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Pumpkin processing by-products: Incorporation of defatted seed flour and ethanolic extract of the peel in gelatin-based biodegradable film

Subprodutos do processamento de abóbora: Incorporação de farinha de semente desengordurada e extrato etanólico da casca em filme biodegradável à base de gelatina

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ABSTRACT: The present study aimed to use pumpkin by-products for the production of gelatin-based biodegradable film (FBs). For this purpose, the incorporation of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP) by casting technique. The FBs were characterized in terms of thickness, visual evaluation, humidity, water solubility, UV/VIS barrier, color parameters, microscopy, Fourier transform infrared spectroscopy (FTIR), biodegradability in soil and the content of phenolic compounds and flavonoids. All the formulations showed film-forming capacity; however, the greater incorporation of DPSF provided heterogeneous films. Thickness and moisture content were not altered by the incorporation of FDSA; however, solubility increased. The incorporation of FDSA and EECA influenced the color parameters, presenting light transmission and opacity of 0.61 to 5.83 (A/mm), respectively, and contributed to the improvement of the UV/VIS barrier. FTIR analysis indicated bands characteristic of gelatin- and lecithin-based films. The addition of FDSA increased the phenolic concentration in the films. The samples incorporated with FDSA and EECA presented lower residue in the biodegradability tests, starting on day 11, inferring that their incorporation accelerates the biodegradation process.

Keywords: Co-products; Packaging; Phenolic compounds; Polymers Pumpkin.

RESUMO: O presente estudo teve como objetivo utilizar subprodutos da abóbora para a produção de filmes biodegradáveis (FBs) à base de gelatina. Para tanto, avaliou-se a incorporação de farinha desengordurada de sementes de abóbora (FDSA) e extrato etanólico de cascas de abóbora (EECA) pela técnica de casting. Os FBs foram caracterizados quanto à espessura, avaliação visual, umidade, solubilidade em água, barreira UV/VIS, parâmetros de cor, microscopia, espectroscopia no infravermelho com transformada de Fourier (FTIR), biodegradabilidade em solo e teor de compostos fenólicos e flavonóides. Todas as formulações apresentaram capacidade filmogênica; entretanto, a maior incorporação de FDSA proporcionou filmes heterogêneos. A espessura e umidade não foi alterada com a incorporação da FDSA, no entanto, a solubilidade aumentou. A incorporação de FDSA e EECA influenciou os parâmetros de cor, apresentando transmissão de luz e opacidade de 0,61 a 5,83 (A/mm), respectivamente, e contribuiu para a melhoria da barreira UV/VIS. A análise FTIR indicou bandas características de filmes à base de gelatina e lecitina. A adição de FDSA aumentou a concentração de fenólicos nos filmes. As amostras incorporadas com FDSA e EECA apresentaram menor resíduo nos testes de biodegradabilidade a partir do 11º dia, inferindo que sua incorporação acelera o processo de biodegradação.

Palavras-chave: Abóbora; Compostos fenólicos; Coprodutos; Embalagem; Polímeros.

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1 INTRODUCTION

The increase in the generation of solid waste that remains for a few years in the environment has caused environmental crises, in addition to economic and social problems, and these impacts are mainly related to the production of plastic packaging (Evode *et al.*, 2021). To minimize the effect caused by plastic packaging, natural polymers can be used in the development of packaging, which have a shorter shelf life when compared to synthetics (Zhang and Sablani, 2021).

Among the polymers with high film production capacity, gelatin has been highlighted due to its biodegradability, low cost and good film-forming properties (Zhang *et al.*, 2026), and the addition of other components in films based on this polymer can improve desirable properties, such as mechanical and moisture migration (Chen *et al.*, 2024), considered the main disadvantages of biodegradable films based on natural polymers.

According to Zhang and Sablani (2021) there is great potential for the incorporation of by-products or food residues to improve the performance of the biodegradable packaging to be developed. Pelissari *et al.* (2013) reported that the use of flour has attracted attention as a packaging material for food, mainly due to the chemical interactions that occur between the components (Gutiérrez e González, 2016), besides being economical, abundant, easily obtainable and renewable.

Pumpkin processing generates by-products, such as peels and seeds, which have high added value. Pumpkin seed flour is rich in crude protein (Lalnunthari *et al.*, 2019) and pumpkin peels can be considered sources of bioactive compounds and antioxidants (Hussain *et al.*, 2021). Phytosterols, tocopherols, phenolic compounds and carotenoids are found in the pell, which motivates the use of this by-product (Massa *et al.*, 2019). The defatted pumpkin seed, obtained after removing the oil, may have 35 to 50% protein contents (Massa *et al.*, 2019; Kumar *et al.*, 2023).

This work aimed to use pumpkin processing by-products to develop gelatin-based biodegradable films (FBs). For this purpose, defatted pumpkin seed flour (DPSF) and pumpkin peel ethanol extract (PPEE) were obtained using ethanol as a solvent and after characterized. The FBs were produced by the casting method with incorporation of different percentages of DPSF and PPEE, and were characterized in relation to visual evaluation and microscopy, thickness, moisture and water solubility, color parameters, light transmission and opacity, barrier UV/VIS, FTIR, content of total phenolic compounds and flavonoids and biodegradability. The development of biodegradable packaging films using these by-products, however, the differential of the present study is to add them to gelatin-based formulations (Lalnunthari *et al.*, 2019).

2 MATERIAL AND METHODS

2.1 MATERIAL

Pumpkins (Curcubita maxima), variety Moranga de Mesa, were purchased at the local market in Umuarama (Paraná, Brazil). To obtain the defatted flour and ethanol extract, ethanol (Panreac, 99.9%) was used as solvent.

Gelatin type A, Bloom 240, GAP 6 (Gelita® from Brazil, Cotia, Brazil), glycerol (Anidrol), and soy lecithin (Êxodo cientifica) were used for the production of biodegradable films.

The following were used in the analysis: ethanol (Anhydrol, 95%), sodium hydroxide (Neon, ⩾ 97%), copper sulfate pentahydrate (Anhydrol, ⩾98.0%), sodium citrate (Anhydrol, 99. 0%), sodium carbonate (Anhydrol, 99.5%), bovine albumin (A7906, Sigma-Aldrich), Folin & Ciocalteu's 2N reagent (47641, Sigma-Aldrich), Quercitin (Sigma - Sigma-Aldrich, ⩾ 95.%), aluminum chloride (Synth, ⩾ 99%), distilled water and plant soil (Class 'A' soil conditioners - Humusfertil).

2.2 RAW MATERIAL PREPARATION

The peels and seeds of the pumpkins were separated manually and subjected to drying (Marconi, MA 035) at 40 ºC for 20 h (Massa *et al*., 2019).

After drying, the seeds and peels were crushed separately using a domestic blender. (Britânia/Diamante Black Filter 900W) and granulometric classification was performed using Tyler type sieves (Bertel, series 1.0) and mechanical stirrer (Marconi, MA 750). The samples of seeds and peels retained on 48 mesh and 100 mesh sieves, respectively, were selected for the extraction process.

2.3 DEFATTED FLOUR: OBTAINING AND CHARACTERIZATION

To obtain the defatted pumpkin seed flour (DPSF), the procedure reported by Stevanato and Silva (2019) was used. The degreased material was kept in an oven (Marconi, MA 035) at 80 °C for 1 hour to remove residual solvent, macerated and graded into 100 mesh (Tyler sieves, Bertel, series 1.0).

DPSF was characterized in relation to content of moisture (925.09 method) and ash (923.03 method) according to the AOAC (2000) and proteins (Wani *et al*., 2006).

The content of total phenolics compounds (TPC) and total flavonoids (TF) were determined according to the methods of Singleton; Orthofer; Lamuela-Raventós (1998) and Freitas *et al*. (2021), respectively. The TPC and TF content were expressed as mg of gallic acid equivalent (GAE) per 100 g of sample and quercetin equivalent (QE) per 100 g of sample.

2.4 ETHANOLIC EXTRACT FROM PUMPKIN PEELS: OBTENTION AND CHARACTERIZATION

To obtain the ethanolic extract of pumpkin peels, 1 g of the sample and 25 mL of ethanol (95%) were added to an Erlemenyer flask (250 mL). Extraction was carried out in orbital stirring equipment (Marconi, MA 830 A) at 50 °C, 140 rpm and 60 min. After the extraction time, the peels were removed by filtration and the filtrate (called ethanolic extract) was stored under refrigeration (7 °C).

The TPC content and TF content were determined as described in item 2.3. To determine the β-carotene content, 0.5 g of ethanolic extract were transferred to a volumetric flask (10 mL) and diluted in ethanol. The absorbance reading of the solution was performed in a spectrophotometer (Shimadzu, UV-1900) at 445 nm. The β-carotene content (μg g^{-1} of extract) was calculated as indicated by Rodriguez-Amaya and Kimura (2004).

2.5 PRODUCTION OF BIODEGRADABLE FILMS

The biodegradable films were produced by the gelatin-based casting technique with incorporation of DPSF and EEPP according to Table 1.

For the production of gelatin films (F1), initially the gelatin and soy lecithin were hydrated in distilled water for 30 min, and kept in an ultrasonic bath (Ultronique Q5.9/40A) at 25 °C for 45 min. After this period, the mixture was solubilized in a water bath (Quimis, 0334M) at 90 °C for 10 min and then the glycerol was added under magnetic stirring (Gehaka, AA 1840) for a period of 40 min.

Table 1. Formulations of gelatin-based biodegradable films with incorporation of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP)

Formulation	Ingredient[1]				
	Gelatin	Soy lecithin	DPSF	EEPP	Plasticizer
F1	3	0.9	0	0	0.9
F2	3	0.9	1	0	0.9
F3	3	0.9	2	0	0.9
F4	3	0.9	0	6	0.9
F5	3	0.9	1	6	0.9
F6	3	0.9	2	6	0.9

[1] concentration were calculated in g per 100 g of filmogenic solution.

In the formulations with the addition of FDSA (F2 and F3) initially the gelatin was hydrated in distilled water (30 min) and solubilized (90 °C, 10 min). Separately, the DPSF was dispersed in distilled water (60 min, under magnetic stirring), then glycerol and soy lecithin were added under magnetic stirring (60 min) and the solution was kept under ultrasound at 25 °C for 45 min. Then, the dispersions (gelatin + DPSF) were mixed under magnetic stirring (20 min). In formulation F4, after solubilization of gelatin (prepared according to F1), the solution was cooled to 30 °C and EEPP was added together with glycerol. The solution was kept in an ultrasonic bath (Ultronique Q5.9/40A) under mechanical agitation for 30 min. For formulations F5 and F6, the solution was previously cooled to 30 °C and the dispersions (gelatin + DPSF) were mixed under mechanical agitation (30 min), where the addition of EEPP was carried out. After preparation, the filmogenic solutions (for all formulations tested) were dispersed on acrylic plates (150 x 150 mm) and subjected to drying (Marconi, MA 035) at 40 °C for 24 h.

2.6 CHARACTERIZATION OF BIODEGRADABLE FILMS

2.6.1 Visual analysis, microscopy and thickness

Visual evaluation was performed in relation to homogeneity (presence of insoluble particles), film formation capacity and handling (ease of removal from the support). Images of the FBs were recorded through a binocular biological microscope (OPTON, TIM – 2008) using the SP10/0.25 – 160/0.17 achromatic objective.

The thickness of the FBs was determined using a digital micrometer (Mitutoyo – IP, 65 model, Japan), through the arithmetic mean of ten random points.

2.6.2 Moisture and water solubility

The moisture of the films was determined in which the samples (2 x 2 cm) were dried in an oven (Marconi, MA 033/1) at 105 °C until constant weight. The dry samples were used to determine the solubility according to Gontard *et al.* (1992), which were solubilized in 50 mL of distilled water and subjected to orbital agitation at 100 rpm (Marconi, MA 830 A) for 24 hours. Subsequently, the samples were dried (105 °C, 24 hours) and the solubility was determined considering the dry film mass before and after shaker incubator.

2.6.3 Color parameters, UV/VIS Barrier, light transmission and opacity

The color parameters were determined in a CR-400 colorimeter (Konica Minolta) using the CIELab system for the parameters L* (luminosity), chroma (a*) and chroma (b*). The matrix angle (h°), which represents the qualitative parameter of color, and the chroma index (C*), which is the quantitative attribute of color, were determined from Equations 1 and 2, respectively.

$$h^{\circ} = tan\,g - 1(^{*}/a^{*}) \tag{1}$$

$$C^{*} = (a^{*2} + b^{*2})^{1/2} \tag{2}$$

The ultraviolet/visible (UV-Vis) barrier property was determined in spectrophotometer (Shimadzu, UV-1900) according to Stoll *et al.* (2016). For the analysis, the FBs were cut into rectangles and placed on the inner wall of a glass cuvette that was used as reference, and the transmittance determined in the range of 200 nm to 700 nm. The transparency of the films was determined from Equation 3, with transmittance data at 600 nm.

$$T = \frac{Abs600}{x} \tag{3}$$

where T is the transparency of the films (mm), Abs600 is the absorbance value at 600 nm and x is the thickness of the films (mm).

2.6.4 Fourier transform infrared spectroscopy (FTIR)

Initially, the FBs were stored in a desiccator containing silica gel for 10 days. Subsequently, the spectra in the infrared region were recorded using a spectrometer (Agilent Cary 630 with a diamond crystal at 21 °C). Sixteen scans were carried out in the spectral range from 650 to 4000 cm^{-1}, with a resolution of 2 cm^{-1}. The Micro Lab program was used to analyze the spectra.

2.6.5 Content of total phenolic compounds and total flavonoids in FBs

The TPC and TF content were determined according to the methodologies reported in Item 2.3, from samples of the films solubilized in distilled water.

2.6.6 Biodegradability

To determine the biodegradability of the films, samples (2 x 3 cm) were dried in an oven with forced air circulation at 60 °C and placed inside mesh screens (4 x 4 cm), and buried 5 cm deep (Stoll *et al*., 2016) using vegetable soil. Samples were taken from the soil at a regular time interval (2 in 2 days) and photographed, later a visual analysis was performed, observing the presence of fungi or molds, fissures or cracks.

2.7 DATA ANALYSIS

Biodegradable films were produced and analyzed in duplicate (n=4). InfoStat® software was used for analysis of variance (ANOVA) and Tukey's test was performed to determine the difference between the means. Results were expressed as mean ± standard deviation.

3 RESULTS AND DISCUSSION

Characterization of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP).

The DPSF showed 5.64 ± 0.33% of moisture, 34.04 ± 1.13% of protein and 6.60 ± 0.01% of ash. The values obtained for TPC and TF content were 104.15 ± 0.63 mg GAE and 20.24 ± 0.30 mg QE per 100 g of DPSF, respectively. The values obtained for TPC, TF and carotenoid content of EEPP were 7.33 ± 0.34 mg GAE 100 g^{-1}, 5.41 ± 0.28 mg QE 100 g^{-1} and 45.10 ± 0.281 µg g^{-1}, respectively.

The protein content of the material indicates its film-forming ability (Lalnunthari *et al*., 2019). According to Alqahtani *et al*. (2021) and Franscisco *et al*. (2024), when incorporated into biodegradable films, plant extracts increase the concentration of active compounds and improve their antioxidant properties.

3.1 CHARACTERIZATION OF BIODEGRADABLE FILMS (BFs)

3.1.1 Visual assessment

Figure 1 presents images of the biodegradable gelatin-based films with the addition of different concentrations of DPSF and addition of EEPP (as Table 1) and the micrographs of each formulation.

In general, the FBs were easy to handle, however, with the increase in the concentration of DPSF, the formulations became heterogeneous, possibly due to the lower solubilization of the flour.

Daudt *et al*. (2016) reported that starch-based biodegradable films, after the addition of pinhão flour, showed irregularities, which were attributed to the presence of components such as lipids, fibers and proteins in the polymer matrix, which despite being present in small concentrations can influence the final structure of the film. However, from Figure 1 it appears that the incorporation of FDSA into gelatin films is possible, as reported by Soo & Sarbon (2018) that incorporated rice flour into gelatin-based films and indicated that these films could be an alternative to edible films for possible application in the food industry.

3.1.2 Thickness, moisture, solubility, color parameters, UV-VIS barrier and transparency

Table 2 shows the results of thickness, moisture, solubility, color parameters, UV-VIS barrier and opacity of the FBs produced. From the data presented in this table, it can be seen that the different combinations of ingredients did not influence the thickness of the BFs. According to Boeira *et al*. (2022) one of the major challenges in producing biodegradable films is controlling the thickness, since the high concentration of polymers makes it difficult to homogenize the material after drying.

From the data in Table 2, it can be seen that the different combinations used in the formulations did not influence the moisture content of the FBs, whose values were similar to those found by Borges *et al*. (2016) for films based on gelatine and lecithin.

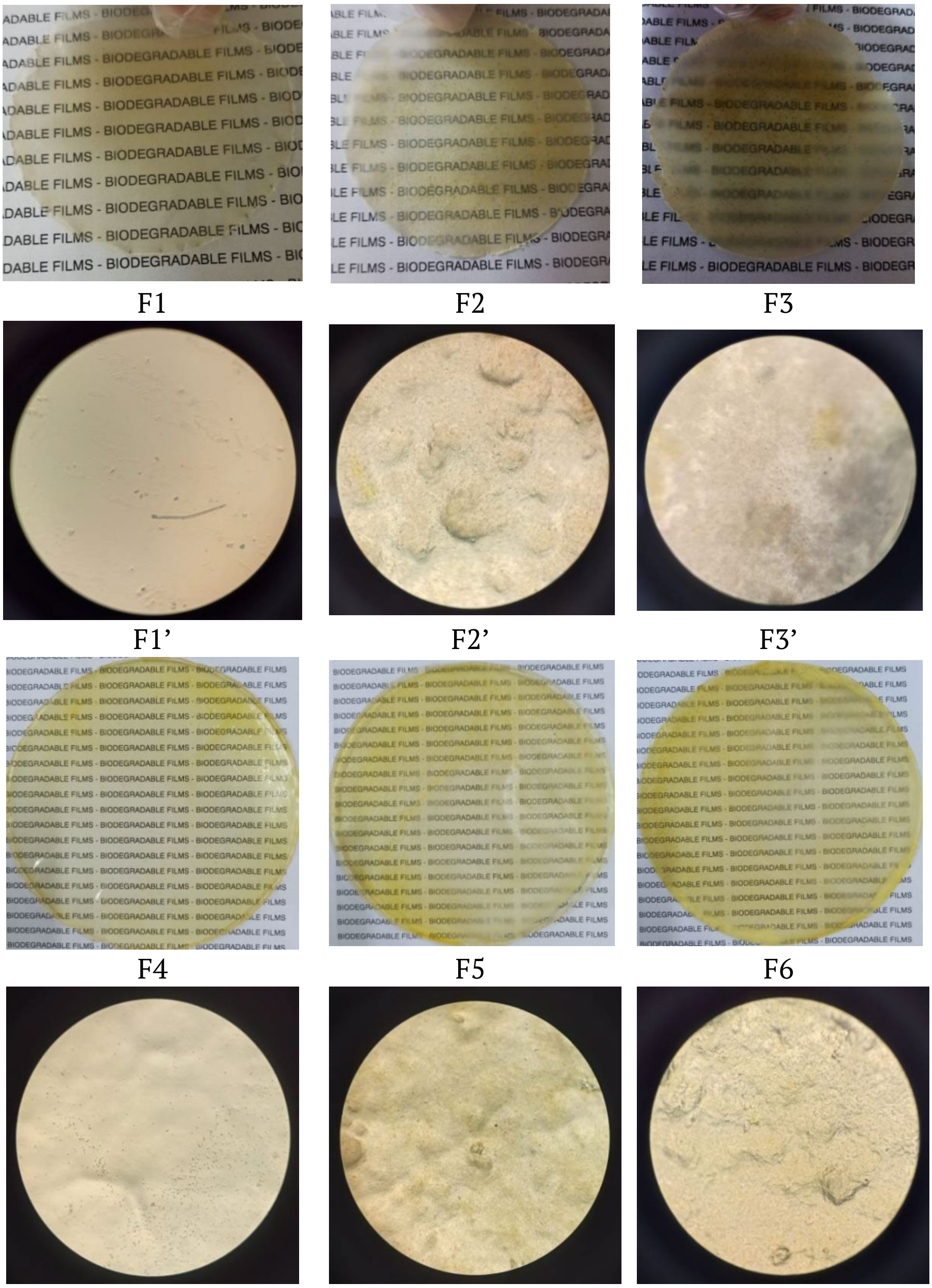

Figure 1. Images of gelatin-based biodegradable films with and without the addition of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP). Formulation (as Table 1). F1’, F2’, F3’, F4’, F5’ and F6’ correspond to the micrographs

Table 2. Moisture, solubility, color parameters and thickness of the gelatin-based biodegradable films in the different formulations evaluated

Property		Formulation[1]					
		F1	**F2**	**F3**	F4	F5	F6
Thickness (mm)		0.162±0.011^a	0.164±0.012^a	0.167±0.006^a	0.166±0.013^a	0.168±0.012^a	0.169±0.010^a
Moisture (wt%)		13.49±1.42^a	13.02±1.83^a	11.78±1.27^a	14.20±0.71^a	13.59±1.19^a	13.74±0.86^a
Solubility (%)		43.63±4.19^a	58.26±1.75^{bc}	56.36±4.16^{bc}	47.57±9.25^{ab}	60.25±9.97^c	59.91±2.79^c
Color parameter	**L***	87.87±0.49^d	88.01±0.55^d	86.26±0.63^{ab}	87.30±0.23^{cd}	86.49±0.46^{bc}	85.39±1.00^a
	a*	1.10±0.03^e	-0.54±0.21^d	-1.25±0.11^c	-4.47±0.31^b	-4.98±0.43^a	-5.15±0.19^a
	b*	-0.72±0.17^a	2.29±0.93^b	8.23±2.37^c	14.91±1.34^d	19.64±1.24^e	21.72±7.90^e
	h°	-31.97±5.81^c	-80.46±8.54^{ab}	-81.85±3.30^a	-73.27±0.48^b	-75.93±0.47^{ab}	-76.92±0.84^{ab}
	C*	1.31±0.12^a	2.08±0.97^a	8.75±2.48^b	15.65±1.37^c	20.46±1.38^d	22.91±1.80^d
Transparency (%)		0.61±0.06^a	4.25±0.11^b	5.56±0.16^d	0.82±0.07^a	4.92±0.27^c	5.83±0.19^d

[1] according to Table 1. Means followed by the same lowercase letter (in each line) do not differ statistically ($p>0.05$)

With the incorporation of DPSF into the FBs, an increase in their solubility was observed, possibly due to the increase in their hydrophilic characteristics. According to Nouraddini *et al.* (2018), who produced films based on eggplant flour and corn starch, the different solubility values are correlated with the presence of hydrophilic groups present in the flour, such as fibers and proteins.

The L* value, which consists of the brightness of the samples, was influenced by the incorporation of DPSF and EEPP. However, the a* value increased after the incorporation of EEPP. The addition of FDSA and ECCA to the FBs caused an increase in the b* chroma value, which corresponds to a tendency towards yellow, due to the characteristic color of DPSF and EEPP, which can be seen visually (Figure 1). Pelissari *et al.* (2013) and Daudt *et al.* (2016) who produced banana and pine nut flour films, respectively, also reported an increase in the b* parameter due to the color of the flour used.

The incorporation of DPSF influenced the values of h° which consists of the matrix angle that represents the qualitative parameter of color and C* which corresponds to the chroma index (C*), which is the quantitative attribute of color. According to Sood & Saini (2022), the C* parameter represents the vividness of the color and films that have a darker color are of interest for use in food products that are sensitive to light.

Transparency (referring to light transmission) and opacity are inversely related, so the higher the transparency, the lower the opacity (Sood & Saini, 2022). According to Deshmukh *et al.* (2021) films with greater opacity can be used as potential materials against the photo-oxidation of food. Thus, as reported in Table 2, F3 and F6 were the films with the highest opacity. The opacity values shown in Table 2 demonstrate that the light transmission and opacity of the FBs were influenced by the addition of DPSF, which may be related to the increase in solids in the formulation.

Figure 2 shows the results for the ultraviolet/visible (UV/Vis) barrier of the FBs obtained, where it can be seen that the formulations with added DPSF showed a better barrier when compared to the gelatine-only formulations (F1 and F4), possibly due to the heterogeneity and opacity of the films. The increase in the barrier is justified by the heterogeneity of the films and their opacity (Li *et al.*, 2021), and the relationship between film opacity and UV/VIS barrier protection was also reported by Rodrigues *et al.* (2020). Given that packaged foods are often exposed to artificial and natural lighting, and that this can alter their properties and even influence their shelf life and quality, it is necessary for packaging to have UV-blocking properties in order to protect photosensitive foods (Aguirre *et al.*, 2021).

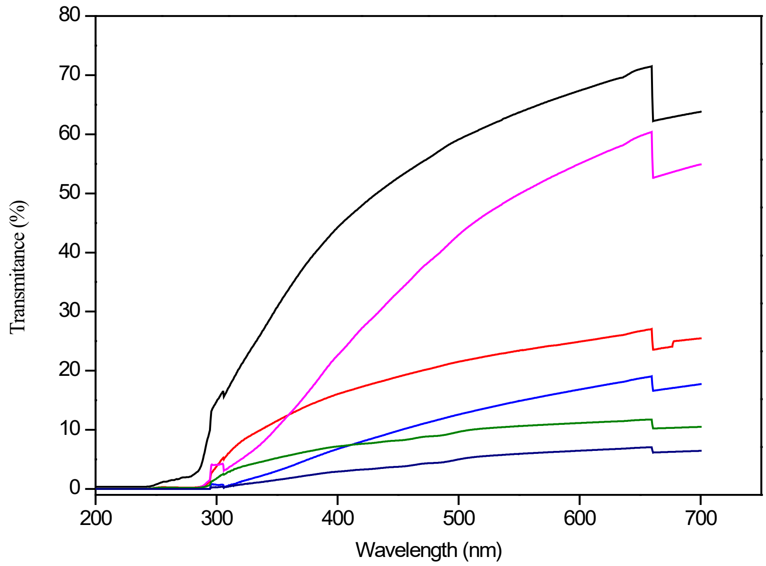

Figure 2. Ultraviolet/Visible (UV/Vis) barrier properties of gelatin-based biodegradable films with the addition of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP) Formulation (as Table 1): F1 ( ), F2 ( ), F3 ( ), F4 ( ), F5 ( ) and F6 ( )

The results of the FTIR analysis of the FBs can be seen in Figure 3, and it can be seen that the structure was not influenced by the different combinations evaluated. The spectra showed bands typical of gelatine-based films at 3266 cm^{-1} (Amide A), 1632 cm^{-1} (Amide I), 1524 cm^{-1} (Amide II), 1229 cm^{-1} (Amide III) and soy lecithin at 2918 cm^{-1}, 2848 cm^{-1} and 1730 cm^{-1} (Borges *et al.*, 2016).

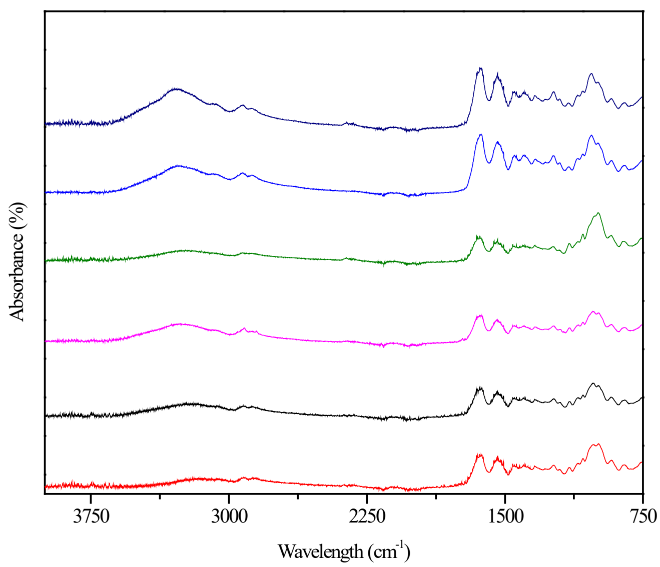

Figure 3. Fourier transform infrared spectroscopy (FTIR) analysis of gelatin-based biodegradable films with the addition of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP). Formulation (as Table 1): F1 ( ), F2 ( ), F3 ( ), F4 ( ), F5 ( ) and F6 ( )

Table 3 shows the TPC and TF content present in FBs developed with the addition of EEPP (F4, F5 and F6), where the increase in these contents with the addition of DPSF can be seen, this is due to the presence of these compounds in the by-product added in the formulation of the films. The results of the phenolic compounds present in the FBs indicate antioxidant properties and suggests the potential to be applied in foods to prevent oxidation reactions and consequently increase the shelf life of products.

Table 3. Contents of total phenolic compounds (TPC) and total flavonoids (TF) of gelatin-based biodegradable film with the addition of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP)

Formulation[1]	TPC content (mg per 100 g film)	TF content (mg per 100 g film)
F4	19.38 ± 0.65[a]	32.95 ± 2.08[a]
F5	29.92 ± 0.82[b]	32.29 ± 0.46[a]
F6	81.18 ± 0.29[c]	42.87 ± 1.20[b]

[1]according to Table 1. Means followed by the same lowercase letter (in each line) do not differ statistically ($p>0.05$)

3.1.3 Biodegradability

Figure 4 shows the biodegradability of gelatin-based biodegradable films with the addition of DPSF and EEPP, performed to simulate natural biodegradation conditions and expose the film to the action of a mixed microflora present in organic soil. From this Figure, after 3 days of the test, it is possible to observe that the FBs began to show changes in their integrity, suggesting the beginning of decomposition, however, after 15 days, regardless of the formulation, the complete biodegradation of the films occurred. For films with the addition of DPSF and EEPP, complete biodegradation was observed after 11 days of incubation. It is possible that during this period there was a greater proliferation of microorganisms and the breaking of hydrogen bonds, facilitating the absorption of water and accelerating this process. According to Chen *et al*. (2021) stronger water absorption is more conducive to the growth of microorganisms and accelerates the biodegradation of films. In addition, the humidity of the soil also contributed to the biodegradation of the films (Stoll *et al*., 2016).

Chen *et al*. (2022) produced residual gelatine films and reported that in the initial stage of biodegradation, the films absorb water from the soil and the hydrogen bonds in the gelatine matrix are partially destroyed, resulting in a state of swelling, then the microflora of the mixture present in the natural soil gradually adheres to the surface of the films and carries out a slow attack process on the films until they are completely consumed, degrading into environmentally friendly zirconium sulphate, CO2 and H2O.

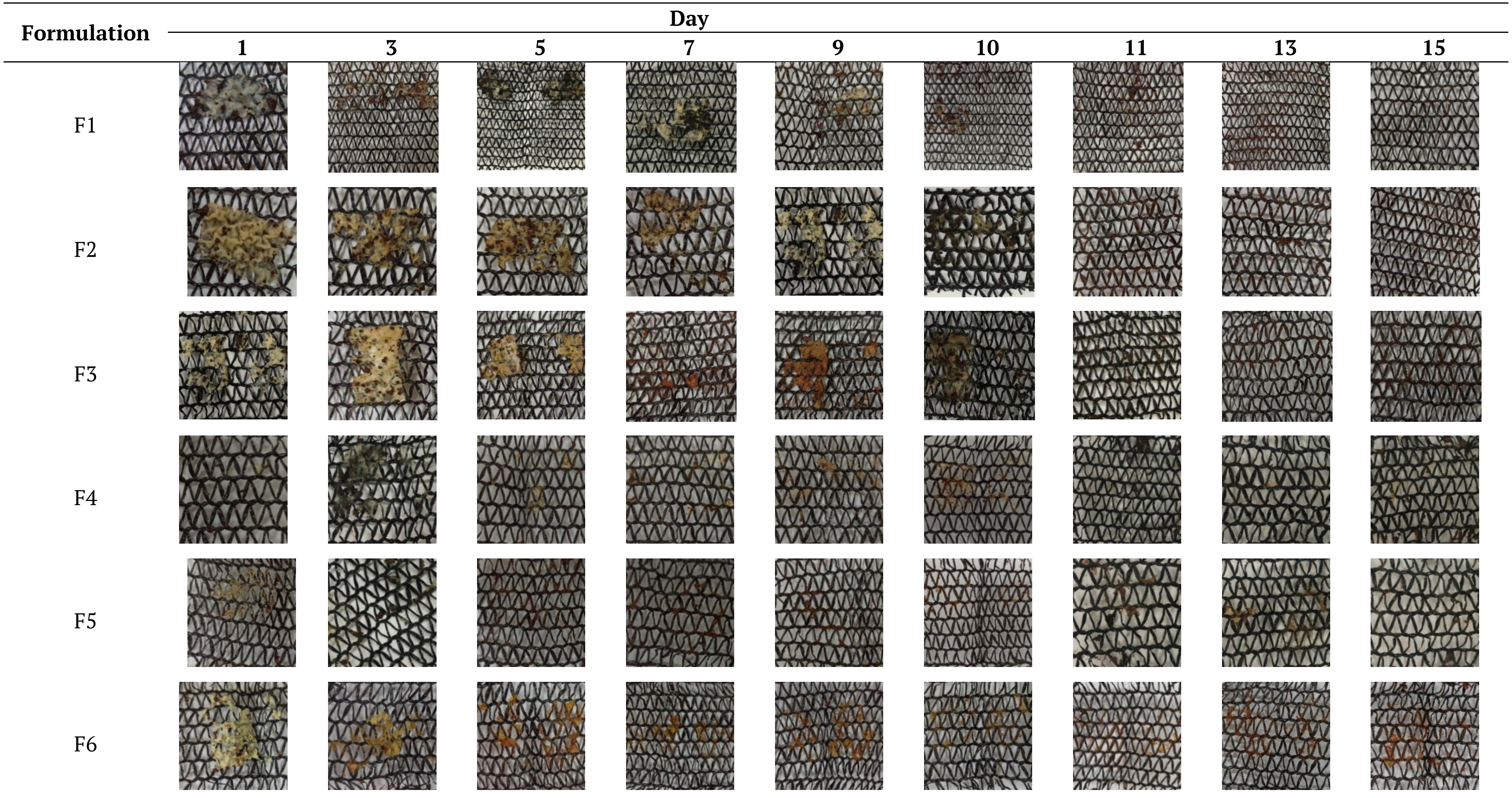

Figure 4. Biodegradability of gelatin-based biodegradable films with the addition of defatted pumpkin seed flour (DPSF) and ethanolic extract of pumpkin peels (EEPP). Formulation (as Table 1)

4 CONCLUSION

Aligning the use of agro-industrial by-products and the impacts caused by the use of synthetic packaging, in this study, gelatine-based films were developed with the incorporation of pumpkin processing by-products. All formulations presented good visual evaluation, however, formulations F3 and F6 were more heterogeneous. The incorporation of DPSF and EEPP improved the physical and mechanical properties of FBs, influencing color, humidity and solubility, UV/VIS barrier and biodegradability. Considering the visual evaluation and other analyzes carried out, the formulations that presented the best properties were those that were incorporated with DPSF and EEPP (F5 and F6), however F6 would need an improvement in its mechanical properties to be an alternative to becoming a packaging. Since the films are more opaque and because they contain antioxidant compounds, the FBs developed are suitable for foods that are sensitive to oxidation. Gelatin-based FBs incorporated with DPSF have the potential to be used in the development of food packaging that contains low humidity.

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REFERENCES

AGUIRRE, A.; MENDEZ, X.; BORNEO, R. Characterization and storage study of chickpea flour films with UV-barrier and Cu-remove properties. **App. Food Res.**, 100011, 2021. DOI: https://doi.org/10.1016/j.afres.2021.100011.

ALQAHTANI, N.; ALNEMR, T.; ALI, S. Development of low-cost biodegradable films from corn starch and date palm pits (Phoenix dactylifera). **Food Biosci.**, v. 42, 101199, 2021. DOI: https://doi.org/10.1016/j.fbio.2021.101199.

ASTM D5338-15. Método de teste padrão para determinar a biodegradação aeróbia de materiais plásticos sob condições de compostagem controladas, incorporando temperaturas termofílicas, **ASTM International, West Conshohocken**, PA, 2021.

BOEIRA, C. P.; FLORES, D. C. B.; ALVES, J. S.; MOURA, M. R.; MELO, P. T. S.; ROLIM, C. M. B.; NOGUEIRA-LIBRELOTTO, D. R.; ROSA, C. S. Effect of corn stigma extract on physical and antioxidant properties of biodegradable and edible gelatin and corn starch films. **Int. J. Biol. Macromol.**, Guildford, v. 208, p. 698-706, 2022. DOI: https://doi.org/10.1016/j.ijbiomac.2022.03.164.

BORGES, J. G.; SILVA, A. G.; CERVI-BITENCOURT, C. M.; VANIN, F. M.; CARVALHO, R. A. Lecithin, gelatin and hydrolzed collagen orally didintegrating films: functional properties. **Int. J. Biol. Macromol.**, Guildford, v. 86, p. 907-916, 2016. DOI: https://doi.org/10.1016/j.ijbiomac.2016.01.089.

CHEN, L.; QIANG, T.; CHEN, X.; REN, W.; ZHANG, H. J. Fabrication and evaluation of biodegradable multi-cross-linked mulch film based on waste gelatin. **Chem. Eng. J.**, Lausanne, v. 419, 129639, 2021. DOI: https://doi.org/10.1016/j.cej.2021.129639.

CHEN, L.; QIANG, T.; CHEN, X.; REN, W.; ZHANG, H. J. Gelatin from leather waste to tough biodegradable packaging film: One valuable recycling solution for waste gelatin from leather industry. **Waste Manag.**, Elmsford, v. 145, p. 10-19, 2022. DOI: https://doi.org/10.1016/j.wasman.2022.04.023.

CHEN, L.; GAO, J.; ZHANG, X.; LI, J.; ZHANG, Y.; QIANG, T. Octadecylamine modified gelatin-based biodegradable packaging film with good water repellency and improved moisture service reliability. **Int. J. Biol. Macromol**., Guildford, v. 279, 135218, 2024. DOI: https://doi.org/10.1016/j.ijbiomac.2024.135218.

CRIZEL, T. M.; RIOS, A. O.; ALVES, V. D.; BANDARRA, N.; MOLDÃO-MARTINS, M.; FLÔRES, S. H. Biodegradable films based on gelatin and papaya peqel microparticles with antioxidant properties. **Food Bioprocess. Tech.**, v. 11, p. 536-550, 2017. DOI: https://doi.org/10.1007/s11947-017-2030-0.

DAUDT, R. M.; AVENA-BUSTILLOS, R. J.; WILLIAMS, T.; WOOD, D. F.; KŸLKAMP-GUERREIRO, I. C.; MARCZAK, L. D. F.; MCHUGH, T. H. Comparative study on properties of edible films based on pinhão (Araucaria angustifolia) starch and flour**. Food Hydrocoll.**, Oxford, v. 60, p. 279-287, 2016. DOI: https://doi.org/10.1016/j.foodhyd.2016.03.040.

DESHMUKH, A. R.; ALOUI, H.; KHOMLAEM, C.; NEGI, A.; HUN, J-H.; KIM, H-S.; KIM, B. S. Biodegradable films based on chitosan and defatted Chlorella biomass: Functional and phyical characterization. **Food Chem.**, London, v. 337, 127777, 2021. DOI: https://doi.org/10.1016/j.foodchem.2020.127777.

DIAS, A. B.; MŸLLER, C. M. O.; LAROTONDA, F. D. S.; LAURINDO, J. B. Biodegradable films based on rice starch and rice flour. **J. Cereal Sci.**, London, v. 51, p. 213-219, 2010. DOI: https://doi.org/10.1016/j.jcs.2009.11.014xr5.

EVODE, N.; QAMAR, S. A.; BILAL, M.; BARCELÓ, D.; IQBAL, H. M. N. Plastic waste and its management strategies for environmental Sustainability. **Case Stud. Chem. Environ. Eng**., New York, v. 4, 100142, 2021. DOI: https://doi.org/10.1016/j.cscee.2021.100142.

FRANCISCO, A. P.; SGANZERLA, G. W.; CASTRO, L. E. N.; BARROSO, T. L. C. T.; SILVA, A. P. G.; ROSA, C. G.; NUNES, M. R.; FORSTER-CARNEIRO, T.; ROSTAGNO, M. A. Pressurized liquid extraction of bioactive compounds from grape peel and application in pH-sensing carboxymethyl cellulose films: A promising material to monitor the freshness of pork and milk. **Food Res. Int.**, Barking, v. 179, 114017, 2024. DOI: https://doi.org/10.1016/j.foodres.2024.114017.

FREITAS, T. S. M.; RODRIGUES, G. M.; FAKHOURI, F. M.; SILVA, C.; CARDOSO, C. A. L.; VELASCO, J. I.; FILGUEIRAS, C. T.; GARCIA, V. A. S. Application of the Box-Behnken experimental design for the extractios of phenolic compounds from araçá-roxo (Psidium myrtoides). **J. Food Process. Preserv.**, Westport, v. 45, e-15260, 2021. DOI: https://doi.org/10.1111/jfpp.15260.

GONTARD, N.; GUILBERT, S.; CUQ, J-L. Edible Wheat Gluten Films: Influence of the Main Process Variables on Film Properties using Response Surface Methodology. **J. Food Sci.**, Champaign, v. 57, p. 190-195, 1992. DOI: https://doi.org/10.1111/j.1365-2621.1992.tb05453.x.

GUTIÉRREZ, T. J.; GONZÁLEZ, G. Effects of exposure to pulsed light on surface and structural properties of edible films made from cassava and taro starch. **Food Bioprocess. Tech.**, v. 9, p. 1812–1824, 2016. DOI: https://doi.org/10.1007/s11947-016-1765-3.

GUTIÉRREZ, T. J. Are modified pumpkin flour/plum flour nanocomposite films biodegradable and compostable?. **Food Hydrocoll.**, Oxford, v. 83, p. 397- 410, 2018. DOI: https://doi.org/10.1016/j.foodhyd.2018.05.035.

HUSSAIN, A.; KAUSAR, T.; DIN, A.; MURTAZA, M. A.; JAMIL, M. A.; NOREEN, S.; REHMAN, H. U.; SHABBIR, H.; RAMZAM, M. A. Determination of total phenolic, flavonoid, carotenoid, and mineral contents in peel, flesh, and seeds of pumpkin (*Cucurbita maxima*). **J. Food Process. Preserv.,** Westport, v. 45, e15542, 2021. DOI: https://doi.org/10.1111/jfpp.15542.

KUMAR, Y.; SANTHOSH, R.; HOQUE, M.; NATH, D.; THAKUR, R.; MADHUBABU, D.; SURYAVANSHI, V.R.; SINGHI, H.; AHMED, J.; SARKAR, P. Development and characterization of defatted pumpkin seed meal and halloysite nanoclay composite films for food packaging. **Packaging Technology and Science**, v.36, p.715-727, 2023. DOI: https://doi.org/10.1002/pts.2751

LALNUNTHARI, C.; DEVI, L. M.; AMAMI, E.; BADWAIK, L. S. Valorization of pumpkin seeds and peels in to biodegradable packaging films. **Food Bioprod. Process**., v. 118, p. 58-66, 2019. DOI: https://doi.org/10.1016/j.fbp.2019.08.015.

LI, J.; JIANG, S.; WEI, Y.; LI, X.; SHI, S. Q.; ZHANG, W.; LI, J. Facile fabrication of tough, strong, and biodegradable soy protein-based composite films with excellent UV-blocking performance. **Compos. B**, v. 211, 108645, 2021. DOI: https://doi.org/10.1016/j.compositesb.2021.108645.

MASSA, T. B.; STEVANATO, N.; CARDOZO-FILHO, L.; SILVA. C. Pumpkin (Cucurbita máxima) by-products: Obtaining seed oil enriched with active compounds from the peel by ultrasonic-assisted extraction. **J. Food Process Eng.**, Westport, v. 42, e13125, 2019. DOI: https://doi.org/10.1111/jfpe.13125.

MUELLER. R-J. Biological degradation of synthetic polyesters-Enzymes as potential catalysts for polyester recycling. **Process Biochem.**, London, v. 41, p. 2124-2128, 2006. DOI: https://doi.org/10.1016/j.procbio.2006.05.018.

NASCIMENTO, T. A.; CALADO, V.; CARVALHO, C. W. P. Development and characterization of flexible film based on starch and passion fruitm esocarp flour with nanoparticles. **Food Res. Int.**, Barking, v. 49, p. 588-595, 2012. DOI: https://doi.org/10.1016/j.foodres.2012.07.051.

NOURADDINI, M.; ESMAIILI, M.; MOHTARAMI. Development and characterization of edible films based on egg plant flour and com starch. **Int. J. Biol. Macromol.**, Guildford, v. 120, p. 1639-1645, 2018. DOI: https://doi.org/10.1016/j.ijbiomac.2018.09.126.

ORSUWAN, A.; SOTHORNVIT, R. Development and characterization of banana flour film incorporated with montmorillonite and banana starch nanoparticles. **Carbohydr. Polym.**, Barking, v. 174, p. 235-242, 2017. DOI: https://doi.org/10.1016/j.carbpol.2017.06.085.

PELISSARI, F. M.; ANDRADE-MAHECHA, M. M.; SOBRAL, P. J. A.; MENEGALLI, F. C. Comparative study on the properties of flour and starch films of plantain bananas (Musa paradisiaca). **Food Hydrocoll.**, Oxford, v. 30, p. 681–690, 2013. DOI: https://doi.org/10.1016/j.foodhyd.2012.08.007.

RODRIGUES, G. M.; FILGUEIRAS, C. T.; GARCIA, V. A. S.; CARVALHO, R. A.; VELASCO, J. I.; FAKHOURI, F. M. Antimicrobial activity and GC-MS profile of cobaiba oil for incorporation into Xanthosoma mafaffa starch-based films. **Polym.**, v. 12, 2883, 2020. DOI: https://doi.org/10.3390/polym12122883.

RODRIGUEZ-AMAYA, D. B.; KIMURA, M. **Harvest Plushandbook for carotenoid analysis**. Harvest Plus Technical monograph series 2, Washington, DC e Cali: International Food Policy Research Institute (IFPRI) e International Center for Tropical Agriculture (CIAT), 2004.

SILVA, M. L. T.; BRINQUES, G. B.; GURAK, P. D. Desenvolvimento e caracterização de bioplásticos de amido de milho contendo farinha de subproduto de broto. **Braz. J. Food Technol.**, Campinas, v. 23, e2018326, 2020. DOI: https://doi.org/10.1590/1981-6723.32618.

SINGLETON, V. L.; ORTHOFER, R.; LAMUELA-RAVENTÓS, R. Analysisof total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. **Methods Enzymol.**, New York, v. 299, p. 152-178, 1998. DOI: https://doi.org/10.1016/S0076-6879(99)99017-1.

SOLANO, A. C. V.; GANTE, C. R. (2014). Development of biodegradable films based on blue corn flour with potential applications in food packaging. Effects of plasticizers on mechanical, thermal, and microstructural properties of flour films. **J. Cereal Sci.**, London, v. 60, p. 60-66, 2014. DOI: https://doi.org/10.1016/j.jcs.2014.01.015.

SOOD, A.; SAINI, C. S. Red pomelo peel pectin based edible composite films: Effect of pectin incorporation on mechanical, structural, morphological and thermal properties of composite films. **Food Hydrocoll.**, Oxford, v. 123, 107135, 2022. DOI: https://doi.org/10.1016/j.foodhyd.2021.107135.

SOO, P. Y.; SARBON, N. M. Preparation and characterization of edible chicken skin gelatin film incorporated with rice flour. **Food Packag. Shelf Life**, v. 15, p. 1-8, 2018. DOI: https://doi.org/10.1016/j.fpsl.2017.12.009.

STOLL, L.; SILVA, A. M.; IAHNKE, A. O. S.; COSTA, T. M. H.; FLORES, S. H.; RIOS, A. O. Active biodegradable film with encapsulated anthocyanins: Effect on the quality atributes of extra-virgin olive oil during storage. **J. Food Process. Preserv.**, Westport, v. 41, e13218, 2016. DOI: https://doi.org/https://doi.org/10.1111/jfpp.13218.

TAPIA-BLÁCIDO, D. R.; SOBRAL, P. J. A.; MENEGALLI, F. C. Effect of drying conditions and plasticizer typeon some physical and mechanical properties of amaranth flour films**.** **LWT,** v. 50, p. 392-400, 2013. DOI: https://doi.org/10.1016/j.lwt.2012.09.008.

WANI, A. A.; SOGI, D. S.; GROVER, L.; SAXENA, D. C. EFFECT of temperature, alkali concentration, mixing time and meal/solvent ratioon the extraction of water melon seed proteins-a response surface approach. **Biosyst. Eng.**, London, v. 94, p. 67-73, 2006. DOI: https://doi.org/10.1016/j.biosystemseng.2006.02.004.

ZHANG, H.; SABLANI, S. Biodegradable packaging reinforced with plant-based food waste and by-products. **Curr. Opin. Food Sci.**, v. 41, p. 61-68, 2021. DOI: https://doi.org/10.1016/j.cofs.2021.05.003.

ZHANG, Q.; ZHANG, J.; PING, Q.; SUI, Z.; LI, H. Performance comparison of glycerol-plasticized type A and type B gelatin films and their suitability for food packaging. **Polym.**, v. 343, 129389, 2026. DOI: https://doi.org/10.1016/j.polymer.2025.129389.

ZHONG, Y.; GODWIN, P.; JIN, Y.; XIAO, H. Biodegradable polymers and green-based antimicrobial packaging materials: **A mini-review. Adv. Ind. Eng. Polym. Res.**, v. 3, p. 27-35, 2020. DOI: https://doi.org/10.1016/j.aiepr.2019.11.002.