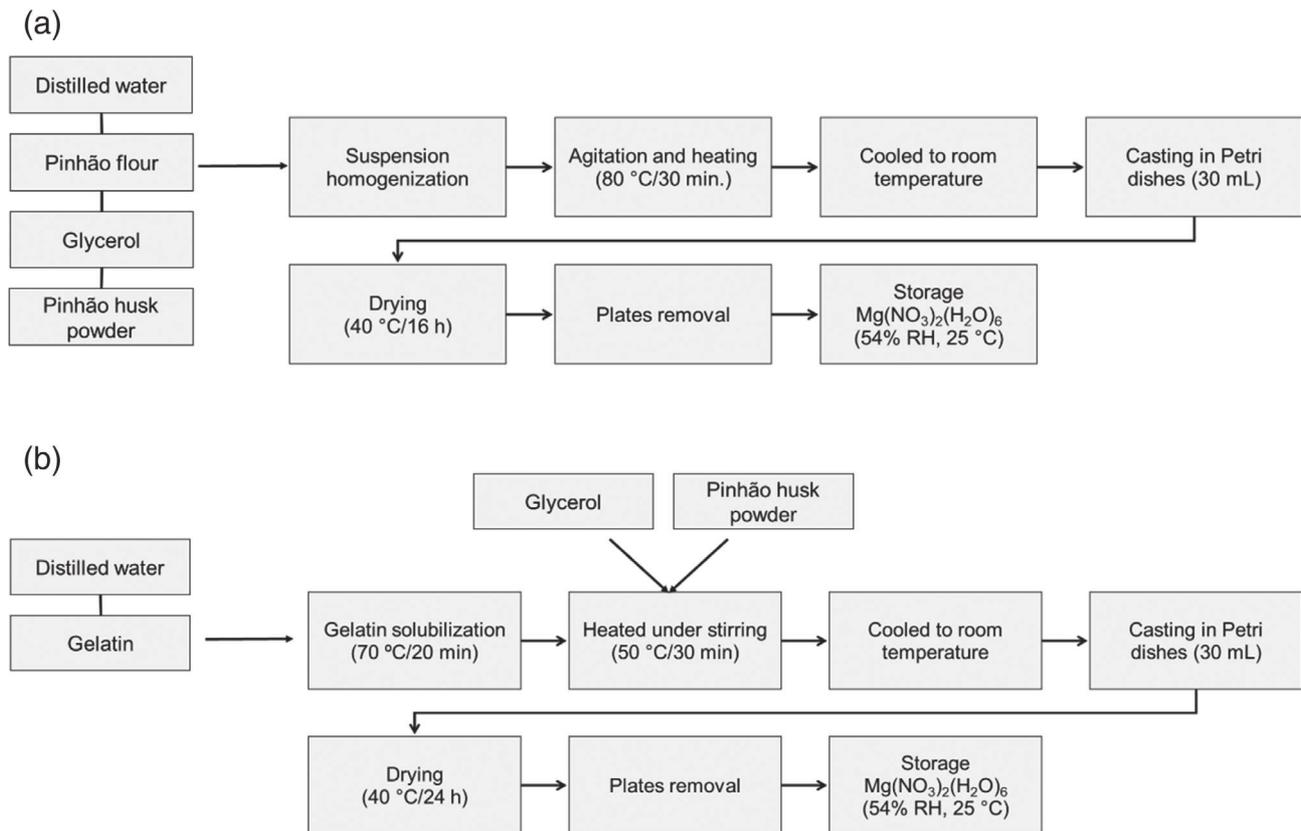


FIGURE 1 Flowchart of the production process of biodegradable pinhão flour-based films (a) and gelatin-based (b).

circulation at 40 °C (Nova Ética, model 400/6ND, Brazil) for 16 h.

For the gelatin-based films, the aqueous suspensions of the films were prepared by solubilizing gelatin (3.5% to 5.0%) in distilled water under stirring (70 °C/20 min) according Figure 1b. Then, glycerol (2.0% to 3.0%) was added as a plasticizer and Pinhão husk powder (0.2% to 0.4%) as a reinforcing agent, and the solution was heated under stirring (50 °C/30 min). Each aqueous suspension (30 mL) was then poured into sterile Petri dishes (15 cm in diameter) and dried in an oven with forced air circulation (40 °C/24 h). After drying, all films were conditioned for 7 days in a desiccator containing a saturated solution of Mg(NO₃)₂(H₂O)₆ (54% RH, 25 °C) for further characterization.

2.5 | Characterization of films

2.5.1 | Visual and tactile aspects

Subjective evaluation of the films' visual and tactile parameters was carried out to verify the films' homogeneity and continuity. Malleability, ease of removal from Petri dishes, and adhesiveness were also evaluated.

2.5.2 | Mechanical properties

The elongation at break (Elo, %) and tensile strength (RT, MPa) of the films were evaluated using a texturometer (Brookfield CT3, Brookfield, USA) equipped with a TA-DGA probe (Double tightening set, Brookfield) according to the standard method D882-18.⁴² The initial separation of the claws and the speed of the probe were 20 mm and 1 mm/s, respectively, with a trigger force of 0.1 N. For each type of film, eight repetitions will be performed. ELO (%) and RT were calculated using Equations 2 and 3. The Young's modulus was obtained by the ratio of stress to strain at the initial linear portion of the curve.

$$\text{ELO}(\%) = \frac{100 \times (L_{\max})}{L_0} \quad (2)$$

$$\text{TS}(\text{MPa}) = \frac{F_{\max}}{A} \quad (3)$$

Where L_{\max} is the strain at break (mm), L_0 is the initial film length (mm), F_{\max} is the maximum load (N), and A is the cross-sectional area of the film (mm²).

2.5.3 | Water vapor permeability

Water vapor permeability (WVP) was determined according to ASTM E96/E96M-16⁴³ with modifications. Samples of the films with 3 cm in diameter were fixed on adapted permeability capsules with an opening of 2.5 cm in diameter (with an exposure area of 0.0013 m²) containing anhydrous calcium chloride (CaCl₂), with 0% relative humidity (RH). The system was placed in a desiccator containing a saturated NaCl solution (70% RH) and maintained at 25°C. The mass gain of the system was monitored at 24 h intervals for 7 days. The evaluations were performed in triplicate. The water vapor permeability was determined by Equation 4.

$$\text{WVP} = \frac{G}{t} \frac{x}{A \cdot P_s \cdot (RH_1 - RH_2)} \quad (4)$$

Where G/t (g.h⁻¹) is the angular coefficient generated by the weight gain of the sample as a function of time, x (mm) is the thickness of the film, A (m²) is the area available for permeation, P_s (KPa) is the saturation pressure of water vapor at the test temperature, RH_1 and RH_2 are the relative humidity's (expressed in fractions) inside the desiccator and the capsule, respectively.

2.6 | Complementary characterization

2.6.1 | Thickness

Film thickness was determined using a digital micrometer (0.001 mm resolution) (Mitutoyo, Japan) measured at 5 points on the films, 4 at the ends, and one point at the center of the film. Film thickness was considered as the arithmetic mean of 10 measurements per formulation.

2.6.2 | Solubility in water

The solubility of the films was evaluated as described by Ge et al.¹⁵ The evaluations were performed in triplicate. The samples were cut into squares (2° × 2° cm) and dried in a conventional natural oven at 100 ± 5°C for 24 h and weighed (W_0). The samples were immersed in 50 mL of distilled water, covered, and stored for 24 h at room temperature (25°C). Then, the residual film samples were dried in an oven at 100 ± 5°C for 24 h to determine the final dry mass (W_1), and the solubility was calculated according to Equation 5.

$$S(\%) = \left(\frac{W_0 - W_1}{W_0} \right) \times 100 \quad (5)$$

2.6.3 | Contact angle

The hydrophobicity of the films was evaluated using the apparent contact angle (CA) technique on a Krüss DSA25 goniometer (Krüss GmbH, Germany) using the sessile drop method. Three droplets of deionized water (surface tension of 72.80 mN/m) with a volume of 5 μL were deposited on the surface of each sample, and the angle was measured immediately after dripping onto the surface of the film. Calculations of angle values were performed using the DSA4 equipment management software (Krüss GmbH, Germany). Measurements were made in triplicate for each film.

2.6.4 | Optical properties

The instrumental color of the films was determined by evaluating the parameters of brightness (L^*), green/red (a^*), and blue/yellow (b^*) with the aid of a digital colorimeter (HunterLab, model MiniScan EZ 4500 L, United States of America) previously calibrated with white and black plates provided by the manufacturer, under the CIELAB system. For the reading of color coordinates, the following conditions were standardized: illuminant D65, viewing angle 8°, and standard observer angle 10. Color parameters were measured concerning the white plate (standard) ($L = 94.40$; $a = -1.29$; $b = 0.70$). The films' total colorimetric difference (ΔE) was calculated using the white calibration plate as a reference, according to Equation 6.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (6)$$

Where $\Delta L^* = L^*_{\text{white plate}} - L^*_{\text{sample}}$, $\Delta a^* = a^*_{\text{white plate}} - a^*_{\text{sample}}$ and $\Delta b^* = b^*_{\text{white plate}} - b^*_{\text{sample}}$.

The opacity of the films was performed as proposed by Nazurah and Nur Hanani.⁴⁴ Rectangular pieces (1 × 4 cm) of films were cut and placed inside the quartz cuvette in a spectrophotometer, using an empty quartz cuvette as a reference. The absorbance was measured at 600 nm, and the film opacity was calculated using Equation 7. All measurements were taken three times.

$$\text{Opacity} = \frac{\text{Abs}_{600\text{nm}}}{x} \quad (7)$$

Where, Abs_{600} is the absorbance value at 600 nm and x is the film thickness (mm).

2.6.5 | Scanning electron microscopy

The morphological structure of the surface of the films was analyzed using scanning electron microscopy (Tesla Vega 3 LMU). The films were fractured manually after freezing with liquid nitrogen, fixed on supports with copper tape, and metalized with a thin layer of gold (Balzers Union, model FL 9496) using a voltage of 15 kV.

2.6.6 | Fourier transform infrared spectroscopy

The films were characterized by Fourier transform mid-infrared spectroscopy (FTIR), conducted in Vertex 70 equipment (Bruker, USA), with attenuated total reflectance (ATR) accessory, equipped with a zinc selenide crystal in the range spectral range of 500–4000 cm^{-1} and resolution of 4 cm^{-1} . All measurements were taken three times.

2.6.7 | Thermogravimetric analysis

The thermogravimetric analysis of the films was carried out using a thermogravimetric analyzer (TGA 4000, Perkin Elmer, USA) in order to evaluate the thermal stability of the films. For this, samples of approximately 5 mg of the films were weighed in alumina crucibles and heated from 20 to 750°C at a rate of 10°C min^{-1} , using nitrogen gas as purge gas (20 mL min^{-1}). TGA and DTG curves

(thermogravimetric derivative) were used for data interpretation. All measurements were taken three times.

2.7 | Statistical analysis

The model equation was fitted to the experimental data to produce the proposed model, and response surface plots. Statistical analysis of results was performed using analysis of variance (one-way ANOVA $p \leq 0.05$), and statistically different means were then analyzed using Tukey's Test for post hoc analysis, with the aid of Statistica 7.0 software (StatSoft, Tulsa, USA).

3 | RESULTS AND DISCUSSION

3.1 | Analysis of the composition of flour and Pinhão husk powder

The Pinhão almonds correspond to 67.54% of the weight of the seed, while the husk corresponds to 32.46% of the weight. A similar yield was observed in a study carried out by Helm et al.,²³ who observed a dry Pinhão yield of 68% for almonds and 32% for husks.

The partial chemical composition of Pinhão flour is composed mainly of starch, water, and considerable amounts of fiber (Table 2). Pinhão flour showed average values of moisture, ash, lipids, and proteins of 9.76%; 2.56%; 1.94%, and 5.12%, respectively. Similar values were reported by Pigozzi et al.²⁶ in raw and cooked Pinhão flour, and by Helm et al.²³ in cooked and dried Pinhão flour (Table 2), and lipid and protein values similar to those obtained by Barreto et al.²⁴ and Jorge et al.²⁵ on Pinhão flour.

TABLE 2 Partial composition of Pinhão flour, husk and values reported by other authors.

	Almonds					Husk			
	Almonds	Barreto et al. ²⁴	Jorge et al. ²⁵	Pigozzi et al. ²⁶	Helm et al. ²³	Husk	Spada et al. ²	Timm et al. ⁴⁶	Dorneles; Noreña ²²
Moisture (%)	9.76 ± 0.23	4.95	—	5.87–7.17	5.0	10.56 ± 0.08	10.3	5.0	0.31
Ash (%)	2.56 ± 0.02	1.75	2.51	2.55–3.22	2.8	1.92 ± 0.08	1.1	—	2.46
Lipids (%)	1.94 ± 0.01	1.56	0.97	1.7–3.06	2.2	0.71 ± 0.01	—	6.0	0.82
Protein (%)	5.12 ± 0.21	5.98	6.36	5.09–5.74	5.5	2.26 ± 0.09	1.69	1.8	9.44
Total Starch (%)	51.28 ± 0.26	80.99	63.75	—	67.0	—	—	—	—
Resistant Starch (%)	3.81 ± 0.13	—	—	—	8.0	—	—	—	—
Total dietary fiber (%)	7.43 ± 0.73	4.77	5.20	—	15.0	69.23 ± 0.73	65.1	46.1	54.03

It was observed that Pinhão flour could be considered a source of total starch, with an average content of 51.28%, where the resistant starch content corresponds to 3.81%. However, the total starch content is lower than that reported in recent studies, which show values of starch present in Pinhão flour between 63.75 and 80.99% (Table 2). Regarding fibers, flour had a considerable content of 7.43%. In addition, the Pinhão flour obtained in this research had a lower content of resistant starch and fiber than that reported by Helm et al.²³ who obtained values of 8.0% and 15% of resistant starch and fiber in the cooked and dried Pinhão flour, respectively. These differences in the partial chemical composition of Pinhão flour can be attributed to possible differences in maturation status, soil, extraction procedures, and/or origin of the plant.⁴⁵

The composition of Pinhão husk powder is mainly composed of fibers (69.23%), carbohydrates (15.33%), and water (10.56%) (Table 2). There are few studies in the literature that carried out the centesimal characterization of the Pinhão husk. The moisture content of Pinhão husk was similar to that observed by Spada et al.² who found a moisture content of 10.3% in Pinhão husk. However, the moisture values obtained in this study were higher than those reported by Timm et al.⁴⁶ and Dorneles; Noreña,²² who obtained moisture values of 5.0% and 0.31% for Pinhão husk and bracts, respectively. Furthermore, Timm et al.⁴⁶ obtained values of lipids and carbohydrates higher than those obtained in this study. However, the Pinhão husk showed a higher value than the studies reported for the total fiber content and similar to the study by Spada et al.² According to Dorneles; Noreña,²² the high fiber content and low lipid content is a common characteristics of the husks of products that have a high tannin content.

3.2 | Visual and tactile aspects

In general, all proposed formulations produced continuous films with good homogeneity and uniform coloring (Table 3). Some films had adhesiveness, making handling difficult and making it less malleable.

The PF1, PF3, PF5, and PF7 formulations for the Pinhão flour-based films and GF3, GF5, and GF7 for the gelatin films were the ones that produced films with a more adhesive appearance, which caused greater difficulty in removing the Petri dishes, in addition to requiring greater care in the handling and packaging of films. This greater adhesiveness can be attributed to a large amount of glycerol in relation to the amount of Pinhão flour/gelatin. On the other hand, the other formulations (PF2, PF4, PF6, PF8, GF1, GF2, GF4, GF6, and GF8) were the easiest film formulations to handle and with

good continuity during drying, possibly due to the higher amount of Pinhão flour and gelatin. In addition, visual differences were noted, mainly in color and roughness, as the amounts of Pinhão husk increased.

3.3 | Mechanical properties

The mechanical properties of the films are characteristics of great importance for measurement when it comes to the integrity of the films. The percentage of elongation (ELO) and tensile strength (TS) of the films showed a difference between the formulations tested for both matrices used ($p < 0.05$) (Table 4). It was observed that all evaluated variables had a significant effect on the mechanical properties of Pinhão flour films and gelatin films (Table S1).

The formulations that used higher amounts of glycerol had lower ELO and TS values (Figures S1 and S2). The Pinhão flour films presented variation from 22.98 to 44.51% for ELO and from 0.60 to 5.06 MPa for TS, while the gelatin films obtained values between 86.37 and 199.86% for ELO and between 2.17 and 3.88 MPa for TS. In the Pinhão flour films, the PF2 and PF6 formulations showed the highest values of ELO (40.26 and 44.51%) and tensile strength (4.39 and 5.06 MPa). As for the gelatin-based films, the GF2 and GF6 formulations, although they do not present the highest ELO values (120.25 and 86.37%), are the ones with the highest RT values (3.61 and 3.88 MPa), which provide flexibility and resistance to the films. For the mechanical properties of ELO and RT, Equations 8, 9, 10, and 11 represent the proposed first-order polynomial model for experimental data, containing only statistically significant terms, with Equations 8 and 9 corresponding to ELO and TS of Pinhão films and Equations 10 and 11 corresponding to ELO and TS of gelatin films:

$$\text{ELO}_{\text{PF}} = 30.498 + 4.868x_1 - 4.143x_2 - 1.168x_3 \quad (8)$$

$$- 3.058x_1x_2 - 1.698x_2x_3$$

$$\text{TS}_{\text{PF}} = 2.371 + 0.926x_1 - 1.121x_2 - 0.306x_1x_2 - 0.086x_2x_3 \quad (9)$$

$$\text{ELO}_{\text{GF}} = 141.488 - 7.512x_1 + 29.150x_2 - 17.740x_3 \quad (10)$$

$$+ 1.515x_1x_2 + 6.045x_1x_3 + 2.902x_2x_3$$

$$\text{TS}_{\text{GF}} = 2.782 + 0.429x_1 - 0.264x_2 - 0.340x_1x_2 - 0.100x_2x_3 \quad (11)$$

Incorporating Pinhão husk powder as a source of fibers to the films provided changes in the mechanical properties, reducing the ELO and increasing the TS of

TABLE 3 Visual appearance of edible Pinhão flour films and gelatin films with different Pinhão husk powder contents. [Color table can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

Pinhão flour films							
Formulation	Appearance	Homogeneous	Difficulty removing plates	Malleability	Manipulation	Adhesiveness	Continuity
PF1		Yes	Little difficulty	Good malleability	Excellent manipulation	Few adhesiveness	Excellent continuity
PF2		Yes	Easy removal	Excellent malleability	Excellent manipulation	Did not present	Excellent continuity
PF3		Yes	Difficult removal	Difficult Malleability	Bad manipulability	Many adhesiveness	Good continuity
PF4		Yes	Easy removal	Excellent malleability	Excellent manipulation	Did not present	Excellent continuity
PF5		Yes	Difficult removal	Difficult Malleability	Bad manipulability	Many adhesiveness	Good continuity
PF6		Yes	Easy removal	Excellent malleability	Excellent manipulation	Did not present	Excellent continuity

TABLE 3 (Continued)

Pinhão flour films							
Formulation	Appearance	Homogeneous	Difficulty removing plates	Malleability	Manipulation	Adhesiveness	Continuity
PF7		Yes	Difficult removal	Difficult Malleability	Bad manipulability	Many adhesiveness	Good continuity
PF8		Yes	Easy removal	Good malleability	Good manipulability	Did not present	Excellent continuity
Gelatin Films							
Formulation	Appearance	Homogeneous	Difficulty removing plates	Malleability	Manipulation	Adhesiveness	Continuity
GF1		Yes	Easy removal	Excellent malleability	Excellent manipulation	Few adhesiveness	Excellent continuity
GF2		Yes	Easy removal	Excellent malleability	Excellent manipulation	Few adhesiveness	Excellent continuity
GF3		Yes	Difficult removal	Difficult Malleability	Bad manipulability	Many adhesiveness	Good continuity
GF4		Yes	Easy removal	Excellent malleability	Excellent manipulation	Few adhesiveness	Excellent continuity

(Continues)

TABLE 3 (Continued)

Gelatin Films							
Formulation	Appearance	Homogeneous	Difficulty removing plates	Malleability	Manipulation	Adhesiveness	Continuity
GF5		Yes	Difficult removal	Moderate Malleability	Bad manipulability	Many adhesiveness	Good continuity
GF6		Yes	Easy removal	Excellent malleability	Excellent manipulation	Few adhesiveness	Excellent continuity
GF7		Yes	Difficult removal	Difficult Malleability	Bad manipulability	Many adhesiveness	Good continuity
GF8		Yes	Easy removal	Good malleability	Good manipulability	Few adhesiveness	Excellent continuity

TABLE 4 Results of mechanical properties (elongation (ELO (%)) and tensile strength (TS (MPa)) and water vapor permeability (WVP) of edible Pinhão flour films and edible gelatin films with different Pinhão husk powder contents.

Formulation	ELO (%)	TS (MPa)	YM (MPa)	WVP (g.mm/kPa.h.m ²)
Pinhão Flour Films				
PF1	27.60 ^{bc} ± 2.66	2.32 ^c ± 0.21	35,79 ^{bc} ± 5,65	0.34 ^c ± 0.03
PF2	40.26 ^a ± 3.89	4.39 ^b ± 0.35	42,25 ^{ab} ± 1,17	0.18 ^d ± 0.02
PF3	26.11 ^c ± 3.39	0.60 ^d ± 0.07	8,25 ^e ± 0,44	0.90 ^a ± 0.05
PF4	32.33 ^b ± 3.74	1.97 ^c ± 0.14	25,81 ^d ± 3,97	0.66 ^b ± 0.03
PF5	25.83 ^c ± 2.20	2.20 ^c ± 0.14	33,09 ^{cd} ± 2,25	0.44 ^c ± 0.06
PF6	44.51 ^a ± 2.20	5.06 ^a ± 0.73	48,04 ^a ± 7,25	0.14 ^d ± 0.02
PF7	22.98 ^c ± 1.70	0.66 ^d ± 0.05	11,88 ^e ± 0,61	1.00 ^a ± 0.05
PF8	24.00 ^c ± 1.72	1.77 ^c ± 0.19	30,39 ^{cd} ± 0,36	0.61 ^b ± 0.04
Gelatin Films				
GF1	145.71 ^{bc} ± 14.54	2.38 ^{bc} ± 0.27	6,59 ^c ± 0,91	0.67 ^c ± 0.05
GF2	120.25 ^{cd} ± 17.31	3.61 ^a ± 0.26	12,21 ^{ab} ± 1,97	0.49 ^d ± 0.04
GF3	199.86 ^a ± 20.18	2.51 ^{bc} ± 0.19	5,04 ^d ± 0,37	0.88 ^{ab} ± 0.09
GF4	171.09 ^{ab} ± 22.13	2.83 ^b ± 0.25	6,65 ^c ± 0,55	0.74 ^{bc} ± 0.05
GF5	97.02 ^{de} ± 10.03	2.17 ^c ± 0.29	8,97 ^b ± 1,12	0.68 ^c ± 0.03
GF6	86.37 ^e ± 7.74	3.88 ^a ± 0.41	18,03 ^a ± 1,72	0.28 ^e ± 0.04
GF7	153.41 ^b ± 15.75	2.35 ^{bc} ± 0.13	6,17 ^c ± 0,69	0.92 ^a ± 0.06
GF8	158.19 ^b ± 14.34	2.39 ^{bc} ± 0.25	6,06 ^{cd} ± 0,74	0.74 ^{bc} ± 0.02

Note: Result expressed as mean ± standard deviation. Different lowercase letters in the same column indicate a significant difference between samples ($p < 0.05$).

the films (Figure S1), a behavior similar to that reported in films with material added as a structure reinforcer.^{8,47} Generally, the best values for these properties were obtained in the PF6 and GF6 formulations, which contain 0.4% Pinhão husk powder. This increase may have been due to the presence of intermolecular interactions between the film components, which could be the main reason for the significant increase in ELO and TS.⁴⁸

Souza et al.³³ reported a decrease in these properties as the glycerol content increased in the development of biodegradable cassava starch films with the addition of clay nanoparticles. A similar study was carried out by Daudt et al.,⁸ who developed Pinhão films with the addition of different concentrations of Pinhão husk powder (0.5, 1.0, 1.5, 2.0, and 2.5%), which obtained ELO values from 4.2 to 19% and TS between 1.5 and 2.5 MPa, values up to 90.56% and 70.35% lower on average for ELO and TS than those obtained in this study. The PF6 and GF6 formulations present a value very close to the values found in conventional packagings, such as low-density polyethylene (LDPE), which is widely used in food. The PF6 formulation showed values up to 49.46% of ELO and 84.33% of TS of LDPE films, while the GF6 formulation showed values of up to 95.97% of ELO and 64.67% of TS of LDPE films; therefore, this formulation can be considered as a promising alternative for use as food packaging.^{49,50}

Regarding Young's modulus values, PF films varied from 8.25 to 48.04 MPa for YM, while GF films obtained values between 5.04 and 18.03 MPa for YM. It can be observed that the higher the concentration of solid matrix in the films (pinhão flour/gelatin and pinhão husk powder), the higher the YM values, making the films more rigid (PF6 and GF6), allowing them to withstand significant deformation forces.⁵¹ A higher concentration of glycerol resulted in a more flexible film with lower YM, as observed in Table 4. Studies involving the addition of pinhão husk powder showed an increase in YM in edible films based on pinhão flour,⁸ as well as in the use of fibers from the pod husk of *Moringa oleifera* in gelatin-based biocomposites.⁴⁷

3.4 | Water vapor permeability

Water vapor permeability (WVP) is an important barrier property to be evaluated in the development of films since it evaluates the ability of water to permeate the polymeric matrix of the film, which can directly interfere with the shelf life of the food.³⁵ During permeability tests, all films remained intact. It was observed that the amounts of Pinhão flour/gelatin and glycerol showed a significant effect. For the Pinhão flour films, it was observed that the use of Pinhão husk powder showed an

interaction effect between the Pinhão flour, while in the gelatin films, an interaction effect of the Pinhão husk powder with gelatin and with glycerol (Table S1).

The WVP of the films differed significantly ($p < 0.05$), with the highest values observed for the films being the PF7 and GF7 formulations (1.00 and 0.92 g.mm/kPa.h.m²) and the lowest for the PF6 and GF6 formulations (0.14 and 0.28 g.mm/kPa.h.m², respectively) (Table 4). The formulations with the highest glycerol content in the formulation had the highest permeability. It can also be observed that the variation in the concentration of Pinhão husk also affects the WVP (Figure S3). For WVP, Equations 12 and 13 represent the proposed first-order polynomial model for experimental data, containing only statistically significant terms, with Equation 12 corresponding to the WVP of Pinhão films and Equation 13 corresponding to the WVP of gelatin films:

$$\text{WVP}_{\text{PF}} = 0.528 - 0.152x_1 + 0.234x_2 - 0.039x_1x_2 - 0.062x_1x_3 \quad (12)$$

$$\text{WVP}_{\text{GF}} = 0.675 - 0.112x_1 + 0.146x_2 + 0.032x_1x_2 - 0.032x_1x_3 + 0.032x_2x_3 \quad (13)$$

A similar behavior was reported by some studies in the literature, where they report that the increase in the concentration of materials as a structure enhancer can lead to a decrease in the WVP of the films.^{10,21,31,47,52,53}

This reduction of the WVP in the films with a higher content of Pinhão husk can also be attributed to possible interactions between the other components of the films, increasing the hydrogen bonding forces between the hydroxyl group in the starch present in the Pinhão flour and the possible ordered distribution of the husk, making permeability difficult of water molecules.^{20,52}

However, in studies carried out by Daudt et al.⁸ and Spada et al.,² the opposite behavior was observed, where the authors reported that the presence of Pinhão husk increased the WVP between 1.6 and 3.8 g.mm/kPa.h.m² for Pinhão flour films, values higher than those obtained in the present study, and between 0.278 and 0.426 g.mm/kPa.h.m² in films based on starch, similar to those obtained. Similar values were obtained by Garavand et al.²¹ in films based on starch-PVA reinforced with chitosan nanoparticles, with WVP values ranging from 0.28 to 0.41 g.mm/kPa.h.m² and by Saranti et al.¹⁸ in gelatin films reinforced with nano emulsion loaded with cloisite Na⁺ and black pepper EO, with PVA values ranging from 0.32 to 0.61 g.mm/kPa.h.m².

3.5 | Additional characterization

3.5.1 | Thickness

The thickness results of the prepared films showed significant differences ($p < 0.05$), with thickness values

Formulation	Thickness (mm)	Solubility (%)	Contact angle (°)
Pinhão Flour Film			
PF1	0.124 ^d ± 0.007	25.25 ^{abc} ± 1.63	46.60 ^{bc} ± 4.97
PF2	0.157 ^b ± 0.005	26.86 ^{ab} ± 1.46	46.03 ^{bc} ± 2.65
PF3	0.130 ^d ± 0.005	28.03 ^a ± 1.58	35.80 ^c ± 2.08
PF4	0.167 ^a ± 0.006	27.14 ^a ± 0.65	46.93 ^b ± 3.44
PF5	0.132 ^{cd} ± 0.007	22.94 ^{bcd} ± 1.30	53.00 ^{ab} ± 3.66
PF6	0.148 ^b ± 0.003	21.28 ^{cd} ± 1.87	60.73 ^a ± 5.32
PF7	0.139 ^c ± 0.007	22.22 ^{cd} ± 0.99	45.03 ^{bc} ± 5.25
PF8	0.166 ^a ± 0.007	19.51 ^d ± 1.44	44.50 ^{bc} ± 1.83
Gelatin Film			
GF1	0.103 ^d ± 0.001	30.63 ^{ab} ± 0.56	94.87 ^a ± 2.21
GF2	0.129 ^c ± 0.004	33.08 ^a ± 1.23	92.83 ^{ab} ± 1.23
GF3	0.096 ^d ± 0.004	25.36 ^c ± 1.85	87.18 ^b ± 1.50
GF4	0.136 ^{bc} ± 0.004	31.46 ^{ab} ± 2.49	93.11 ^{ab} ± 2.25
GF5	0.107 ^d ± 0.007	28.29 ^{bc} ± 0.35	87.23 ^b ± 2.51
GF6	0.140 ^{ab} ± 0.009	32.36 ^{ab} ± 1.68	91.10 ^{ab} ± 1.48
GF7	0.100 ^d ± 0.002	29.65 ^{abc} ± 1.43	86.70 ^b ± 5.19
GF8	0.151 ^a ± 0.004	32.72 ^{ab} ± 1.93	88.30 ^{ab} ± 2.76

Note: Result expressed as mean ± standard deviation. Different lowercase letters in the same column indicate a significant difference between samples ($p < 0.05$).

TABLE 5 Thickness, solubility and contact angle results of edible Pinhão flour films and edible gelatin films with different Pinhão husk powder contents.

between 0.124 and 0.167 for the Pinhão flour-based films and between 0.096 and 0.140 for the gelatin films (Table 5). It can be seen that by increasing the concentration of Pinhão husk powder in the film formulations, there was an increase in thickness, emphasizing that the amount of solids directly influenced it in the film-forming solution.⁵⁴

All films were considered homogeneous since they presented low values of standard deviations. However, the increase in thickness can influence other properties, such as mechanical properties, permeability to water vapor, and opacity.³⁵ The same behavior is reported by other authors when adding composites as reinforcers.^{8,40,47,53,55}

3.5.2 | Solubility and contact angle

One of the important characteristics of food packaging is solubility, where films with high water solubility result in low resistance when applied to a food matrix.⁵⁶ The films prepared in this study did not lose their integrity in aqueous solution at the end of 24 h, gelatin-based films being the most soluble. The solubility remained between 19.51% (PF8) and 28.03% (PF3) for Pinhão films and between 25.36% (GF3) and 33.08% (GF2) for gelatin-based films (Table 5).

Adding higher levels of Pinhão husk resulted in a decrease in solubility in water for the Pinhão films ($p < 0.05$). In contrast, for the gelatin films, this variation did not promote significant variations in the solubility values. The fact that gelatin films are more soluble than Pinhão flour films is due to the greater availability of hydroxyl groups with increasing glycerol concentration.^{39,57}

This lower solubility of PF films can be attributed to the favorable interaction between the components, maintaining the compounds, avoiding the generation of large pores, and decreasing their solubility.^{3,10} In addition, the decrease in the solubility of the films may be related to the presence of the fibers and bioactive compounds present in the Pinhão husk, which may be related to the formation of crosslinks in the polymeric matrix that contributed to the reduction of the hydrophilic character and polar groups in the film.^{13,58}

The solubility of the films in the present study was lower than that reported by Spada et al.,² who observed solubility in starch-based films with the addition of Pinhão husk between 30 and 36%. The values obtained are still within those reported by other studies in the literature. For example, Da Silva et al.⁵⁹ obtained solubility values in pectin films and babaçu coconut mesocarp between 13.89 and 50.64%; while Ruiz-Martínez et al.³⁹ when evaluating the effect of agave nanoclays and

TABLE 6 Results of optical properties of edible Pinhão flour films and edible gelatin films with different Pinhão husk powder contents.

Formulation	L*	a*	b*	ΔE	Opacity
Pinhão Flour Film					
PF1	73.47 ^a ± 0.79	9.53 ^d ± 0.50	27.66 ^e ± 0.82	35.81 ^e ± 1.23	4.43 ^{ab} ± 0.32
PF2	71.77 ^a ± 1.33	9.27 ^d ± 0.83	30.02 ^d ± 1.37	38.52 ^d ± 2.05	3.73 ^{bc} ± 0.23
PF3	65.60 ^b ± 0.94	14.80 ^c ± 0.58	34.09 ^c ± 0.89	46.94 ^c ± 1.40	3.84 ^{bc} ± 0.34
PF4	65.35 ^b ± 0.25	13.91 ^c ± 0.09	34.88 ^c ± 0.14	47.36 ^c ± 0.25	3.01 ^c ± 0.23
PF5	59.16 ^c ± 1.08	20.04 ^b ± 0.59	37.96 ^b ± 0.74	55.55 ^b ± 1.31	5.15 ^a ± 0.45
PF6	57.74 ^c ± 0.21	19.68 ^b ± 0.12	38.11 ^b ± 0.22	56.43 ^b ± 0.28	4.27 ^{ab} ± 0.30
PF7	52.36 ^d ± 0.93	25.18 ^a ± 0.53	39.62 ^a ± 0.51	63.12 ^a ± 0.94	5.10 ^a ± 0.43
PF8	57.93 ^c ± 1.93	19.71 ^b ± 0.69	37.18 ^b ± 0.08	55.70 ^b ± 1.13	4.53 ^{ab} ± 0.35
Gelatin Film					
GF1	76.61 ^a ± 2.19	4.59 ^{cd} ± 0.37	18.91 ^c ± 2.29	25.38 ^d ± 3.12	2.86 ^a ± 0.11
GF2	71.64 ^{ab} ± 1.60	5.48 ^c ± 0.63	23.37 ^b ± 1.72	32.07 ^c ± 2.40	1.29 ^d ± 0.13
GF3	67.05 ^{bc} ± 1.20	3.50 ^d ± 0.41	21.18 ^{bc} ± 0.87	33.64 ^{bc} ± 1.33	2.20 ^{bc} ± 0.18
GF4	72.95 ^a ± 2.08	4.85 ^{cd} ± 0.43	20.32 ^{bc} ± 1.54	28.93 ^{cd} ± 2.38	1.24 ^d ± 0.11
GF5	64.23 ^c ± 2.59	11.50 ^a ± 1.39	30.41 ^a ± 2.61	43.47 ^a ± 3.91	2.01 ^c ± 0.11
GF6	64.87 ^c ± 1.73	10.92 ^{ab} ± 0.89	30.99 ^a ± 1.31	43.28 ^a ± 2.30	2.20 ^{bc} ± 0.21
GF7	66.56 ^c ± 3.91	9.68 ^b ± 1.15	28.11 ^a ± 2.50	39.87 ^{ab} ± 4.08	2.57 ^{ab} ± 0.13
GF8	66.85 ^{bc} ± 3.27	9.38 ^b ± 1.02	29.69 ^a ± 3.06	40.68 ^a ± 4.09	2.15 ^c ± 0.02

Note: Result expressed as mean ± standard deviation. Different lowercase letters in the same column indicate a significant difference between samples ($p < 0.05$).

microfibers on gelatin-based films, reported values between 19.7 and 30.7% of solubility.

The water contact angle test ($^{\circ}$) of the biodegradable films incorporated with Pinhão husk powder was significant ($p < 0.05$). The Pinhão films presented values between $\theta = 35.80^{\circ}$ (PF3) and $\theta = 60.73^{\circ}$ (PF6), while the gelatin films presented values between $\theta = 86.70^{\circ}$ (GF7) and $\theta = 94.87^{\circ}$ (GF2) (Table 5).

According to the results obtained, all samples of Pinhão films exhibited contact angle values lower than 65° , which are indicative of hydrophilic surfaces,⁶⁰ with the PF6 sample being the one that presented the highest hydrophobic character among the analyzed films ($\theta = 60.73^{\circ}$). Gelatin films generally have a more hydrophobic character. This hydrophobicity of the gelatin films may be due to the rearrangement of the hydrophobic part of the gelatin molecules in the air during gelation.⁶¹ This hydrophobic character of the PF6 and GF6 formulations may also be associated with the lower WVP obtained in these formulations.

The contact angle values obtained in our study were within those reported by Spada et al.,² who reported contact angle values ranging from $\theta = 22^{\circ}$ to 103° between cassava starch with Pinhão husk powder treated and untreated. The increase in surface hydrophobicity of the PF6 film can be attributed to the presence of phenolic compounds present in the Pinhão husk powder, which may have migrated to the surface of the film during the drying process, as well as in the levels of lipids, proteins and, probably, lignin present in the Pinhão husk.^{20,60} Similar results were observed in bovine gelatin films reinforced with bionanocomposites of gelatin-cellulose nanocrystals, which obtained contact angle values with water between 80.7° and 95.2° .⁶²

3.5.3 | Optical properties

As expected, adding different amounts of Pinhão husk powder promoted changes in the values of the color parameters (Table 6). The films exhibited significant differences for all color parameters ($p < 0.05$). As expected, adding more Pinhão husk powder (0.4%) resulted in films with lower L^* values and darker films with higher a^* , b^* , and ΔE values. Similar results were reported in films made with Pinhão flour⁸ and starch,² as well as in gelatin films incorporated with agave microfibers and nanoclays³⁹ and with orange peel powder.⁶³

The a^* and b^* values indicate that the films are more reddish and yellow, giving them an orange-looking color. This behavior for the color parameters can be attributed to the presence of phenolic compounds, flavonoids and

tannins, and other extractive substances in the Pinhão husk.^{22,46}

The opacity of the films showed the same behavior as the color parameters, and it was observed that the films with a larger quantity of Pinhão husk promotes a higher opacity. The films showed opacity values ranging between 3.01 (PF4) and 5.15 (PF5) and between 1.24 (GF4) and 2.86 (GF1), showing a significant difference ($p < 0.05$) (Table 6). The ability to block UV can be attributed to the presence of phenolic compounds in the film structure due to the addition of Pinhão husk, as the phenolic rings of bioactive compounds can increase the UV protection property of the films.^{64,65} In addition, several other factors can influence the opacity of the films, such as the presence of additives, processing conditions, thickness, and internal and superficial heterogeneity of the film structure.⁵⁴

Opacity is a property that, in addition to impacting appearance, can influence the shelf life of the packaged product. Although transparent films are more favorable because they allow the consumer to see the products, more opaque films with a darker color can be a viable alternative for different types of food since they can protect food against the adverse effects of light, increasing the shelf life of photosensitive foods.^{52,66}

3.5.4 | Scanning electron microscopy

Analyzing the micrographs of the Pinhão flour and gelatin films reinforced with Pinhão husk powder, it can be noted that, in general, the surfaces of the formulations of all analyzed films showed a rough, irregular surface and without cracks, probably due to the good interaction between all components (Figure 2). The PF6 and GF6 films were the ones that presented the most regular, cohesive, and homogeneous surfaces among all the studied films.

It was observed that the presence of Pinhão husk powder made the films rougher and more irregular due to the presence of fibers inside the matrix of the films. The presence of roughness in the films may be related to the presence of phenolic compounds present in the Pinhão husk powder during the preparation of the films, which may have migrated to the surface of the film during the drying step.⁶⁰

In addition, the greater compaction of the surface of the PF6 and GF6 films may have occurred due to the intermolecular interaction in the matrix of the films that was favored by the composition of the formulations. In contrast, in the other formulations, the polymeric chains' entanglement in the covalent and non-covalent bond

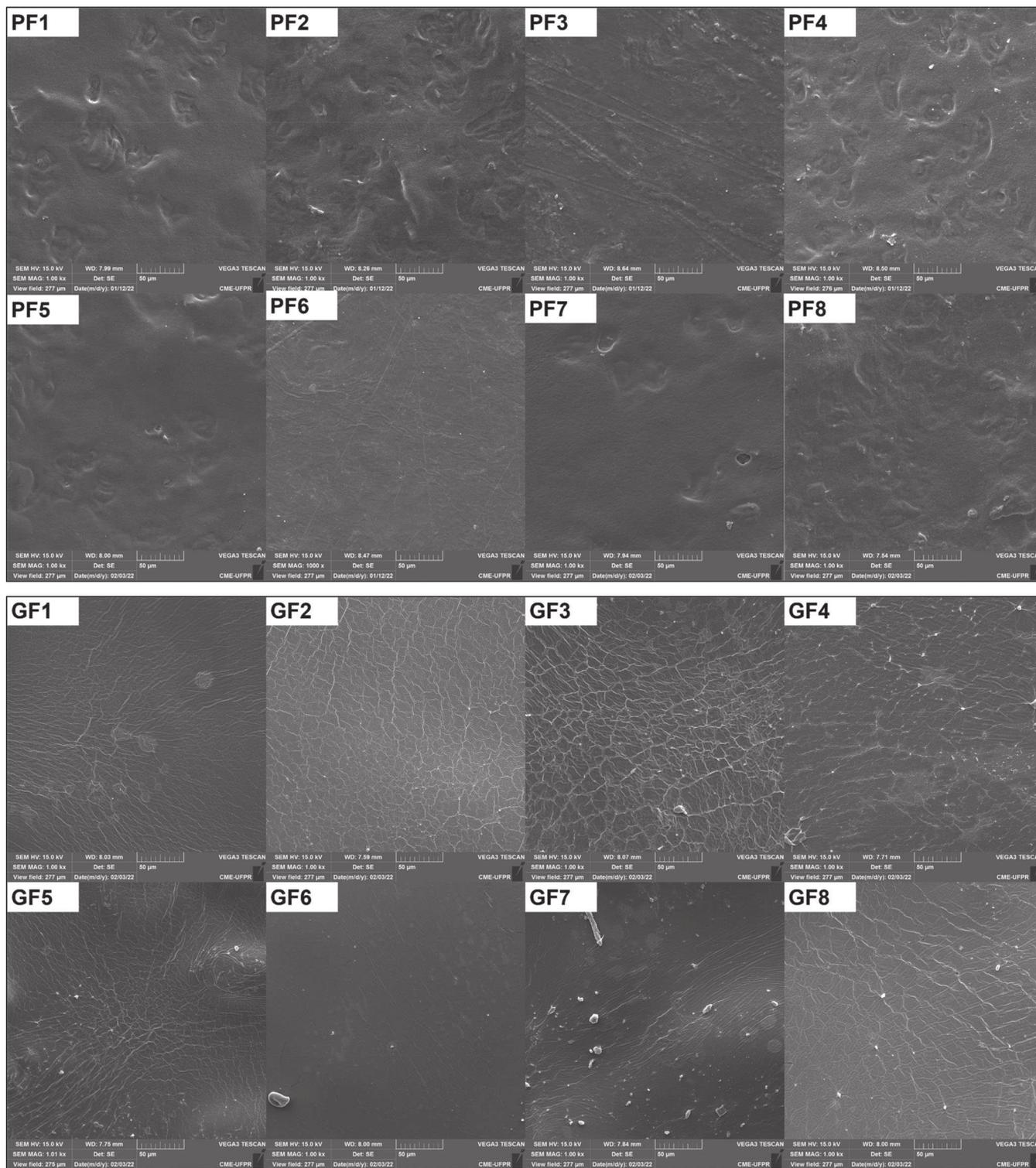


FIGURE 2 SEM of the surfaces (1000 \times) of edible Pinhão flour (PF) films and edible gelatin films (GF) incorporated with different Pinhão husk powder contents.

pathways may have promoted surface roughness.⁶⁷ The addition of Pinhão husk powder to the matrix of Pinhão flour and gelatin films can generate a more resistant interface and, thus, there is an increase in the mechanical strength and water resistance of the films.²⁰

Similar characteristics regarding the roughness of the films were reported in films based on Pinhão flour reinforced with Pinhão husk powder,⁸ cassava starch reinforced with grape stalks¹⁰ and chicken skin gelatin incorporated into rice flour.⁶⁷

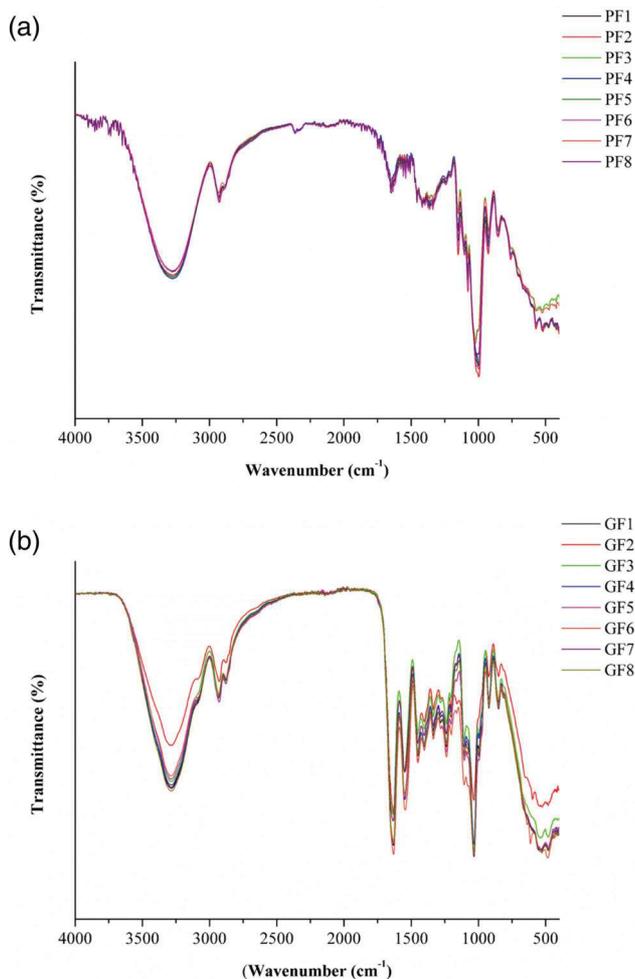


FIGURE 3 FTIR spectra of edible Pinhão flour films (a) and edible gelatin films (b) with different Pinhão husk powder contents. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

3.5.5 | Fourier transform infrared spectroscopy

The spectra of the films formulated with Pinhão flour with the addition of Pinhão husk powder are shown in Figure 3a, in which it is possible to observe a similar absorption behavior ($500\text{--}4000\text{ cm}^{-1}$) for the films. It is possible to observe pronounced peaks in the range of 3290 cm^{-1} , where they are correlated with the elongation of the -OH groups characteristic of the formation of hydrogen bonds.⁸ In this region, the PF1, PF2, and PF6 films showed less intense peaks, which may be related to the lower percentage of glycerol in the respective formulations.

The CH_2 groups are identified in the peaks in the region of 2930 cm^{-1} , whose peaks are between 2800 and 3000 cm^{-1} correlated with the proportion of amylose and amylopectin; the higher the intensity, the higher the amylose content.²⁷ In the spectral region between 2335

and 2800 cm^{-1} , asymmetric and symmetric C-H stretching vibrations were observed for aliphatic portions in polysaccharides present in Pinhão.⁶⁸

Peaks were identified in the region around 1640 cm^{-1} , whose region is correlated to the amide group of proteins.⁶⁹ According to Ramos et al.,⁷⁰ the region between 800 and 1150 cm^{-1} is related to the absorption bands of molecules of glycerol. The peaks in this region, mainly at 995 cm^{-1} were characteristic of alkene groups for C-H binding,² were less intense for samples PF3 and PF7. The proportion of Pinhão flour and glycerol for these films is approximately equal to 1, which may be affecting this intensity. In addition, the Pinhão husk is also affecting these peaks, where the lower concentration of powder in the husk generated a lower peak intensity in this absorption region.

As for the gelatin films, absorption peaks in the range of 3285 , 1634 , 1238 , and 1056 cm^{-1} were found in all samples (Figure 3b). The peak at 3285 cm^{-1} is attributed to the elongation of the O-H and N-H groups on the amide A and phenol functional groups in the film structure. It is possible to identify a reduction in peak intensity for the GF2 film, which has a higher proportion of gelatin than the other films. This may be related to a greater gelatin-gelatin interaction to the detriment of hydrogen bonds.³² In addition, the films showed the characteristic bands of gelatin at wavenumbers 1634 cm^{-1} , 1540 cm^{-1} , and 1238 cm^{-1} correlated with amide I (illustrating the C=O stretching vibration coupled with bending in the plane of the N-H bond and elongation of the C-N bond), amide II (representing N-H bending and C-N elongation) and amide III (associated with in-plane vibration of the C-N and N-H groups of the long-linked amine). The peak at 1056 cm^{-1} is also highlighted, where it signifies the interaction between the plasticizer (OH groups of glycerol) and the structure of the gelatin film.⁷¹ Similar results have been reported for gelatin films containing additives.^{14,72,73}

3.5.6 | Thermogravimetric analysis

The thermal stability of biofilms over a wide temperature range (20 to 750°C) was evaluated using thermogravimetric analysis (TGA) and its first derivative (DTG) (Figure 4). Thermal decomposition showed multiple stages of mass loss, with three stages of decomposition according to the DTG curves (Figure 4c,d).

The first stage of mass loss occurs between 50 and 130°C . This loss corresponds to the elimination of free water and low molar weight volatile compounds present in the sample by evaporation/dehydration,^{8,10} with more significant mass loss observed for the films PF7 with

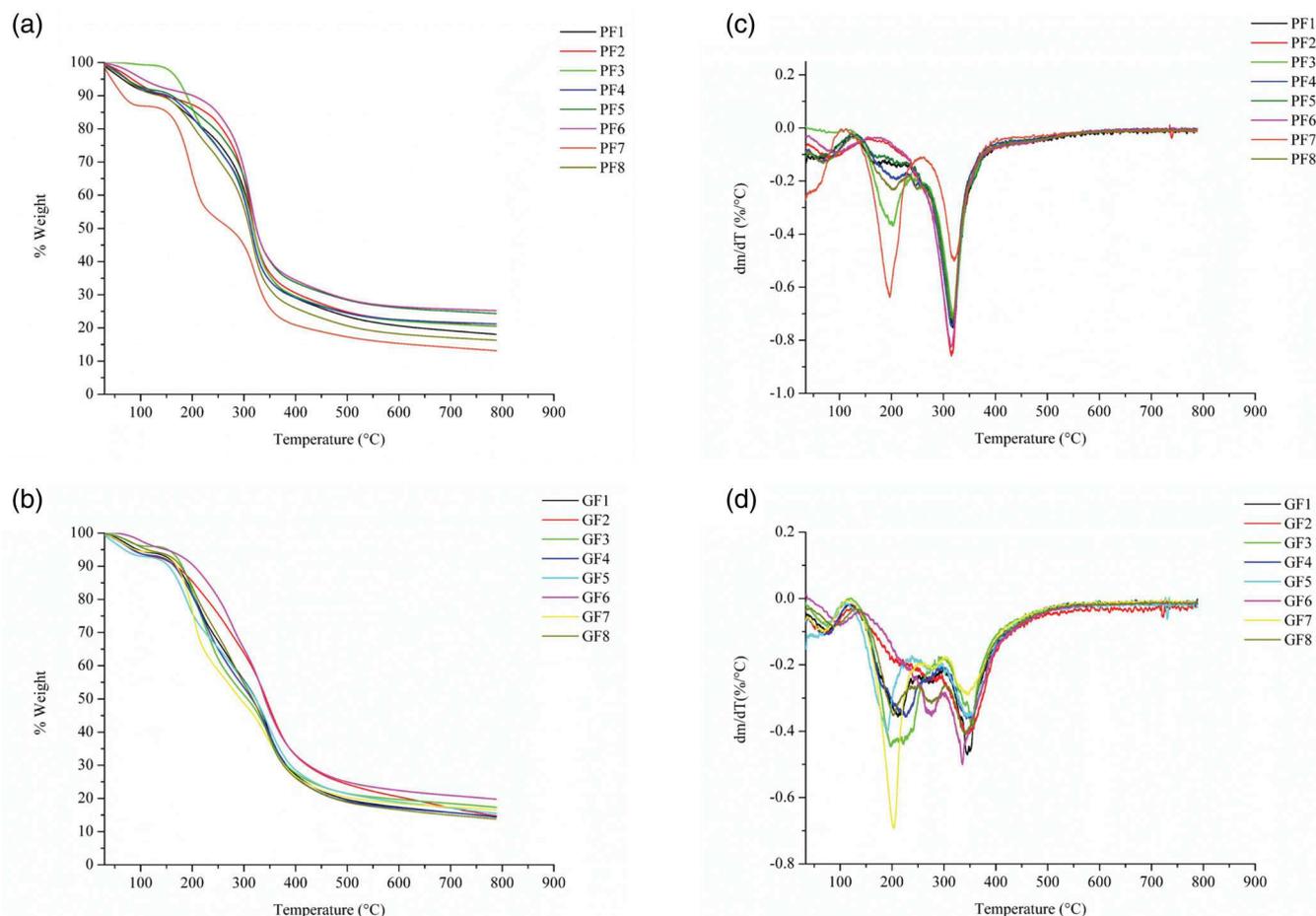


FIGURE 4 TGA and DTG of edible Pinhão flour films (a,c) and edible gelatin films (b,d) with different Pinhão husk powder contents. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.52333)]

13.09% and GF5 with 9.35%. The second stage of mass loss for the PF and GF films occurred between temperatures of 150 and 240°C, this stage being associated with the degradation of glycerol, as well as the evaporation of low molecular weight components that may be present in the Pinhão husk.^{8,10,55} In this stage of thermal decomposition, the films that presented the most significant mass loss were those that presented the highest values of glycerol in their composition, with emphasis on the films PF3 (25.22%), PF7 (34.29%), GF3 (33.04%), and GF7 (34.63%). This event shows the films are stable at temperatures below 150°C, losing only free water and low molecular weight volatile compounds.

The third event is observed approximately between 250 and 500°C and presents the most significant mass loss of the three stages, ranging from 36.48 (PF7) to 58.42% (PF2) of the loss for the PF films, while the GF films ranged from 39.33 (GF7) to 57.08% (GF6). This step corresponds to the degradation of the starch components for the PF films, which is wholly decomposed, along with the other components that are in the flour and Pinhão husk, such as protein, lipid, and fiber, up to 350°C.^{8,10,55}

In contrast, for the GF films, this stage is mainly related to the degradation of the gelatin chains, with two distinct peaks being observed in the DTG curves, corresponding to the pyrolysis of components with different thermal stability in this stage,^{61,74} in addition to the degradation of cellulosic substances present in the Pinhão husk.^{8,10,55,74} The PF and GF films showed behavior similar to thermal decomposition. They exhibited multiple stages of weight loss, similar to that reported in the literature for Pinhão husk,⁸ starch-based films^{10,40,55} and gelatin films^{39,61,74} with reinforcements.

4 | CONCLUSIONS

The results of this study confirm that Pinhão husk can be used as a reinforcing material in the production of films based on starch (Pinhão flour) and gelatin. The incorporation of Pinhão husk powder positively influenced the increase in tensile strength and decrease in WVP and solubility, in addition to altering the thermal and microstructural properties of the films. In addition, ATR-FTIR

and SEM could generate correlations with the physical and mechanical properties, noting that the use of Pinhão husk powder efficiently improved the uniformity and cohesion of the film structures. The thermal stability study revealed that the films are stable below 150°C, losing only free water and low molecular weight volatile compounds.

According to the results, the formulations PF6 (5.0% Pinhão flour, 1.2% glycerol, and 0.4% Pinhão husk powder) and GF6 (5.0% gelatin, 2.0% glycerol, and 0.4% Pinhão husk powder) were the ones that presented the best WVP values, and mechanical properties related to ELO and TS, respectively. Since these formulations presented values relatively close to those of low-density polyethylene (LDPE) (ELO = 49.46% and 95.97%; and TS and 84.33% and 64.67%), respectively, these results justify the use of Pinhão husk powder as reinforcement for films, suggesting that these films have potential application as biodegradable, edible packaging for ecologically correct foods, reducing the accumulation of synthetic plastics in nature.

AUTHOR CONTRIBUTIONS

Deocleciano Cassiano de Santana Neto: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft (equal). **Thainnane Silva Paiva:** Methodology (supporting). **Rogério Willian Silva dos Santos:** Methodology (supporting). **Cristiane Viera Helm:** Methodology (supporting). **Fábio Anderson Pereira da Silva:** Investigation (equal); methodology (equal); resources (supporting); supervision (supporting); writing – review and editing (supporting). **Luiz Mário de Matos Jorge:** Conceptualization (equal); investigation (equal); methodology (equal); resources (equal); supervision (equal); writing – review and editing (supporting). **Regina Maria Matos Jorge:** Conceptualization (lead); data curation (equal); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (equal); project administration (lead); resources (lead); software (equal); supervision (lead); validation (equal); writing – review and editing (supporting).

ACKNOWLEDGMENTS

The authors wish to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)-Finance Code 001 for the financial support and doctoral scholarship awarded to D.C. Santana Neto and the National Council for Scientific and Technological Development (CNPq) (process number: 88882.381653/2019-01); (grant numbers: 312215/2017-7 and 315598/2020-4), as well as the Center for Electronic Microscopy of the UFPR (CEM), for the image analysis. We are also grateful for the scientific cooperation of the EMBRAPA Florestas/Colombo-PR for

donating the Pinhão seeds, for the Gelnex© for donating the bovine gelatin and the Federal University of Paraná for its support in carrying out this research.

CONFLICT OF INTEREST STATEMENT

The authors declare there are no conflict of interest and also that there is no data available from an open source, as a repository.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Deocleciano Cassiano De Santana Neto  <https://orcid.org/0000-0003-1017-1383>

Thainnane Silva Paiva  <https://orcid.org/0000-0002-0991-6096>

Rogério Willian Silva Dos Santos  <https://orcid.org/0000-0001-7728-226X>

Cristiane Vieira Helm  <https://orcid.org/0000-0002-7518-9069>

Fábio Anderson Pereira Da Silva  <https://orcid.org/0000-0002-4302-6070>

Luiz Mário De Matos Jorge  <https://orcid.org/0000-0003-1869-1957>

Regina Maria Matos Jorge  <https://orcid.org/0000-0003-3561-3555>

REFERENCES

- [1] R. W. S. Dos Santos, D. C. De Santana Neto, M. S. Leite, R. M. M. Jorge, T. L. P. Dantas, *Packag. Technol. Sci.* **2023**, 36, 281.
- [2] J. C. Spada, C. L. Luchese, I. C. Tessaro, *J. Polym. Environ.* **2018**, 26, 2686.
- [3] M. D. Hazrol, S. M. Sapuan, E. S. Zainudin, N. I. A. Wahab, R. A. Ilyas, *Polymer* **2022**, 14, 1590.
- [4] Plastics Europe Plastics – the Facts **2022**.
- [5] W. D. J. B. Duarte, *Revista Ibero-Americana de Humanidades, Ciências e Educação* **2022**, 8, 22.
- [6] L. Huang, Q. P. Li, H. Li, L. Lin, X. Xu, X. Yuan, J. B. Koongolla, H. Li, *Mar. Pollut. Bull.* **2023**, 186, 114399.
- [7] C. López-De-Dicastillo, J. Gómez-Estaca, R. Catalá, R. Gavara, P. Hernández-Muñoz, *Food Chem.* **2012**, 131, 1376.
- [8] R. M. Daudt, A. J. G. Sinrod, R. J. Avena-Bustillos, I. C. Küilkamp-Guerreiro, L. D. F. Marczak, T. H. McHugh, *Food Hydrocolloids* **2017**, 71, 60.
- [9] S. A. Mir, B. N. Dar, A. A. Wani, M. A. Shah, *Trends Food Sci. Technol.* **2018**, 80, 141.
- [10] E. J. G. Laureanti, T. S. Paiva, I. De Souza Tasso, I. D. Dallabona, C. V. Helm, L. M. De Matos Jorge, R. M. M. Jorge, *J. Appl. Polym. Sci.* **2021**, 138, 1.
- [11] V. Muralidharan, S. Gochhayat, S. Palanivel, B. Madhan, *Environ. Sci. Pollut. Res.* **2022**, 1, 1.

- [12] A. Bermúdez-Oria, G. Rodríguez-Gutiérrez, F. Rubio-Senent, Á. Fernández-Prior, J. Fernández-Bolaños, *Meat Sci.* **2019**, *148*, 213.
- [13] E. Da Santos, T. C. D. A. Sousa, S. Neto, D. C. De, C. V. B. Grisi, V. C. D. S. Ferreira, F. A. P. Da Silva, *LWT* **2022**, *154*, 112837.
- [14] S. Shankar, L. F. Wang, J. W. Rhim, *Food Packag. Shelf Life* **2019**, *21*, 100363.
- [15] L. Ge, M. Zhu, X. Li, Y. Xu, X. Ma, R. Shi, D. Li, C. Mu, *Food Hydrocolloids* **2018**, *83*, 308.
- [16] H. Du, T. Min, X. Sun, X. Bian, Z. Zhu, Y. Wen, *Food Biosci.* **2022**, *50*, 102168.
- [17] G. Song, S. Lin, Y. Wu, J. Shen, J. Wu, W. Zhu, S. Yu, J. Li, S. Wang, *J. Food Eng.* **2023**, *343*, 111362.
- [18] Saranti, T. F. d. S.; Melo, P. T. S.; Cerqueira, M. A.; Aouada, F. A.; De Moura, M. R. *Polymers* **13**, 4298 **2021**
- [19] J. Lin, D. Pan, Y. Sun, C. Ou, Y. Wang, J. Cao, *Food Sci. Nutr.* **2019**, *7*, 4140.
- [20] G. R. De Carvalho, G. S. Marques, L. M. D. M. Jorge, R. M. M. Jorge, *Braz. J. Chem. Eng.* **2021**, *38*, 341.
- [21] Y. Garavand, A. Taheri-garavand, F. Garavand, F. Shahbazi, D. Khodaei, I. Cacciotti, *Appl. Sci.* **2022**, *12*, 1111.
- [22] M. S. Dorneles, C. P. Z. Noreña, *J. Food Process. Preserv.* **2020**, *44*, 1.
- [23] C. V. Helm, M. De Matos, G. G. De Lima, W. L. E. Magalhães. *Produção de farinha de pinhão funcional com compostos bioativos*. Embrapa Florestas, Colombo **2020**.
- [24] A. G. Barreto, L. D. A. Sobral, A. F. Guerra, R. I. Nogueira, R. Catie, B. De Godoy, S. Pereira, *6th European Drying Conference Liège*, Belgium **2017**, p. 19.
- [25] T. Jorge, J. De Dea Lindner, S. M. V. Mejia, B. Mattioni, J. Rotta, S. Morés, A. De Francisco, E. S. Sant'Anna, *J. Inst. Brew.* **2018**, *124*, 365.
- [26] L. Pigozzi, W. G. Sganzerla, A. Paula De Lima Veeck, L. Costa De Conto, *Chem. Eng. Trans.* **2019**, *75*, 145.
- [27] R. M. Daudt, R. J. Avena-Bustillos, T. Williams, D. F. Wood, I. C. Külkamp-Guerreiro, L. D. F. Marczak, T. H. McHugh, *Food Hydrocolloids* **2016**, *60*, 279.
- [28] C. L. Luchese, J. M. Frick, V. L. Patzer, J. C. Spada, I. C. Tessaro, *Food Hydrocolloids* **2015**, *45*, 203.
- [29] P. S. Müller, D. Carpiné, F. Yamashita, N. Waszczynskyj, *J. Food Sci.* **2020**, *85*, 2832.
- [30] J. C. Spada, E. M. Da Silva, I. C. Tessaro, *Revista Brasileirade Ciências Agrárias* **2014**, *9*, 365.
- [31] D. Sutay Kocabaş, M. Erkoç Akçelik, E. Bahçegül, H. N. Özbek, *Ind. Crops Prod.* **2021**, *171*, 113847.
- [32] Z. Riahi, R. Priyadarshi, J. W. Rhim, R. Bagheri, *Food Hydrocolloids* **2021**, *112*, 106314.
- [33] A. C. Souza, R. Benze, E. S. Ferrão, C. Ditchfield, A. C. V. Coelho, C. C. Tadini, *LWT - Food Sci. Technol.* **2012**, *46*, 110.
- [34] M. Guimarães, V. R. Botaro, K. M. Novack, F. G. Teixeira, G. H. D. Tonoli, *Ind. Crops Prod.* **2015**, *70*, 72.
- [35] Z. A. M. Adilah, B. Jamilah, Z. A. Nur Hanani, *Food Hydrocolloids* **2018**, *74*, 207.
- [36] M. Fazeli, M. Keley, E. Biazar, *Int. J. Biol. Macromol.* **2018**, *116*, 272.
- [37] M. P. Silveira, H. C. Silva, I. C. Pimentel, C. G. Poitevin, A. K. Costa Stuart, D. Carpiné, L. M. Matos Jorge, R. M. M. Jorge, *J. Appl. Polym. Sci.* **2019**, *137*, 48726.
- [38] K. Wegrzynowska-Drzymalska, D. T. Mlynarczyk, D. Chelminiak-Dudkiewicz, H. Kaczmarek, T. Goslinski, M. Ziegler-Borowska, *Int. J. Mol. Sci.* **2022**, *23*, 9700.
- [39] I. G. Ruiz-Martínez, D. Rodrigue, J. Solorza-Feria, *Polym. Bull.* **2022**, *79*, 1437.
- [40] G. S. Marques, G. R. De Carvalho, N. P. Marinho, G. I. B. De Muniz, L. M. De Matos Jorge, R. M. M. Jorge, *J. Appl. Polym. Sci.* **2019**, *136*, 1.
- [41] AOAC, *Official methods of analysis*, 18th ed., Association of Official Analytical Chemists, Washington, DC, USA **2005**.
- [42] ASTM ASTM D882-18. Standard Test Method for Tensile Properties of Thin Plastic Sheeting. **2012**.
- [43] ASTM *Standard Test Methods for Water Vapor Transmission of Materials*. West Conshohocken, PA **2016**.
- [44] R. N. F. Nazurah, Z. A. Nur Hanani, *Carbohydr. Polym.* **2017**, *157*, 1479.
- [45] F. J. O. G. Da Costa, C. L. Leivas, N. Waszczynskyj, R. C. Bueno de Godoi, C. V. Helm, T. A. D. Colman, E. Schnitzler, *Thermochim. Acta* **2013**, *565*, 172.
- [46] T. G. Timm, G. G. De Lima, M. Matos, W. L. E. Magalhães, L. B. B. Tavares, C. V. Helm, *J. Food Process. Preserv.* **2020**, *44*, 44.
- [47] N. Lehman, R. Phengthai, W. Chinpa, *J. Polym. Environ.* **2018**, *26*, 1405.
- [48] H. N. M. Chambi, C. R. F. Grosso, *Ciencia e Tecnol. de Alimentos* **2011**, *31*, 739.
- [49] F. M. B. Coutinho, I. L. Mello, L. C. Maria, *Polímeros: Ciência e Tecnologia* **2003**, *13*, 1.
- [50] Y. Kusumastuti, N. R. E. Putri, D. Timotius, M. W. Syabani, *Heliyon* **2020**, *6*, e05280.
- [51] C. Zhang, S. S. Nair, H. Chen, N. Yan, R. Farnood, F. Li, *Yi Carbohydr. Polym.* **2020**, *230*, 115626.
- [52] E. Sogut, H. Cakmak, *Food Hydrocolloids* **2020**, *106*, 105861.
- [53] A. Riaz, C. Lagnika, M. Abidin, M. M. Hashim, W. Ahmed, *J. Polym. Environ.* **2020**, *28*, 411.
- [54] H. C. Silva-Rodrigues, M. P. Silveira, C. V. Helm, L. M. Matos Jorge, R. M. M. Jorge, *J. Appl. Polym. Sci.* **2020**, *137*, 1.
- [55] G. R. De Carvalho, G. S. Marques, L. M. De Matos Jorge, R. M. M. Jorge, *J. Appl. Polym. Sci.* **2019**, *136*, 1.
- [56] W. G. Sganzerla, G. B. Rosa, A. L. A. Ferreira, C. G. Da Rosa, P. C. Belling, L. O. Xavier, C. M. Hansen, J. P. Ferrareze, M. R. Nunes, P. L. M. Barreto, A. P. De Lima Veeck, *Int. J. Biol. Macromol.* **2020**, *147*, 295.
- [57] E. M. Ciannamea, P. M. Stefani, R. A. Ruseckaite, *Food Hydrocolloids* **2014**, *38*, 193.
- [58] I. Choi, S. E. Lee, Y. Chang, M. Lacroix, J. Han, *LWT* **2018**, *93*, 427.
- [59] D. C. Da Silva, I. A. Lopes, L. J. S. Da Silva, M. F. Lima, A. K. D. Barros Filho, H. A. Villa-Vélez, A. A. Santana, *Int. J. Biol. Macromol.* **2019**, *130*, 419.
- [60] D. Piñeros-Hernandez, C. Medina-Jaramillo, A. López-Córdoba, S. Goyanes, *Food Hydrocolloids* **2017**, *63*, 488.
- [61] Y. Liu, S. Liu, J. Liu, X. Zheng, K. Tang, *J. Appl. Polym. Sci.* **2022**, *139*, 52119.
- [62] L. S. F. Leite, C. M. Ferreira, A. C. Corrêa, F. K. V. Moreira, L. H. C. Mattoso, *Carbohydr. Polym.* **2020**, *238*, 116198.
- [63] H. Taghavi Kevij, M. Salami, M. Mohammadian, M. Khodadadi, Z. Emam-Djomeh, *Polym. Bull.* **2021**, *78*, 4387.
- [64] C. U. López-Palestina, C. L. Aguirre-Mancilla, J. C. Raya-Pérez, J. G. Ramirez-Pimentel, A. Vargas-Torres, A. D. Hernández-Fuentes, *CYTA - J. Food* **2019**, *17*, 142.
- [65] N. Ghadiri Alamdari, S. Salmasi, H. Almasi, *Food Bioprocess Technol.* **2021**, *14*, 2380.
- [66] D. P. Arbeláez, J. A. Mina Hernández, J. H. Grande-Tovar, J. I. Castro, D. Paola Navia-Porras, J. Andrés Arbeláez Cortés, J.

- Hermisul, M. Hernández, C. D. Grande-Tovar, J. A. A. C. Co, *Molecules* **2022**, *27*, 2264.
- [67] P. Y. Soo, N. M. Sarbon, *Food Packag. Shelf Life* **2018**, *15*, 1.
- [68] S. D. S. Barros, W. A. G. Pessoa Jr., A. Cruz Júnior, Z. V. Borges, C. M. Poffo, D. M. Regis, F. A. De Freitas, L. Manzato, *Res. Soc. Dev.* **2021**, *10*, e270101018836.
- [69] J. Liao, S. Deng, H. Essawy, X. Bao, H. Wang, G. Du, X. Zhou, *Materials* **2022**, *15*, 15.
- [70] Ó. L. Ramos, I. Reinas, S. I. Silva, J. C. Fernandes, M. A. Cerqueira, R. N. Pereira, A. A. Vicente, M. F. Poças, M. E. Pintado, F. X. Malcata, *Food Hydrocolloids* **2013**, *30*, 110.
- [71] N. Syahida, I. Fitry, A. Zuriyati, N. Hanani, *Food Packag. Shelf Life* **2020**, *23*, 100437.
- [72] H. Haghighi, M. Gullo, S. La China, F. Pfeifer, H. W. Siesler, F. Licciardello, A. Pulvirenti, *Food Hydrocolloids* **2021**, *113*, 106454.
- [73] M. Tavassoli, M. A. Sani, A. Khezerlou, A. Ehsani, D. J. McClements, *Food Hydrocolloids* **2021**, *118*, 106747.
- [74] I. G. Ruiz-Martínez, D. Rodrigue, M. L. Arenas-Ocampo, B. H. Camacho-Díaz, S. V. Avila-Reyes, J. Solorza-Feria, *Foods* **2022**, *11*, 1573.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: D. C. De Santana Neto, T. S. Paiva, R. W. S. Dos Santos, C. V. Helm, F. A. P. Da Silva, L. M. De Matos Jorge, R. M. M. Jorge, *J. Appl. Polym. Sci.* **2024**, *141*(15), e55233. <https://doi.org/10.1002/app.55233>