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Effect of *amaranthus viridis* e *bidens pilosa* leaves extract on properties of soy protein-locust bean gum active films

Efeito do extrato das folhas de Amaranthus viridis e Bidens pilosa nas propriedades dos filmes ativos de proteína de soja e goma de alfarroba

Efecto del extracto de hoja de amaranthus viridis y bidens pilosa sobre las propiedades de las películas activas de proteína de soya y goma de algarrobo

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Agatha Natasha Melo da Silva
ORCID: https://orcid.org/0000-0003-1469-4376
State University of Maringá, Brazil
E-mail: ra110512@uem.br
Laís Ravazzi Amado
ORCID: https://orcid.org/0000-0002-0069-102X
São Paulo State University, Brazil
E-mail: laisravazziamado@gmail.com
Beatriz Cervejeira Bolanho Barros
ORCID: https://orcid.org/0000-0003-1191-4740
State University of Maringá, Brazil
E-mail: beatrizbolanho@yahoo.com.br
Otavio Akira Sakai
ORCID: https://orcid.org/0000-0002-3502-5107
Federal Institute of Paraná, Brazil
E-mail: otavio.sakai@ifpr.edu.br
Angela Maria Picolloto
ORCID: https://orcid.org/0000-0002-8969-9844
State University of Maringá, Brazil
E-mail: angela.picolloto@gmail.com
Keila de Souza Silva
ORCID: https://orcid.org/0000-0002-9718-1826
State University of Maringá, Brazil
E-mail: kssilva@uem.br

Abstract
The present study aimed to manufacture active biodegradable films from soy protein isolate (SPI), locust bean gum (LBG) and caruru and blackjack leaf extract. The interaction between the compounds was evaluated through the analysis of FTIR. The effect of extract addition on the microstructure, mechanical properties, antioxidant capacity, UV radiation absorption capacity, color and opacity of the film were evaluated. All extracts interacted with the protein, however only two treatments interacted with the LBG present in the film. Addition of extracts in the film provided an increase in antioxidant activity. The addition of the blackjack extract increased the tensile strength of the films, but reduced their elasticity. The addition of caruru extract, however, not favoring the resistance of the films. All the extracts darkened the films and the caruru extract provided more greenish films than all the other made films. The films containing blackjack extract presented a higher intensity of light absorption in the spectral range of ultraviolet A and C.
Keywords: Active films; BlajJack extract; Caruru extract; Soy protein isolate film.

Resumo
O presente estudo teve como objetivo a fabricação de filmes biodegradáveis ativos a partir de isolado protéico de soja (SPI), goma de alfarroba (LBG) e extrato de folhas de caruru e blackjack. A interação entre os compostos foi avaliada através da análise de FTIR. Avaliou-se o efeito da adição do extrato na microestrutura, propriedades mecânicas, capacidade antioxidante, capacidade de absorção da radiação UV, cor e opacidade do filme. Todos os extratos interagiram com a proteína, porém apenas dois tratamentos interagiram com o LBG presente no filme. A adição de extratos no filme proporcionou um aumento na atividade antioxidante. A adição do extrato de blackjack aumentou a resistência à tração dos filmes, mas reduziu sua elasticidade. A adição de extrato de caruru, porém, não favoreceu a resistência dos filmes. Todos os extratos escureceram os filmes e o extrato de caruru proporcionou filmes mais

esverdeados do que todos os outros filmes feitos. Os filmes contendo extrato de blackjack apresentaram maior intensidade de absorção de luz na faixa espectral do ultravioleta A e C.
Palavras-chave: Filmes ativos; Extrato de blakjack; Extrato de caruru; Filme isolado de proteína de soja.

Resumen
El presente estudio tuvo como objetivo fabricar películas biodegradables activas a partir de proteína aislada de soya (SPI), goma de algarrobo (LBG) y extracto de hojas de caruru y blackjack. La interacción entre compuestos se evaluó mediante análisis FTIR. Se evaluó el efecto de la adición del extracto sobre la microestructura, propiedades mecánicas, capacidad antioxidante, capacidad de absorción de radiación UV, color y opacidad de la película. Todos los extractos interactuaron con la proteína, pero solo dos tratamientos interactuaron con la LBG presente en la película. La adición de extractos en la película proporcionó un aumento en la actividad antioxidante. La adición de extracto de blackjack aumentó la resistencia a la tracción de las películas, pero redujo su elasticidad. La adición de extracto de caruru, sin embargo, no favoreció la resistencia de las películas. Todos los extractos oscurecieron las películas y el extracto de caruru proporcionó más películas verdes que todas las demás películas hechas. Las películas que contenían extracto de blackjack mostraron una mayor intensidad de absorción de luz en el rango espectral de ultravioleta A y C.
Palabras clave: Películas activas; Extracto de blakjack; Extracto de caruru; Película aislada de proteína de soya.

1. Introduction

Natural polymers as proteins and polysaccharides have been studied for several researchers as an alternative to manufacture biodegradable package and to reduce the use of non-degradable and non-renewable materials in packaging industry (Silva *et al*., 2018; Amado, 2019; Silva *et al*., 2016). Some researchers have related that this biodegradable package also can serve as carrier of antioxidant compounds aiming to improve the capacity of prevent oxidative degradation in food (Ahmed *et al*., 2017; Han *et al*., 2018).

Locust Bean Gum (LBG) is a polysaccharide extracted from the seed endosperm of the carob tree plant that presents ability to form very viscous and stable aqueous solution at relatively low concentration (Dakia *et al.*, 2008). Soy protein is a cheap and abundant raw material, obtained from residues generated by the industries producing soybean oil (Oliveira, 2021). Both locust bean gum and soy protein have been used to form edible films due to ability to provide a good barrier to oxygen Cho *et al.* (2007) and Ciannamea (2014), also, serve as carrier of additives and bioactive components (Barak and Mudgil, 2014; Yuan *et al.*, 2017; Adilah, 2018).

Some researchers have indicated that polysaccharide-protein complexes result in films with better properties than films containing polysaccharides or proteins separately (Silva *et al.*, 2018; Amado, 2019). Silva *et al.*, (2016) observed that LBG addition increased the elasticity and reduced oxygen and light permeability of the films based on whey protein isolate.

Interest in the use of natural antioxidants has gained more and more space due to the growing concern of consumers with their health Chang-Bravo (2014). Plant extracts have been seen as a safe, natural and low-cost source of antioxidants when compared to synthetic antioxidants, which can have a toxic effect C̆anadanovic´-Brunet (2006). Natural plant extracts have a high content of natural bioactive compounds, which have antioxidant activity and are capable of affecting the behavior of many cellular systems (Rawel *et al.*, 2002). When used in the preparation of biodegradable films it can result in active films with the ability to prevent or limit oxidation in packaged foods (Domínguez-Pacheco, 2017; Han *et al*., 2018).

Some properties of biodegradable films, such as structural and mechanical properties can be influenced by the addition of active substances Benbettaïeb (2017). Bioactive compounds added to biodegradable films could act as a cross-linkers interacting with the functional groups of proteins (Rawel *et al.*, 2002) and, this way, they could influence on functional and physical properties of films according to their nature and concentration (López-Hernández *et al*., 2018). Adilah (2018), for instance, observed that films-based soy protein isolate become stronger with the addition of mango kernel extract. (Nabilah *et al.*, 2021) observed that mangosteen (*Garcinia mangostana* L.) pericarp extract (MPE) addition increased the antioxidant

properties of the fish gelatin, soy protein isolates, and corn starch films, however the highest value of DPPH-scavenging activity was observed in SPI films due to the bioavailability of polyphenol compounds in soy protein film. Moreover, the antioxidant properties of phenolic acids, combined to oxygen barrier of the films can reduces oxidation reactions of the food packaged by these films (Han *et al.*, 2018).

The presence of antioxidants in extracts from *Bidens pilosa* L. and *Amaranthus viridis* L. was previously reported (Krishnaiah, 2011).

Bidens pilosa (blackjack) of the *Asteraceae* family, is present in almost all of Brazil, mainly in the South and Midwest regions and is a great source of phytochemicals, rich in phenolic compounds, protein, magnesium and high copper content (Kelen *et al.*, 2015). (Cortes-Rojas *et al.*, 2013) verified the antioxidant activity of the hydroethanolic extract prepared by different extraction methods, manipulating different parts of the plant, such as flowers / leaves, stem and root. The study showed that the extracts of the leaves / flowers of blackjack have better antioxidant activity than root extract.

Amaranthus viridis L., known as caruru in Brazil, contains various nutrients such as Vitamin A, Vitamin B, Vitamin C and minerals such as calcium, phosphorus and iron Sowjanya (2014). *A. viridis* extract is indicating as natural antioxidant due to its good radical scavenging activity.

Bidens pilosa and *Amaranthus viridis* L. are highly invasive plant species, considered as weed infestation in agricultural fields (Kissmann, 1997; Satorre *et al.*, 2020). Effective weed control is essential to reduce huge economic losses and low quality crop yields and, for this reason, herbicides are used to control the growth of blackjack and caruru in fields. The employment of *Bidens pilosa* and *Amaranthus viridis* extract in complex films based on soy protein + LBG can be an alternative to obtain biodegradable active films, encourage a commercial application for these weed and consequently reduce the use of herbicides in fields.

The aim of this work was to develop biodegradable active films with SPI + LBG + extract of two plant species (*Bidens pilosa* or *Amaranthus viridis L.*) and evaluate the effect of extract addition on the microstructure, mechanical and antioxidant properties, UV radiation absorption capacity and opacity of the packaging.

2. Methodology

2.1 Materials

Soy protein isolate (SPI) with 91% total protein, 6.5% of moisture, 3.5% ash, 0.4% fat, approximately, was kindly supplied by Maxsoy® fibras & ingredientes (Hortolândia, São Paulo, Brazil). Locust bean gum (LBG) (GRINDSTED ® LBG 246, São Paulo, Brazil) was kindly supplied by Danisco (Cotia, Brazil). Glycerol was supplied by Sigma, Co (St. Louis MO, USA).

2.2 Plant extracts

Bidens pilosa (blackjack) and amaranthus viridis l. (caruru) leaves, both collected on land in the city of Umuarama (Paraná, Brazil), located at 23 ° 45'59.0 "s 53 ° 19'30.0" w (world geodetic system, 1984), were sanitized, cut and dried in a drying oven with air circulation (marconi ma035) at 37 ° c for 22 hours and then macerated until powder was obtained. the species identified by dr. milaneze-gutierre m.a. in a copy of voucher (number 36367 for bidens pilosa and 36364 for amaranthus viridis l) were deposited in the herbarium huem, in Maringá, Paraná, Brazil.

Extraction of bioactive compounds from the leaves was performed using hydroethanolic solvent (50% ethanol), ratio leaf: solvent of 1:10, for 30 minutes at 25 ° C in an ultrasonic bath (ECO-SONICS, Q3.8, 40 kHz, 88 Watts, São Paulo, SP).

The extracts obtained were centrifuged (HERMLE, Z 326 K - Germany) at 3000 rpm for 5 minutes. The supernatant was collected and added to film-forming solution.

2.3 Film preparation

An aqueous stock solution of 1% (w/w) LBG was prepared by stirring at 25°C for 1hour and after heated at 80°C for 30 minutes.

The aqueous filmogenic solution was prepared with 5% (w/w) soy protein isolate (SPI), 0.4% (w/w) of the LBG stock solution and 2% (w/w) glycerol. The pH was adjusted to 11 with NaOH (20%) and then the solution was heated at 65ºC for 10 minutes in thermostatic bath (Symmetric, SI/6Aneis/18L, Brazil) to complete dissolution of soy protein [23, 24]. Then, the mixtures were stirred at room temperature for 1 hour and after thermally treated at 80 °C for 20 minutes in thermostatic bath (Symmetric, SI/6Aneis/18L, Brazil) to denature the protein fraction. Next, solutions were cooled until 25 °C to insert the extract. Two concentrations of Caruru (C) and Blackjack (B) extract were tested: 5% (5C, 5B) and 7% (7C, 7B) (w/w). SPI-LBG film without extract addition was adopted as control. The solutions were distributed in plastic trays and dried in an oven at 37 °C. SPI film, without LBG and extract, was manufactured and analyzed aiming to understand how LBG addition influences the interaction between extract and SPI and how this interaction influences the mechanical properties, microstructure and photoacoustic films.

Dried films with and without extract were equilibrated in desiccator contained magnesium nitrate- 6-hydrate saturated solution (53% relative humidity) at 25°C for one week before carry out the analyses.

2.4 Fourier-transform infrared spectroscopy analysis

Spectra analysis of the SPI films and SPI-LBG films with and without extracts were carried out in the infrared spectrophotometer, model Cary 630 FTIR, brand Agilent Technologies, using an attenuated total reflectance accessory (ATR) with a diamond ATR crystal. All the spectrum was performed between 4000 to 400cm^{-1}, scanning 64 scans and 4cm^{-1} precision. Analyzes were performed in triplicate.

2.5 Microstructure

The microstructure of the SPI films and SPI-LBG films with and without extract (caruru and blackjack) were studied by Scanning Electron Microscopy – SEM (SEI model Quanta 250) using a working distance (WD) between 9.5 mm and an accelerating voltage of 5 kV. Protein films were coated with a sputtered carbon thin film and analyzed at 1000× magnification.

2.6 Mechanical properties

Mechanical properties of the SPI films and SPI-LBG films, with and without extracts addition, were evaluated at 25 °C according to standard method ASTM D882-12 (ASTM D882-12, 2012) using a texture analyzer (TA.XT Plus, Stable Micro Systems, Surrey, UK). Rectangular samples (130 mm x 25 mm) preconditioned (53% UR) were cut and fixed by grips, apparent samples was 100 mm x 25 mm and the initial distance between grips was 80 mm. During the traction tests the speed was fixed at 0.8 $mm \cdot s^{-1}$. Maximum strength, elongation-at-break and Young's modulus were obtained using software `s EXPONENT, version 5.1.1.0.

2.7 Determination of total phenolic compounds (TPC) and antioxidant activity (AA)of the films

The extraction procedure of the SPI-LBG films with and without extracts addition was performed in triplicate, in a proportion of 1:10 (w/v) of film:hydroethanolic solvent (50% ethanol). The cut film was mixed with extracting solution and crushed using a Turratec equipment (Tecnal, TE-102 model—Piracicaba, Brazil) for 5 minutes, then taken to the ultrasonic bath (LojaNetLab SSBu – 3,8 L, 100 W) for 30 minutes at 25°C. The solution was centrifuged (HERMLE, Z 326 K – Germany) at 3000 rpm for 5 minutes and the collected supernatant was standardized in volumetric flasks and packed in refrigerated flasks until the day of TPC and AA analysis.

Quantification of total phenolic compounds (TPC) was performed using the Spectrophotometric method of Folin-Ciocalteu, as described by Fabra *et al.*, (2018) with some modifications. The analysis was performed by mixing 0.5 mL of the extract, 2.5 mL of the aqueous solution Folin-Ciocalteau 10%- and 2-mL sodium carbonate aqueous solution (7.5%). The mixture was homogenized and incubated at 50 °C for 5 minutes in a thermostatic bath (Symmetric, SI/6Aneis/18L, Brazil). The absorbance was measured at 760 nm in a spectrophotometer (UV - VIS Femto, 700 Plus), using a mixture of 0.5 ml of water, 2.5 mL of Folin-Ciocalteau 10% and 2 mL of sodium carbonate aqueous solution (7.5%). All results of this analysis were expressed in mg gallic acid/ g film.

Determination of antioxidant activity of the films, the methods 2.2-defenil-1-picril-Hidrazil (DPPH) and the ferric antioxidant power reducer (FRAP) were performed.

The determination of the antioxidant capacity of the films by the DPPH method was carried out according to Rodríguez *et al.*, (2020), with adaptations. The analysis was performed mixing 0.1 mL of the extract with 3.9 mL of 0.1 mM DPPH in ethanol solution. The mixture was homogenized and left to stand for 30 minutes in the absence of light, and then its absorbance was measured at 517 nm in a spectrophotometer (UV - VIS Femto, 700 Plus).

To assess antioxidant capacity of films using FRAP method, the methodology described by Ekrami *et al*., (2019) was used, with modifications. The analysis was performed mixing 90 µL of the extract with 2.7 mL of the reagent FRAP (25 ml of 0.3 mM acetate buffer pH 3.6; 2.5 ml of 10 mM TPTZ solution in 40 mM HCl; 2.5 ml of 20 mM ferric chloride) and 0.27 mL of distilled water, in the absence of light. The mixtures were incubated at 37 °C for 30 minutes in a water bath and after cooling, the absorbance readings were taken at 595 nm in a spectrophotometer (UV - VIS Femto, 700 Plus) using the FRAP reagent as white.

The analytical curves of DPPH and FRAP methods were prepared with Trolox solutions with concentrations between 0.05 to 0.70 mM and 0.20 to 0.75 mM, respectively, and the results were expressed in µM of Trolox per g of film.

2.8 Photoacoustic Spectroscopy (PAS)

Photoacoustic spectroscopy was used to study the wavelengths (λ) of the absorption bands of the SPI films and SPI-LBG films with and without extracts. The spectra were found in the emission range of ultraviolet (UV) and visible (VIS), with wavelength variation from 200 to 800nm. The power of the xenon arc lamp (Oriel, model 68820) used in the experiment was equal to 700 W. The emitted radiation was modulated by a mechanical chopper (SR 540), which in turn generates a reference signal of the modulation frequency through a stable rotating paddle. The selected modulation frequency corresponded to 18 Hz. Modulated light was diffracted, for each λ selected by a scanning monochromator (Oriel Instruments, model 77250) in the Oriel 77296 grid for ultraviolet-visible. The light is transmitted to a diffraction crack 3 mm thick, reaching a sample located inside the photoacoustic cell. The emergence of higher diffraction orders is annihilated using band filters.

The modulated light when striking the sample produces a pressure variation, generating the photoacoustic signal. This signal is transmitted to the *lock-in* preamplifier by means of the microphone (Brüel & Kjaer, model BK 2669) coupled to the photoacoustic cell generating data of the magnitude of the photoacoustic signal and the phase that are sent to a microcomputer.

The photoacoustic spectra must be normalized by the emission spectrum of the lamp, since it does not emit equally in all wavelengths. Conventionally, radiation spectral are normalized with perfectly absorbing samples, such as ultrapure coal (Coelho *et al.*, 2006; Coelho *et al*., 2010). In this work, it was used the crucible graphite charcoal produced in the laboratories of the spectroscopy group of the Department of Physics of the State University of Maringá.

2.9 Optical properties

Color measurement was evaluated in five replicates using a colorimeter (Konica Minolta, model CR-400) previously calibrated on white surface. The response was expressed in the form of the parameters L* (lightness or darkness), a* (redness or greenness) and b* (yellowness or blueness).

The visible light barrier properties of the SPI-LBG films with and without extract were measured in triplicate, using a quartz colorimetric cell and an UV-VIS (GENESYS 10S UV-VIS, Thermo Scientific, EUA), according to Eq. (1).

$$op = \frac{A600}{X} \quad (1)$$

where Op is opacity (mm^{-1}); A600 is absorbance at 600 nm; X is mean thickness of films (mm).

2.10 Film thickness

Thickness of the films was measured using a digital micrometer (Mitutoyo, Japan).

2.11 Statistical analyses

The data expressed in mean ± standard deviation were statistically analyzed by an analysis of variance (ANOVA) and Tukey's test at a 5% significance level, using Statistic 7.0 software version 7.0 (StatSoft, Inc, Tulsa, USA).

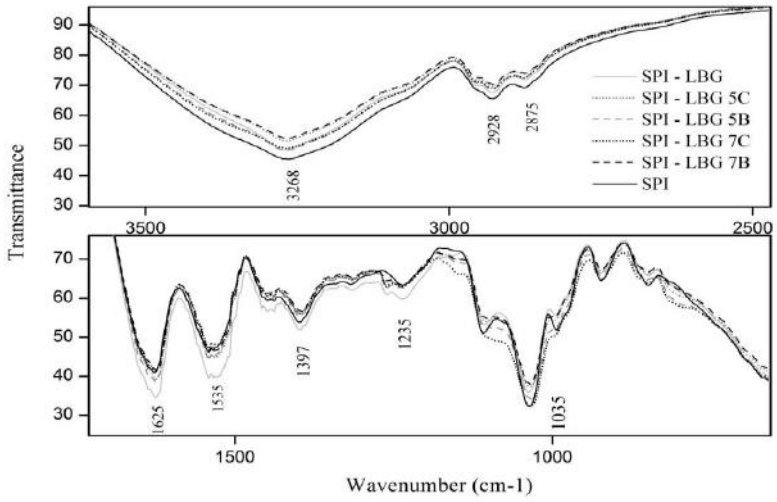
3 Results and Discussion

3.1 Fourier-transform infrared spectroscopy analysis

Figure 1 presents the FTIR spectra of SPI and SPI + LBG film with or without blackjack (B) and caruru (C) extract addition. FTIR spectra indicate several functional groups present in different positions. In the Figure 1a, the absorption bands correspond to at axial deformations of groups free O-H at 3268 cm^{-1} and C-H stretching at 2928 cm^{-1}. In the Figure 1b, the absorption bands correspond to C=O stretching (amide I) at 1625 cm^{-1}, N–H bending (amide II) at 1535 cm^{-1}, and C–N stretching (amide III) at 1235 cm^{-1}.

It can be seen that locust bean gum (LBG) addition did not influence the frequencies of the absorption bands shown in Figures 1a and 1b, however the intensity of the transmittance of all absorption bands highlighted in the figures changed, indicating that there was interaction between protein and polysaccharide and that this interaction promoted structural changes in the SPI film.

Figure 1 - FTIR spectra of SPI films and SPI-LBG films without and with 5 and 7% of Caruru (C) and Blackjack (B) extract. The spectra were divided into two range of interest: figure (a) - 3500 to 2700 cm^{-1} and figure (b) - 1800 to 600 cm^{-1}.



Source: Authors.

Intensity of the absorption bands corresponds to 3268 cm^{-1} are assigned to molecules that have the presence of O-H in their formulation, such as polysaccharides and phenolic groups (Liang, 2018). The addition of 5% caruru extract and 7% blackjack extract increased the band intensity at 3268 cm^{-1} (compared SPI-LBG film), indicating interaction, through hydrogen bonds, between the phenolic compounds present in extract (5C and 7B) and LBG. Other concentrations of extracts did not have a good interaction with the polysaccharide since the intensity band at 3268 cm^{-1} was not altered.

The blackjack (5B and 7B*)* and caruru extracts (5C and 7C) addition in the SPI-LBG film promoted changes in the absorption intensity of protein amides I, II and III (Figure 1b), indicating that there was interaction between protein and phenolic compounds present in the extracts.

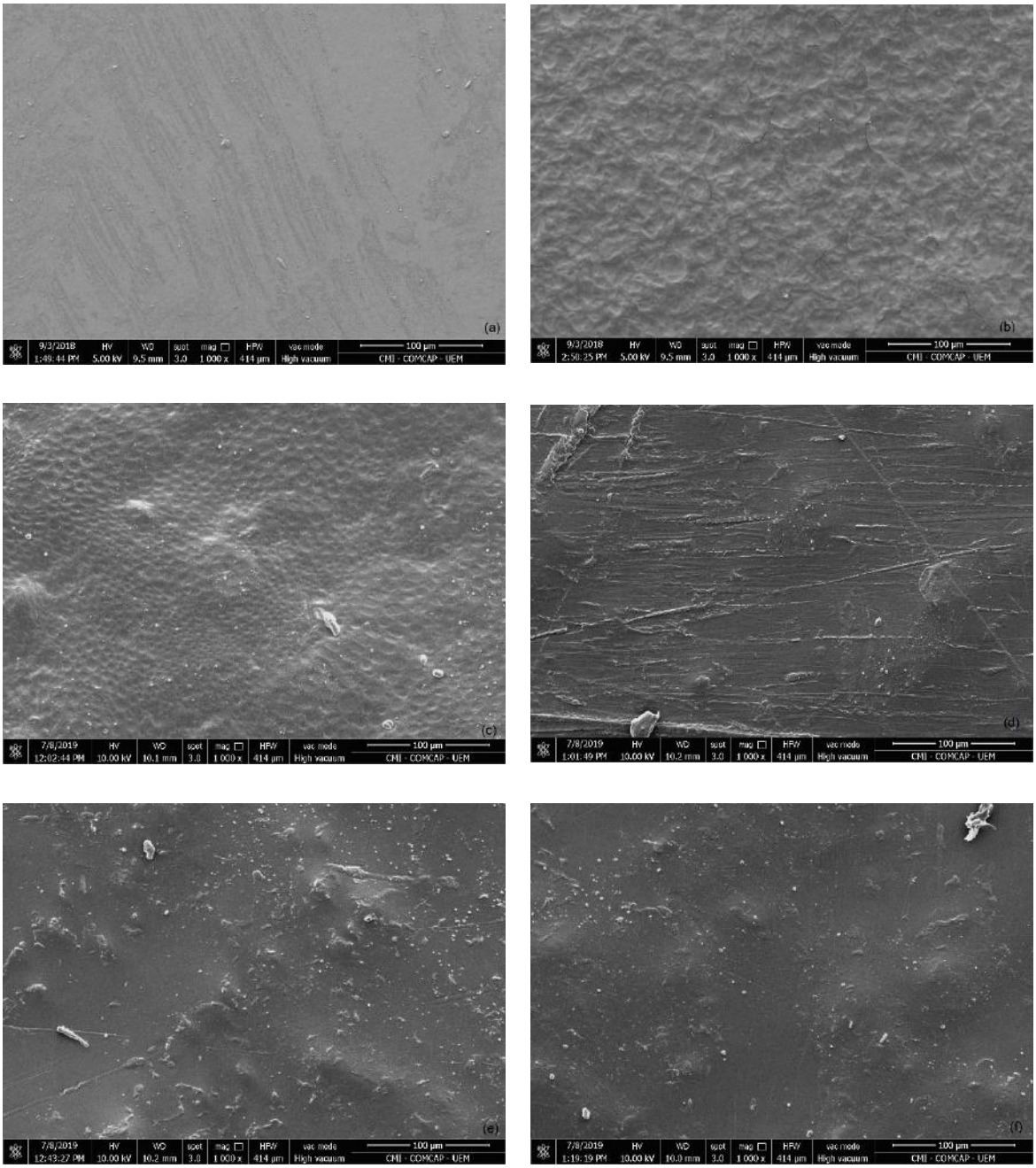
3.2 Microstructure

Study of the superficial microstructures of films reveals information about the continuity, integrity, homogeneity and structural organization of the filmogenic matrix (Souza et al.,2010). SEM images studied were obtained in magnifications of 1000 times and are shown in Figure 2.

The micrograph of the SPI film (Figure 2a) demonstrates the smooth surface structure, without cracks or roughness, compact, cohesive and with no microspores. In Figure 2b, it is possible to verify that the addition of LBG made the microstructure of the SPI film rougher, with the presence of micropores and agglomerates.

The addition of the studied extracts to the SPI-LBG matrix influenced the microstructure in different ways. The addition of 5% caruru extract (Figure 2c) resulted in a structure with greater amount of pores than films without extract. These pores were reduced and the structure became more homogeneous as extract concentration increased (Figure 2e). The incorporation of 5% blackjack extract in the SPI-LBG film (Figure 2d), however, resulted in a continuous and homogeneous surface without agglomerates, suggesting that there was a good dispersion between film matrix and blackjack extract. No major changes were observed in the microstructure of the film when the concentration of blackjack extract was increased to 7%.

Figure 2 - Surface structure (x 1000) of the SPI film (a); SPI-LBG film (b); SPI-LBG film with caruru extract addition: 5% (c) and 7% (e); SPI-LBG film with blackjack extract addition: 5% (d) and 7% (f).



Source: Authors.

3.3 Mechanical properties

Tensile strength (TS) and elongation at break (EB) were studied to determine the strength and flexibility of the film. YM was deduced from the slope of stress-strain curve and it shows the stiffness of the film (Table 1).

Table 1 - Tensile Strength (TS), Elongation-at-Break (EB) and Young's Modulus (YM) for SPI film and SPI-LBG film with or without different concentration of the blackjack (B) and caruru (C) extracts.

Treatment	TS (MPa)	EB (%)	YM (MPa)
SPI	3.61 ± 0.41 ^{e}	30.26 ± 3.52 ^{bc}	49.56 ± 5.16 ^{c}
SPI-LBG	5.43 ± 0.43 ^{c}	42.76 ± 5.48 ^{a}	55.91 ± 6.42 ^{c}
SPI-LBG-5B	6.23 ± 0.36 ^{ab}	30.52 ± 3.54 ^{bc}	166.65 ± 16.88 ^{a}
SPI-LBG-7B	6.67 ± 0.63 ^{a}	35.81 ± 3.83 ^{b}	110.05 ± 9.07 ^{b}
SPI-LBG-5C	4.45 ± 0.31 ^{d}	42.89 ± 3.89 ^{a}	49.05 ± 5.51 ^{c}
SPI-LBG-7C	5.57 ± 0.46 ^{bc}	25.54 ± 2.62 ^{c}	117.06 ± 17.74 ^{b}

Mean ± SD. Means with the same letter for the same response variable did not differ significantly at $p \leq 0.05$ according to the Tukey test. Source: Authors.

Mechanical property SPI films were improved with LBG addition. When the polysaccharide was added, TS and EB of SPI film were 50 and 40%, respectively, higher than film without gum.

Interaction between blackjack extract and SPI-LBG matrix, observed in FTIR analyzes (Figure 1), favored the tensile strength of composite films, but not its elasticity. The addition of 5% blackjack extract significantly increased ($p < 0.05$) to TS (15%) and MY (almost 3 times), but reduced in 28% the capacity of the SPI-LBG film to extend before breaking when compared to the composite film without extract. Increase of the amount of extract added did not change significantly the mechanical property (TS and EB) of the film. Probably, the phenolic compounds of the blackjack acted as crosslinking agent, increasing intermolecular interaction with the functional groups of SPI molecules and contributing to a greater tensile strength. Han *et al.*, (2018) added licorice residue extract to the SPI film and noted similar results, of increasing TS and reducing EB with the addition of the extract, and reported that the increase in strength can be attribute to the generation of a stable network due to the extract's crosslinking with the SPI matrix. It was also observed that blackjack extract addition reduced elongation at break of the composite films to values close to SPI film indicating that, probably the interaction between extract and SPI-LBG matrix influenced the interaction between protein and polysaccharide, since the plasticizing effect promoted by LBG addition, represented by higher EB value of the composite films (42.76 ± 5.48) in comparison with SPI film (30.26 ± 3.52), was reduced with extract addition.

The interaction between caruru extract and the SPI-LBG matrix, observed in the FTIR analysis, unlike the interaction observed for blackjack extract, did not improve the mechanical properties of the composite films. Addition of 5% caruru extract reduced in 18% the resistance to break of SPI-LBG film, but did not change its elasticity and Young Module. The interaction between phenolic compounds from the caruru extract with the polysaccharide, observed in treatment 5C (Figure 1a), may have favored the maintenance of the elasticity of the films without extract. When this interaction is reduced, observed in the 7C treatment, the TS and YM of the composite films increases to values close to SPI-LBG films but its elasticity reduces significantly.

These observed results can also be related to the microstructure of the film obtained by scanning electron microscopy analysis (Figure 2). The increase in the heterogeneity of the composite film with the addition of 5% caruru resulted in a reduction in the tensile strength and elasticity of the material. However, it is noted that the increase in the amount of extract reduces the heterogeneity and improves the mechanical properties of the SPI-LBG film. In the case of blackjack, the addition

of 5% of the extract was sufficient to increase the homogeneity of the structure of the SPI-LBG films and also to increase their mechanical resistance.

3.4 Antioxidant activity

Table 2 shows the total phenolic compounds (TPC) and antioxidant activity (AA) of the SPI-LBG films with and without caruru or blackjack extract addition.

Table 2 - Total phenolic compounds (TPC) and antioxidant activity (FRAP and DPPH) of the SPI-LBG films without and with different concentrations of Caruru (C) or Blackjack (B) extracts.

Treatment	TPC (mg GAE/100g)	FRAP (mmol/100g)	DPPH (mmol/100g)
SPI-LBG	539.84±5.42^{a}	29.57±0.60^{a}	23.74±0.34^{a}
SPI-LBG-5B	623.59±16.30^{b}	34.61±4.23^{c}	35.05±0.68^{b}
SPI-LBG-7B	623.57±1.55^{b}	36.06±0.56^{d}	33.47±1.36^{b}
SPI-LBG-5C	624.24±5.42^{b}	32.76±0.30^{b}	34.77±2.37^{b}
SPI-LBG-7C	716.39±12.43^{c}	32.98±0.07^{b}	38.70±1.02^{c}

Mean ± SD. Means with the same letter did not differ significantly at $p < 0.05$ according to the Tukey test. Source: Authors.

The TPC and AA found in the control film may be associated with the presence of isoflavones and amino acids that are good scavengers of free radicals, such as tyrosine, cysteine, histidine and tryptophan (Ciannamea *et al.*, 2016).

The data for antioxidant activity of the films produced show that the lowest values obtained for TPC, DPPH and FRAP were observed in the control film. Addition of extracts of caruru (5C, 7C) or blackjack (5B, 7B) promoted a significant increase in the TPC and in antioxidant potential of the films.

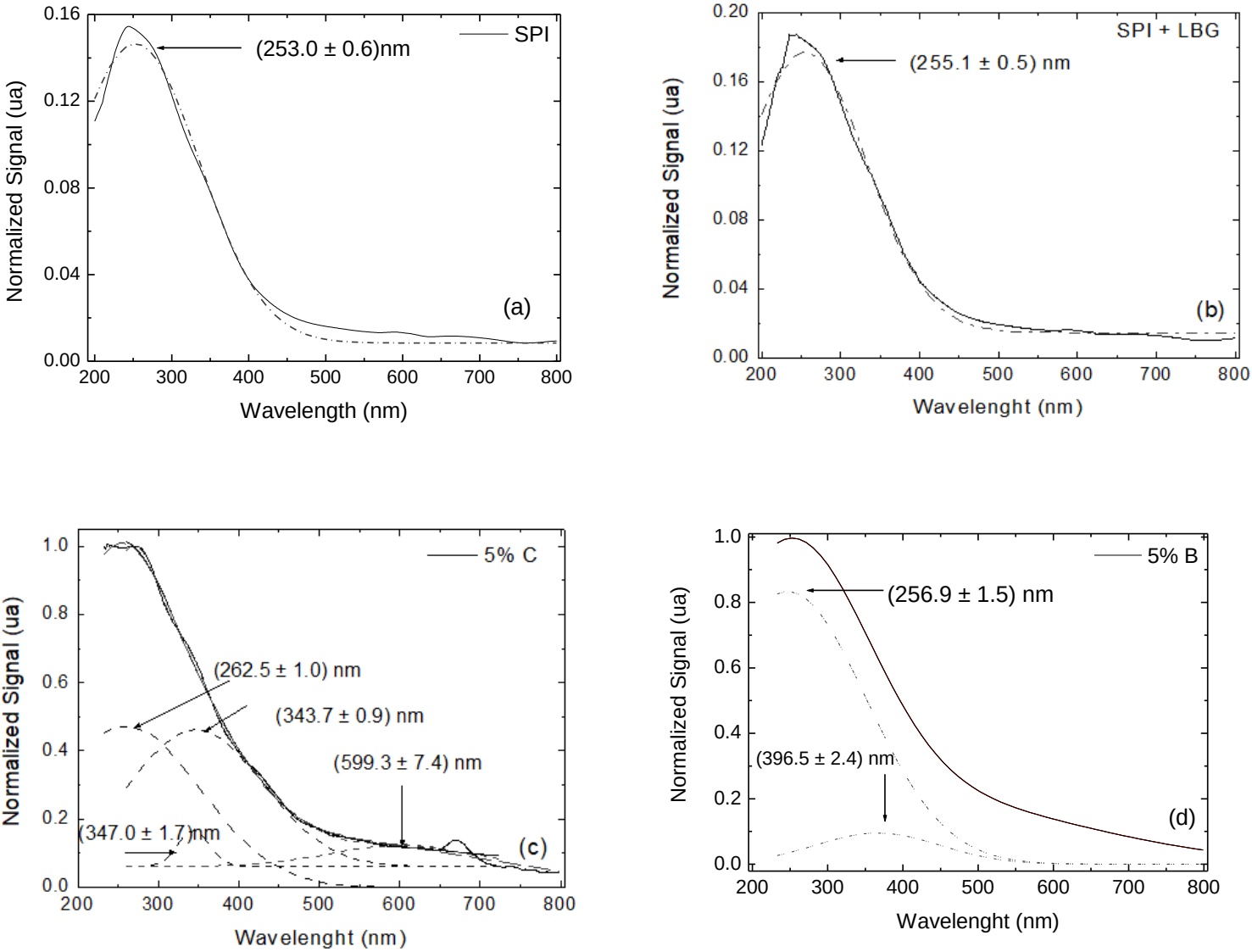
The films produced with the addition of 5% Caruru or blackjack extract showed similar values of TPC and AA by the DPPH method ($p > 0.05$). In higher concentrations, 7% extract, the film produced with Caruru (7C) presented higher values for TPC and AA by the DPPH method, in relation to the other elaborated films ($p < 0.05$). On the other hand, for the FRAP method, the 7B film showed greater activity when compared to the other films ($p < 0.05$). A Pearson's correlation analysis shows a positive (0.88) and significant ($p < 0.05$) correlation between the TPC and the AA evaluated by the DPPH method, which demonstrates that these compounds are the main responsible for the sequestration of free radicals, evaluated by this in vitro test. While FRAP method, other compounds may be involved, which are likely to be present in higher concentration in the blackjack extract.

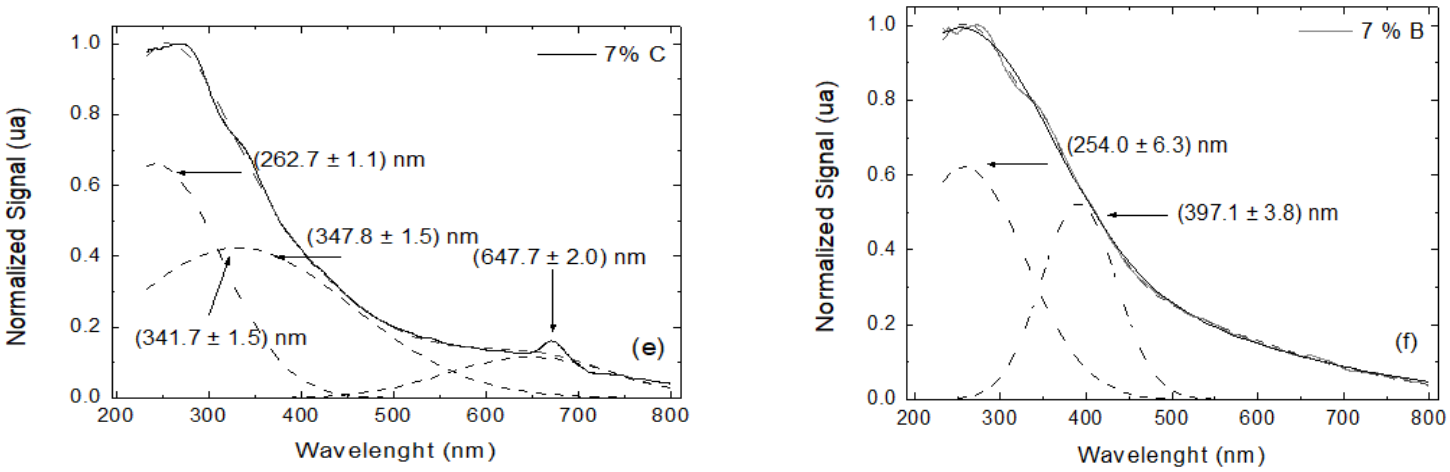
3.5 Photoacoustic Spectroscopy (PAS)

The detailed analysis of the absorption peaks was made after a Gaussian adjustment (analysis; fit multi-peaks Gaussian) of the spectra, using a computer program. Through this fit, the values of wavelength in the center of the plateau (λ_c), width (ω), and area (A) of each band are found. Each material has intrinsic absorption intervals. Initially, the spectra were placed on the same baseline, which was done by establishing a minimum point, so that the vertical axis in the graphs would start close to zero. The individual fits are represented in the graphs of this work by means of the dashed Gaussian curves.

The normalized photoacoustic spectra of the SPI and SPI + LBG films are shown in Figures 3.a and 3.b. It was possible to observe similarities between their profiles, indicating that the locust bean gum possibly absorbed the radiation spread in the same wavelength as the soy protein isolate. The wavelength on the plateau of the SPI film band was equal to λ_c = 253.0 ± 0.6 nm, and for the SPI+LBG film, the wavelength on the band plateau was equal to λ_c = 255 ± 0.5 nm, making them equivalent according to the measurement confidence interval assessment.

Figure 3 - Photoacoustic signal spectrum SPI film (a); SPI-LBG film (b); SPI-LBG film with caruru extract addition: 5% (c) and 7% (e); SPI-LBG film with blackjack extract addition: 5% (d) and 7% (f).





Source: Authors.

It is usual to apply spectroscopic methods to assess the purity of soy protein in solutions used in the development of manufactured products (Alburquerque *et al.*, 2013; Yuan *et al.*, 2017). Soy proteins absorb in the UV-C region. This absorption is associated with the electronic transitions $n - \pi^*$ characteristic between the C = O bonds, referring to the carboxylic groups existing in the amino acids. Two main aromatic amino acids are responsible for UV absorption: tyrosine and tryptophan. Optical absorption in the spectral range between 230 to 300 nm is an effect of the linkages of the aromatic side chains of these amino acids, and by lower intensity bonds formed by disulfides at wavelengths close to 260 nm (Goldfarb *et al.*, 1951). In a recent study Alburquerque e*t al.*, (2013) spectral scans were performed to characterize the photoacoustic profile of vegetable oils before and after applying heat to them. For soybean oil, the results showed that the central absorber wavelengths were equivalent to 300 nm before heating, and 257 nm after heating. Previous spectroscopic studies carried out on the SPI sample highlighted its maximum optical absorption at 280 nm.

It is very important that the food packaging is absorbent in the UV – C region due to the germicidal effect of this radiation. When interacting with radiation packaging, UV-C has the ability to inactivate the proliferation of microorganisms such as viruses and bacteria that may be present in the packaging and be a source of contamination for packaged foods. Currently, there are treatments applied in controlled regulated doses that use the exposure of packaging to UV-C as a food preservation technology (Alothman et al., 2009; Bu *et al*., 2013; Balbinot e Borges, 2020). Interaction of packaging with UV-C radiation is more efficient close to 260 nm because its germicidal action is highly lethal for most microorganisms in the absorbing wavelength of cellular DNA structures. At approximately 260 nm, the interaction with possible microorganisms in the material causes photochemical deterioration of DNA, altering cell transcription and reproduction, which consequently leads to the cell death of microorganisms (Cutler & Zimmeerman, 2011). In June 2020, SIGNIFY and the National Laboratories for Emerging Infectious Diseases (NEIDL) at Boston University in the United States published results of research that validates the effectiveness of UV-C sources in inactivating SARS-COV 2 (Storm *et al.*, 2020).

The effect of addition two different concentrations (5% and 7%) of caruru and blackjack extracts in SPI-LBG film was analyzed in the present study. Results presented in Figure 3.c revealed a wide absorption range of the film in the visible ultraviolet region. Through Gaussian deconvolution, the main optical absorption wavelengths (263 nm, 344 nm, 347 nm and 599 nm) were observed. The absorption at 263 nm can indicate that the interaction of SPI-LBG with the caruru extract caused a slight displacement of the spectral band in comparison to the film without extract. The 344 nm and 347 nm peaks may be associated with the presence of phenolic acids (with a peak close to 335 nm) and mainly flavonoids of biomolecules known as

quercetin, whose absorptive potential is established in the range between 330 and 370 nm (Dóka *et al.*, 2004; Molina *et al.*, 2014, Domínguez-Pacheco *et al.*, 2017). Peak obtained at 600 nm may be the result of the presence of chlorophyll B molecules (Charland and Leblanc, 1993; Delosme, 2003; Guskos *et al.*, 2013). Figure 3.d shows the spectrum of the SPI + LBG film with 5% blackjack extract addition. In the result, two optical bands are noted, one referring to the film without extract and the second band, with a plateau at 397 nm, can be associated with the presence of carotenoids Hernández-Aguilar *et al.*, (2019) and chlorophyll A from blackjack. Carotenoids are secondary responsible for plant pigmentation and have adequate antioxidant activity (Pushkala *et al.*, 2012).

Results represented by the photoacoustic spectra of the SPI-LBG films with 7% caruru and blackjack extract (Figures 3.e and 3.f respectively) indicated that the increased concentration of the extracts maintained the optical absorption in the films established in the main plateaus. The graphs indicate that there was only a tendency for the band to shift to red, λ_c = (647.7 ± 2.0), in the 7C film.

The radiation absorption property in the ultraviolet B and A spectral range (280 to 400 nm), observed in films with the addition of extract, demonstrates that these types of packaging can protect fatty foods against oxidative reactions that favor the deterioration of these foods (Verduin *et al.*, 2020).

Table 3 shows the main wavelengths found in the plateaus of the absorption bands and the respective areas obtained under the Gaussian curves. The area under the spectral bands can be associated with the intensity of the relative light energy absorbed by the materials converted into photoacoustic signals (Morato *et al.*, 2013). Results indicate that there was an increase of 18.43% in the area of optical absorption of the SPI film with the LBG addition.

Table 3 - Characterization of photoacoustic properties of the SPI films and SPI-LBG films with and without extracts.

Treatment	Plateau (nm)	Spectral Band Number	Band area (ua)
SPI	253.0 ± 0.6	1	131.3 ± 0,2 253,0± 0,6 131.3 ± 0.2
SPI-LBG	255.1 ± 0.5	1	155.5 ± 1.0
SPI-LBG-5B	256.9 ± 1.5	1	218.2 ± 14.0
	396.5 ± 3.4	2	115.5 ± 19.4
SPI-LBG-7B	254.0 ± 6.25	1	176.3 ± 9.3
	397.1 ± 3.8	2	227.8 ± 9.2
SPI-LBG-5C	262.5 ± 1.0	1	73.6 ± 9.2
	343.7 ± 0.9	2	5.2 ± 0.8
	347.0 ± 1.7	3	83.3 ± 10.7
	599.3 ± 7.4	4	13.6 ± 1.2
SPI-LBG-7C	262.7 ± 1.1	1	116.4 ± 1.0
	347.8 ± 1.5	2	9.27 ± 0.06
	341.7 ± 1.1	3	131.8 ± 1.2
	647.7 ± 2.0	4	25.9 ± 1.4

Mean ± SD. Means with the same letter for the same response variable did not differ significantly at $p \leq 0.05$ according to the Tukey test. Source: Authors.

When studying the Gaussian profiles of the films in which the extracts were incorporated, it can be observed that the area found under the characteristic band of the protein in SPI-LBG-5B film (256.9 nm) presented a magnitude of approximately 40.32% higher in relation to the film without extract (255.1 nm), and in the SPI-LBG-7B film, this increase in the magnitude of the area under the curve at 254 nm was equal to 13.38%. These results indicate that the interaction between blackjack extract and SPI-LBG matrix increased the film's ability to absorb energy in the UV-C region.

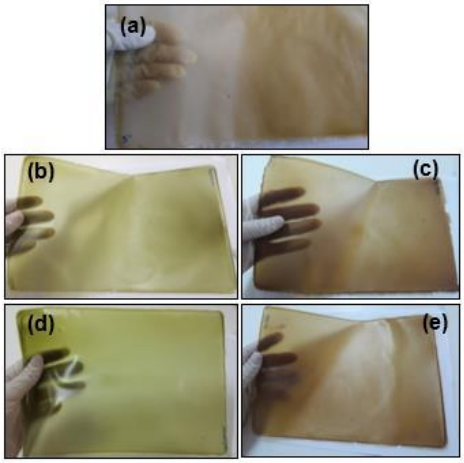
Unlike blackjack extract, the interaction between caruru extract and SPI-LBG matrix resulted in a reduction of the film's ability to absorb light in the UV-C region. The amplitude of the spectral band area of the SPI-LBG film with 5% caruru extract addition was about 52.67% smaller in relation to the film without extract, whereas in the film developed with 7% caruru extract this attenuation of the area was close to 25.14%.

Comparing the areas of the spectral bands of SPI-LBG film with 5 or 7% blackjack extract (approximately 397 nm) with the films SPI-LBG + 5% caruru extract (with overlapping bands in 343.7 and 347 nm) and SPI-LBG + 7% caruru extract (bands 341.7 and 347.8 nm), it can be verified that the potential energy absorber in the spectral range of ultraviolet B and A (280 to 400 nm) of the films containing blackjack extract are very promising, as they presented a higher intensity of light absorption in this wavelength range. In films developed with caruru extract, the optical absorption band was also detected, tending to visible red radiation (between 600 to 700 nm), although of low intensity.

3.6 *Optical properties and visual appearance*

The manufactured films visual appearance is presented in the Figure 4. The color parameters (L*, a* and b*) and the opacity of the films with extract and without extract are shown in Table 4.

Figure 4 - Images of the SPI-LBG film (a); SPI-LBG film with caruru extract addition: 5% (b) and 7% (d); SPI-LBG film with blackjack extract addition: 5% (c) and 7% (e).



Source: Authors.

Table 4 - Color and opacity of SPI + LBG films with different concentrations of caruru (C) and blackjack (B) extracts.

	L*	a*	b*	Opacity
SPI-LBG	85.37±0.88^{a}	-2.54±0.36^{a}	22.38±0.33^{a}	3.44±0.27^{a}
SPI-LBG-5B	62.97±2.82^{b}	4.46±1.52^{de}	33.16±1.27^{bc}	3.73±0.33^{a}
SPI-LBG-7B	64.04±2.19^{b}	4.31±0.90^{e}	35.28±0.97^{d}	4.15±0.27^{a}
SPI-LBG-5C	67.31±3.20^{b}	-9.50±0.22^{b}	30.70±1.63^{b}	4.26±0.61^{a}
SPI-LBG-7C	67.52±4.20^{b}	-12.88±0.62^{c}	32.77±2.18^{bc}	4.33±0.81^{a}

Mean ± SD. Means with the same letter for the same response variable did not differ significantly at $p \leq 0.05$ according to the Tukey test. Source: Authors.

The results in Table 4 were expressed as mean ± standard deviation and analyzed using Analysis of Variance (ANOVA) and Tukey's Test, considering a significance level of $p<0.05$, using the STATISTICA program (Realese7).

Caruru and blackjack extract addition significantly darkened the SPI+LBG film. The addition of 5 and 7% of caruru extract resulted in films 3.7 and 5 times more greenish than films without extract. The greenish color can be attributed to the presence of chlorophyll in the film confirmed by the photoacoustic analysis (Table 3). Blackjack extract addition, however, provided more reddish films that may be related to the presence of carotenoids in the film. The presence of this compound was also confirmed by the photoacoustic analysis. Extract addition intensified the yellowish color of the films being that SPI-LBG-7B film was 58% more yellowish than SPI-LBG film.

When analyzing the opacity results, it was noted that the addition of the extract did not significantly change the transparency of the films and did not increase the light absorption capacity at the 600nm wavelength. Despite this result, Table 3 shows that SPI-LBG films with extract addition are capable of absorbing light at other wavelengths, which indicates that these films have a greater ability to protect packaged food against photooxidation of nutrients than the films without extract.

4. Conclusion

It was possible to obtain active films based on soy protein + locust bean gum using blackjack and caruru extract. FTIR results showed that all extracts interacted with the protein, but only two treatments interacted with LBG present in the film. The addition of the extracts increased the antioxidant activity of the films and their content of phenolic compounds. The addition of blackjack extract provides a continuous and homogeneous surface, increased the tensile strength of the films, but reduced their elasticity. Addition of caruru extract, however, increases the amount of pores of the surface, not favoring the resistance of the films.

Soy protein films (with and without extract and polysaccharide) absorbed in the UV-C region, which facilitates the disinfection of the film through UV-C radiation. Interaction of films with UV-C radiation reduces the microbial load on the packaging, with the possibility of inactivating even the SARS-COV 2 virus, before being used to package food. All films with extract showed an increase in the UV light absorption capacity, being that the films containing blackjack extract presented a higher intensity of light absorption in spectral range of ultraviolet A and C.

Regarding the color of the films, all the extracts darkened the films and the caruru extract provided more greenish films than all the other films produced.

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