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Antioxidant films based on gelatin capsules and minimally processed beet root (*Beta vulgaris* L. var. Conditiva) residues

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ABSTRACT: Biocomposite films were prepared by incorporating different concentrations of beet root residue powder (BRP) (2, 4, 8, and 12 g BRP/100 g water) into films based on residues of gelatin capsules (GCR) (40 g GCR/100 g water). Control films had no BRP added. A complete mechanical, physicochemical, barrier, optical, and antioxidant characterization of all films was performed. Among all the films considered, BRP12 was found to present the most adequate properties and was further investigated. SEM micrographs showed that BRP12 presented a less homogeneous surface in comparison with the control film, but they showed similar thermal stability. After 15 days of soil degradation, the films lost over 75% of weight. The films were effective on protecting sunflower oil from primary oxidation process, and BRP12 showed higher protection than control film. Therefore, this study suggests that the formulated films could act as promising antioxidant materials and contribute to environmentally friendly technologies. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43094.

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INTRODUCTION

The waste disposal of petroleum-based plastics, which do not naturally decompose, has become an increasing environmental concern. One alternative to replace these synthetic materials is the use of biopolymers such as proteins, carbohydrates, and lipids, to develop biodegradable products. In this sense, packaging films made of renewable resources have received attention because of their advantageous and ecofriendly characteristics including complete degradation by microorganisms, biocompatibility, and potential food applications.[1] In addition, biodegradable films can be produced from industrial residues and thus, further cooperate with the development of sustainable technologies. Packaging films have been successfully manufactured from industrial wastes, which are consequently turned into added value products. Sedlařík *et al.*[2] have developed biocomposites based on dairy industry waste and a synthetic biodegradable polymer, and Çokaygil *et al.*[3] incorporated pectin jelly extracted from orange peels into starch films.

Residues generated by the processing of fruits and vegetables are the most well-studied sources of antioxidants and dietary fibers.[4] Taking this into account, residues from the minimal processing of beet root (*Beta vulgaris* L. var. Conditiva) could be a source of active and reinforcing agents for biopolymers because they contain both natural antioxidants and fiber. This antioxidant potential is due to the significant content of phenolic compounds and betalains,[5] and the natural fiber content is essentially cellulose fibrils incorporated into a lignin matrix, as for all plant fibers.[6] Although this material presents advantageous nutritional and functional characteristics, it is usually discharged and inevitably collaborates with the large amount of global food waste.

Another interesting industrial residue is that generated by the processing of nutraceutical gelatin capsules. This material is primarily composed of gelatin, glycerol, and water, but it is not reused by industries and implies waste treatment and disposal. In fact, proteins from diverse sources are used as biopolymers to develop biodegradable materials because of characteristics such as film formation and relative abundance.[7] Nonetheless, to the best of our knowledge, this study is the first to explore the use of gelatin capsules residue as a bio-based matrix in conjunction with residues from minimally processed beet root to develop active biodegradable films.

Therefore, based on the potential use of industrial residues and application of biodegradable films, the aim of this study was to investigate the effect of beet root residues on the mechanical, physicochemical, barrier, optical, and antioxidant properties of gelatin-based biodegradable films. The thermal, morphological, and degradation properties of a selected film were also evaluated, along with its protective effect against sunflower oil primary oxidation.

EXPERIMENTAL

Materials

Peels, stalks, and shavings obtained from the minimal processing of beet root (*B. vulgaris* L. var. Conditiva) were donated by Degasperi Wholesaler (Rio Grande do Sul, Brazil). Gelatin capsule residue, derived from the production of linseed oil nutraceutical capsules, was supplied by the Chemical Pharmaceutical Tiaraju Laboratory (Rio Grande do Sul, Brazil). The gelatin used to produce the capsules was of bovine source and molecular weight about 110 kDa. Radical 2,2-diphenyl-1-picrilidrazil (DPPH) was purchased from Sigma-Aldrich (São Paulo, Brazil). Sunflower oil (Cargill Agrícola SA, Brazil) and soil (Vida Ecological Development Ltda) were purchased at a local market. All other chemicals used in this study were of analytical grade.

Preparation and Characterization of Beet Root Residue Powder

The residues obtained from the minimal processing of beet root were first sanitized with sodium hypochlorite (200 ppm; 15 min), centrifuged, and sliced in a food processor (model Philips RI7762/91; Walita, Brazil) until they reached a homogeneous size to be dried in an oven with forced air circulation (60°C; 12 h) (model B5AFD; DeLeo, Brazil). The material was ground in a knife mill (model SL-31; Solab, Brazil) and then sieved (115 mesh; model Tamis; Bertel, Brazil). A powder (BRP) with particle diameters smaller than 125 mm was obtained from the beet root residues. Previous analyses had been performed according to AOAC methods[8] to determine the contents of total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF). The results were expressed as gram per 100 g on a dry basis (DB). In addition, the microstructure of BRP was evaluated by a scanning electron microscopy (SEM) (model JSM 6060; JEOL, Japan). The BRP was packed in a vacuum sealer (model F 200 Flash; Fastvac, Brazil) and stored at 25°C in the dark until the day of use.

Characterization of Gelatin Capsule Residue

The composition of the gelatin capsule residue (GCR) was analyzed following the AOAC methods[8] for protein, lipid, ash, and moisture content (MC). The results were expressed as gram per 100 g on a DB. This material was stored under refrigeration (5°C) before further use.

Film Preparation

The filmogenic solution (FS) was prepared by melting and dissolving 40 g GCR/100 g distilled water at 60°C for 20 min in a water bath. Preliminary experiments were performed to establish the ideal concentration of GCR and the maximum concentration of BRP to be added without interfering preparation, handling or homogeneity of the films. After the FS was cooled to 40°C, BRP was added at concentrations of 2, 4, 8, and 12 g BRP/100 g water, and the solution was gently stirred (model 713-D; Fisatom, Brazil). After approximately 3 min, the powder was well dispersed in the FS, and no phase separation between the components was detected, regardless the BRP concentration. Film without the incorporation of BRP was used as control. A vacuum pump was used to remove air bubbles. The solution was then cast onto polystyrene Petri dishes (0.113 g cm^{-2}) and dried in a ventilated oven (model B5AFD; DeLeo) at 35°C for 14 h. The films were stored for at least 48 h under a controlled relative humidity of 58% at 25°C (maintained by a saturated NaBr solution) prior to characterization.

Film Characterization

Thickness and Mechanical Properties. Film thickness (FT) was determined with a digital micrometer (model IP40; Digimess, Brazil) with 0 to 25 mm resolution and 0.001 accuracy. Measurements were performed at five random positions for each sample, and the average value was calculated. The tensile strength (TS; MPa), percent elongation at break (EAB; %), and Young's Modulus (YM; MPa) were evaluated for 10 strips of each film (100 mm × 25 mm). A texture analyzer (TA.XT2i e Stable Micro Systems, United Kingdom) was used to perform the mechanical analysis, according to the ASTM D882-09[9]: initial grid separation of 50 mm and crosshead speed of 0.8 mm s^{-1}.

Moisture Content and Water Solubility. The MC was gravimetrically determined by oven-drying (model TLK48; DeLeo, Brazil) three samples of each film (2 cm in diameter) at 105°C for 24 h. It was calculated as the percentage of weight loss during drying relative to the initial weight. The water solubility (WS) was measured according to Colla *et al.*[10] with slight modifications. The previously dried films were immersed in 30 mL of distilled water, and the mixture was maintained under soft stirring for 24 h at 25°C using a shaker (model NT145; Novatecnica, Brazil). This property was expressed as the percentage of solubilized matter from dry films after immersion.

Swelling. The films were analyzed according to Cao *et al.*[11] Samples (2.5 cm × 2.5 cm) were previously weighed (w_1) in air-dried conditions and then immersed in deionized water (25°C ± 2°C). After 2 min, the films were taken from the water, the excess of water was removed with the help of filter paper, and the samples were weighed (w_2). The adsorbed water was calculated by the following equation:

$$\text{Swelling } (\%) = [(w_2 - w_1)/w_1] \times 100$$

where w_1 and w_2 are the weights of the air-dried and wet samples, respectively.

Water Vapor Permeability. Evaluation of the water vapor permeability (WVP) of films was based on ASTM standard E96[12] and on the procedure described by Talja *et al.*[13] with slight modifications. The films were fixed onto aluminum permeation cells (inner diameter: 63 mm; height: 25 mm) containing granular anhydrous $CaCl_2$. The temperature was maintained at 25°C to achieve a relative humidity gradient of 0/75%. The progress of mass gain was followed gravimetrically at intervals of 1, 2, 12, and 24 h.

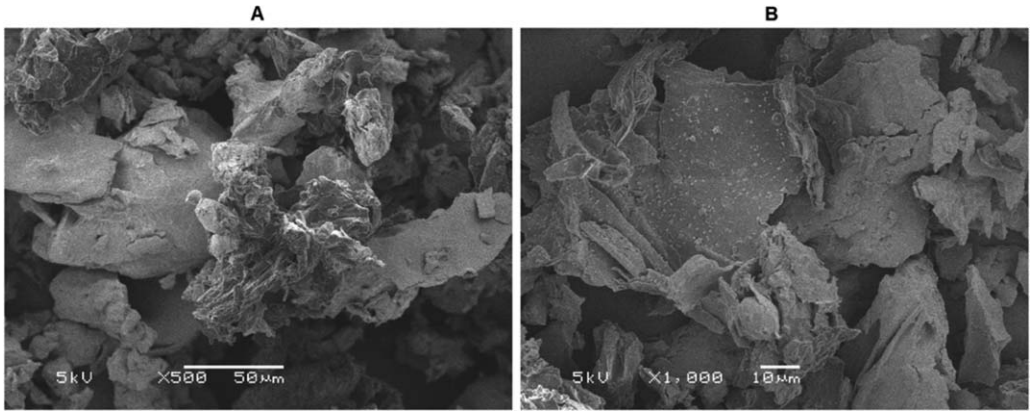

Figure 1. Scanning electron micrographs of beet root powder: (A) 500× magnification and (B) 1000× magnification.

Color Parameters. The CIELab color parameters, L^* (lightness/brightness), a^* (redness/greenness), and b^* (yellowness/blueness), were determined for the surface of the films using a colorimeter (model CR-300; Minolta, Co., Ltd., Japan). The films were placed on a white plate ($L_0^* = 97.83$, $a_0^* = 0.13$, and $b_0^* = 1.66$) as a standard background. The total difference in color (ΔE^*) was calculated as follows[14]:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

Light Transmission and Opacity. Light transmission measurements were taken at wavelengths from 200 to 800 nm.[15] A UV–vis spectrophotometer (model UV-1800; Shimadzu, Japan) was used to evaluate the light barrier properties of the films. The opacity value was calculated by the following equation[16]:

$$\text{Opacity value (A/mm)} = (-\log T_{600})/x$$

where T_{600} is the fractional transmittance at 600 nm, and x is the FT (mm).

DPPH Radical Scavenging Activity. Analysis of the capacity of the antioxidant components present in the films to scavenge the stable radical DPPH was adapted from the method described by Huang *et al.*[17] Typically, 0.1 mL of sample solution is used; but in this case, 1 cm^2 of each film sample was added to 3.9 mL of a methanolic DPPH solution (0.06 mmol/L). The mixture was kept in the dark under orbital agitation at 80 rpm (model NT145; Novatecnica, Brazil). After 8 h, a spectrophotometer (model UV-1800; Shimadzu, Japan) was used to measure the absorbance of the mixture, at a wavelength of 517 nm. A methanolic DPPH solution without a film sample served as the blank. This property was calculated as follows:

$$I\ (\%) = [(A_b - A_f) \div A_b] \times 100$$

where I is the percentage inhibition of the DPPH radical, A_b the blank absorbance, and A_f the film absorbance.

Thermal Stability. The selected and control films were evaluated on a thermogravimetric analyzer (TGA; model TGA-50; Shimadzu, Japan) operated at a rate of 10°C min^{-1} from room temperature to 600°C. Nitrogen was used as the purge gas.

Film Surface Characteristics. A scanning electron microscope (model JSM 6060; JEOL, Japan) was used at an accelerating voltage of 5.0 kV to visualize the morphology of the upper (drying surface) and lower (in contact with the Petri dish) surfaces of the selected and control films. All samples were stuck onto cylindrical bronze stubs with double-sided adhesive and then thinly sputtered with gold. Micrographs were observed at a magnification of 1000×.

Indoor Soil Burial Degradation. The soil burial degradation test was performed for the selected and control films according to the methodology described by Martucci and Ruseckaite[18] with some modifications. Plastic boxes (6 cm × 6 cm × 6.5 cm) were added of natural organic soil and used as the degradation medium for films. The film samples were cut into rectangles (2 cm × 3 cm) and dried at 60°C (model TLK48, DeLeo, Brazil) until constant weight (m_0). Then, they were placed into an aluminum mesh and buried at the depth of 4 cm from the surface of the soil. Every 2 days, water was added to the soil to maintain the humidity at approximately 40%. The degree of degradation of the films was determined after 5, 10, and 15 days as the weight loss (WL; %), by the equation:

$$\text{WL}\ (\%) = [(m_t - m_0)/m_0] \times 100$$

where m_o is the initial mass and m_t the remaining dried mass at time t.

Effect of the Film on the Retardation of Sunflower Oil Oxidation. The selected and control films were cut into rectangles of 110 mm × 60 mm, bent, and sealed (model F 200 Flash; Fastvac, Brazil) on both edges. The top remained open until 8 mL of sunflower oil containing no artificial antioxidants was added. The controls also consisted of sunflower oil packed in closed plastic bottles (PLA) and in open-glass Petri dishes (GLA). The bags and controls were stored for 35 days at 35°C and 54% RH and exposed to fluorescent lights with an intensity of 900–1000 lux (Luxometer; model MS6610; V&A Instrument, China). Samples of oil were collected after 3, 7, 14, 21, 28, and 35 days to determine the peroxide value (PV). The CIELab color parameters, L^*, a^*, and b^*, were determined for the oil packed in the films to investigate the transference of pigments from the material into the food product.

Statistical Analysis. All analyses were performed in triplicates. One-way analysis of variance (ANOVA) was performed on the data. Tukey's test of multiple comparisons was applied to analyze differences between the means of the properties of the

Table I. Chemical Composition (g/100g DB) of the Gelatin Capsule Residue (GCR)

GCR	
Moisture (%)	28.98 ± 0.25
Protein	65.95 ± 1.89
Lipid	0.77 ± 0.06
Ash	0.27 ± 0.00
Glycerol	33.01

Mean values ± standard deviation ($n = 3$).

films. The level of significance was established at $P < 0.05$. The software Statistica 12.0 (Statsoft, Inc., Tulsa, OK) was used for data analysis.

RESULTS AND DISCUSSION

Characterization of Beet Root Residue Powder

The content of TDF in BRP was 65.97 g/100 g (DB), which comprised 46.01 g of DF and 19.96 g of SDF. The content of TDF in BRP was very similar to that of other fibrous agro-industrial residues, such as malt bagasse, oat hulls, rice hulls, and fibrous residues from banana pseudo-stems,[19] whereas the content of SDF was approximately 10 times more abundant.

The SEM micrographs of BRP are shown in Figure 1. Irregular clumps and both smooth and rough surfaces were observed. Fibers without chemical or physical treatment tend to present smooth surfaces because of the presence of hemicellulose, pectin, and/or lignin that form a smooth and thick outer layer. However, these materials can be removed from the fiber bundles during grinding and expose the fiber microstructure, which results in rough and flaky fiber structures.[20]

Characterization of Gelatin Capsule Residue

As demonstrated in Table I, the major components of GCR were protein, glycerol, and water. The content of protein is related to the presence of gelatin in the residue, whereas glycerol is the plasticizer agent.

Film Characterization

Thickness and Mechanical Properties. Results for FT, TS, EAB, and YM are shown in Table II. Results for FT ranged from 0.133 to 0.267 mm. The increasing concentration of BRP resulted in greater FT, which may be attributed to the distribution of BRP in the gelatin matrix and the increasing solid content of the film composition. In general, values of EAB increase as the level of plasticizer increases.[21] The higher proportion of gelatin and, consequently, of glycerol in the control film formulation produced the most flexible films and achieved the highest EAB value (282.75%), while the increasing concentration of BRP caused a decrease in EAB values. The same effect was observed for YM, which indicates that the control film was less stiff and films BRP8 (674.72 MPa) and BRP12 (731.58 MPa) were the most rigid. In this study, the TS property was not significantly influenced by the addition of BRP, and values averaged 2.48 MPa.

Similar results were observed by Iahnke *et al.*[22] who developed biocomposites based on gelatin and carrot residue fiber. The addition of the fiber in the films increased YM and decreased EAB. Despite the addition of the fiber improved many of other film properties, it was not able to act as a reinforcement agent regarding the tensile properties. In another study, the addition of 10% and 20% of sugar cane fiber in starch films increased the YM and decreased EAB and TS. Increasing fiber loading usually provides greater TS values. However, the opposite effect may take place when the interactions between the fiber and the matrix are weak and hinder the stress transference from the fiber to the matrix.[23] This fact is one possible explanation for the present results, since the interactions between the constituents of the biocomposite are mainly done via hydrogen bonding.

The mechanical strength of biopolymers is influenced by the cohesion of the constituents of the polymer matrix. The formation of strong and/or numerous bonds between polymeric chains results in strong cohesion, which hinders their separation.[24] The BRP, which is insoluble in the gelatinous solution, decreases the cohesion of the film-forming materials and, consequently, prevents the action of the fiber as a reinforcement agent. This behavior can be also explained by the large stiffness contrast between the fiber, which is very stiff, and the gelatin-based matrix, which is soft, that promotes large stress concentrations at the interface of the fiber/matrix.[25] Therefore, the utilization of fiber treatments such as bleaching, alkalization, and addition of coupling agents are an alternative to promote improved fiber/matrix interface and enhance strength of the biocomposite.[6]

Moisture Content and Water Solubility. The control film exhibited the highest MC (24.64%) and WS (44.54%) values, which results are very similar to that found for fish skin gelatin film (MC: 24.59% and WS: 46.55%).[26] The addition of 4%,

Table II. Film Thickness (FT), Tensile Strength (TS), Elongation at Break (EB), and Young's Modulus (YM) of Films Based on GCR Incorporated with Different Concentrations of BRP

Sample	FT (mm)	TS (MPa)	EAB (%)	YM (MPa)
Control	0.133 ± 0.011^{e}	2.57 ± 0.14^{a}	282.75 ± 16.39^{a}	202.21 ± 17.34^{d}
BRP2	0.168 ± 0.013^{d}	2.48 ± 0.31^{a}	229.08 ± 7.52^{b}	365.49 ± 53.53^{c}
BRP4	0.198 ± 0.012^{c}	2.45 ± 0.21^{a}	181.67 ± 13.55^{c}	532.98 ± 60.16^{b}
BRP8	0.216 ± 0.014^{b}	2.43 ± 0.07^{a}	137.63 ± 9.18^{d}	674.72 ± 79.14^{a}
BRP12	0.267 ± 0.016^{a}	2.45 ± 0.13^{a}	117.96 ± 7.36^{e}	731.58 ± 79.38^{a}

Mean values ± standard deviation ($n = 3$). Different superscript letters in the same column indicate statistically significant differences ($P < 0.05$).

Table III. Moisture Content (MC), Water Solubility (WS), Swelling (SL), and Water Vapor Permeability (WVP) of Films Based on GCR Incorporated with Different Concentrations of BRP

Sample	MC (%)	WS (%)	SL (%)	WVP (g mm h^{-1} m^{-2} kPa^{-1})
Control	24.64 ± 0.74^{a}	44.54 ± 2.91^{a}	190.15 ± 2.80^{a}	0.566 ± 0.047^{a}
BRP2	14.61 ± 0.52^{b}	44.88 ± 1.74^{a}	189.77 ± 5.02^{a}	0.464 ± 0.003^{b}
BRP4	14.12 ± 0.29^{bc}	39.07 ± 0.48^{b}	172.86 ± 3.84^{b}	0.453 ± 0.015^{b}
BRP8	14.14 ± 0.08^{bc}	39.45 ± 0.48^{b}	170.76 ± 4.72^{b}	0.444 ± 0.019^{b}
BRP12	13.57 ± 0.15^{c}	39.58 ± 0.69^{b}	166.58 ± 9.74^{b}	0.437 ± 0.005^{b}

Mean values ± standard deviation ($n = 3$). Different superscript letters in the same column indicate statistically significant differences ($P < 0.05$).

8%, and 12% BRP caused a significant decrease ($P < 0.05$) in the values of these properties (Table III). Ahmad *et al.*[27] prepared films from fish gelatin that presented high solubility, which was decreased by the incorporation of rice flour. The ease of solubilization of the gelatin-based film was attributed to its high content of hydrophilic amino acids, whereas the composite films had improved water resistance due to their intermolecular interactions.

The BRP is mainly composed of insoluble fibers and consisted of smaller portions of carbohydrates, proteins, lipids, and ash. The interaction between its hydrophilic groups via hydrogen bonds with gelatin reduces the availability of polar groups that would easily interact with water molecules and facilitate the film solubility in water. Besides, the presence of hydrophobic groups in the BRP contributes to increase the film hydrophobicity and thus, decrease the WS.[28]

Swelling Test. One of the main drawbacks of applying gelatin films as food packaging is their tendency to swell or dissolve when in contact with the surface of highly moist foodstuffs, and, therefore, the combination of gelatin with other agents is a possibility to improve this property.[29] The data presented in Table III suggested that the SL degree of control film (190.15%) was decreased when BRP was incorporated to the film matrix in concentrations of 4% (172.86%), 8% (170.76%), and 12% (166.58%), which is in agreement with results obtained by WS test. Gelatin-based films tend to swell because of their hydrophilic character, which facilitates the transport of water molecules into the polymer matrix. The addition of agents that increase the hydrophobicity of the system, and the interactions made between the fiber and matrix via hydrogen bonding contribute to reduce the availability of hydrophilic amine functions that interact with water and, thus, to reduce the water entrance into the film.[30]

Water Vapor Permeability. Table III shows the effect of BRP presence on WVP of the GCR-based film. The obtained value for control film (0.566 g mm h^{-1} m^{-2} kPa^{-1}) was lower than that reported for cold water fish skin gelatin (0.826 g mm h^{-1} m^{-2} kPa^{-1}) studied by Hosseini *et al.*,[31] but higher than those found for films made of gelatin from tuna skins (0.165 g mm h^{-1} m^{-2} kPa^{-1}) and bovine hides (0.220 g mm h^{-1} m^{-2} kPa^{-1}) studied by Gómez-Estaca *et al.*[32] In general, WVP should be as low as possible to reduce the moisture transfer between the food and its surroundings. The use of strategies to improve this property is a valuable approach, since protein-based films, such as gelatin films, are limited in their application as a food packaging materials due to their high WVP.[33,34]

Many factors may influence the WVP of films, such as their solubility coefficient, integrity of film matrix, ratio between crystalline and amorphous zones, thickness, polymeric chain mobility, and interactions between the functional groups of polymers. The hydrophilicity of protein molecules and plasticizers commonly added to the films matrix contribute to the poor WVP property of gelatin-based films since the transmission of water trough a film commonly occurs trough its hydrophilic part.[35,36]

The addition of BRP into the GCR-based film contributed to decrease the values of WVP (~0.450 g mm h^{-1} m^{-2} kPa^{-1}) and improved this barrier property, but there were no significant differences among the WVP of films with different fiber concentrations BRP2, BRP4, BRP8, and BRP12. The results are in agreement with those found for MC, WS, and swelling and suggest that the substitution of gelatin–water interactions by the interactions between the fiber and hydrophilic groups of the

Table IV. Color of Films Based on GCR Incorporated with Different Concentrations of BRP

Sample	L^*	a^*	b^*	ΔE^*
Control	91.34 ± 0.48^{a}	0.66 ± 0.05^{e}	-0.64 ± 0.04^{e}	6.63 ± 0.05^{e}
BRP2	53.59 ± 1.13^{b}	32.06 ± 0.61^{a}	24.59 ± 0.55^{a}	57.96 ± 0.31^{d}
BRP4	36.53 ± 0.57^{c}	30.26 ± 0.99^{b}	18.25 ± 0.68^{b}	70.79 ± 0.49^{c}
BRP8	26.97 ± 0.13^{d}	14.78 ± 0.33^{c}	5.02 ± 0.14^{c}	72.12 ± 0.43^{b}
BRP12	24.91 ± 0.39^{e}	6.45 ± 0.63^{d}	1.93 ± 0.14^{d}	98.47 ± 1.13^{a}

Mean values ± standard deviation ($n = 3$). Different superscript letters in the same column indicate statistically significant differences ($P < 0.05$).

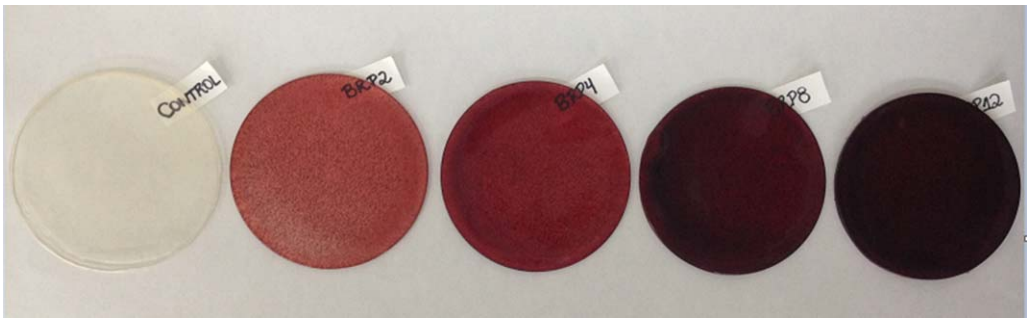

Figure 2. Visual appearance of the GCR-based films incorporated with 2%, 4%, 8%, and 12% BRP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

gelatin chain took place at the same extent for all the BRP film formulations.

Color Parameters. The color properties of the films are indicated in Table IV. The L^*, a^*, and b^* parameters of the control film were 91.34, 0.66, and −0.66, respectively. As shown in Figure 2, its appearance was visually the most transparent and colorless and had similar values to those of a tilapia scale gelatin film ($L^* = 90.29$, $a^* = -1.22$, and $b^* = 2.26$).[37] The addition of different concentrations of BRP (Figure 2) caused a linear decrease in the lightness parameter, which agreed with the opacity results. In films with BRP, lower concentrations of BRP resulted in lower a^* and b^* values, which suggests that color pigments such as betalains present in BRP influenced these color parameters. ΔE^* varied depending on the concentration of BRP incorporated into the films. A greater amount of BRP increased the color difference, which reached 98.47 for BRP12.

Light Transmission and Opacity. Light transmission at different wavelengths from 200 to 800 nm and opacity at 600 nm of the studied films are shown in Table V. All of the film formulations exhibited very low light transmission at 200 and 280 nm, which indicates that they acted as barriers against UV light. These results agree with previous studies on protein-based films, which attributed this characteristic to the content of aromatic amino acids that absorb UV light.[38,39] In the visible range, transmittance for the control film was greater than 80% at 600 nm, whereas all of the biocomposite films demonstrated decreased light transmission with increasing concentrations of BRP. As a consequence, films with BRP exhibited higher opacity, and the pure gelatin-based film (0.90 A mm^{-1}) was similar to a fish gelatin film (0.97 A mm^{-1}) with a more transparent appearance.[40]

DPPH Radical Scavenging Activity. Figure 3 shows the antioxidant activity of all studied films determined by DPPH and expressed as the percentage inhibition of the DPPH radical (I). The incorporation of BRP into the film formulation positively affected the antioxidant property assayed by this method when compared with the control film (6.3%). The highest I was observed for BRP12 (42%), and a greater concentration of BRP improved this property. These inhibition values can be attributed to the contents of betalain and phenolic compounds that remained in the BRP after processing. DPPH is a nonbiological molecule, and this analysis may indicate the presence of bioactive compounds that are present in the biopolymer matrix and can act as antioxidants.

Selection of a Film Formulation

Results for FT, TS, percent EAB, YM, MC, WS, swelling, WVP, color, light transmission, transparency, and DPPH radical scavenging capacity were carefully analyzed, and a single film formulation with incorporated BRP was selected for further evaluation of its thermal stability, film surface characteristics, degree of degradation of the film, and effect on the retardation of sunflower oil oxidation. In general, films BRP8 and BRP12 had very similar properties, such as TS, YM, MC, WS, and WVP, which are important in determining the applications of the films. These properties were improved or maintained at the same level in films with 8% and 12% BRP added when compared with the control film. However, BRP12 demonstrated better DPPH radical scavenging activity and higher opacity, which

Table V. Light Transmittance and Opacity of Films Based on GCR Incorporated with Different Concentrations of BRP

	Light transmittance (%) at different wavelengths (nm)*									
Sample	200	280	300	350	400	500	600	700	800	Opacity**
Control	0.02	0.88	36.50	60.52	73.10	79.77	82.64	82.52	83.00	0.90 ± 0.08^{e}
BRP2	0.04	0.03	0.84	5.17	15.91	21.01	43.10	54.48	58.16	2.18 ± 0.11^{d}
BRP4	0.02	0.03	0.04	0.44	3.21	4.73	19.71	31.80	135.78	3.58 ± 0.22^{c}
BRP8	0.02	0.01	0.02	0.04	0.13	0.29	4.58	11.31	184.79	6.19 ± 0.15^{b}
BRP12	0.02	0.01	0.02	0.03	0.00	0.02	0.75	6.08	9.66	7.99 ± 0.46^{a}

*Each value represents the mean value of three determinations.
**Mean values ± standard deviation ($n = 3$). Different superscript letters in the same column indicate statistically significant differences ($P < 0.05$).

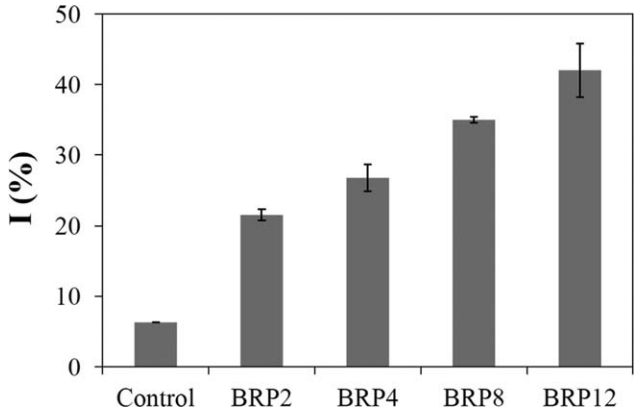

Figure 3. DPPH radical scavenging activity (I) of GCR-based films with BRP at different concentrations. Control: film without BRP. The bars represent mean values ± standard deviation.

may have contributed to the protective and antioxidant properties of that film; it was, therefore, the selected formulation.

Thermal Stability. The TGA thermogram in Figure 4 illustrates the thermal degradation behavior of the selected film BRP12 and the control film. Three main stages of weight loss (Δw) were observed for both of the films, and their behavior was similar. The occurrence of three stages of weight loss for gelatin-based films is reported in the literature.[27] The first stage ranged from approximately 30°C to 150°C, and the weight loss was most likely due to the loss of free water. Similar results were observed for fish skin gelatin films.[7] At this point, BRP12 showed 13.48% of weight loss, while control film lost 10.00%, indicating that for this temperature range the addition of BRP caused a greater weight loss in the films. This behavior was extended to 260°C; however, from this temperature until the end of the experiment, the weight loss of BRP was lower than control films, which suggests that the interactions of the components of the biocomposite film led to a stiffer and more compact structure and to an increase in the carbon residue content.[39] The second stage was observed at a temperature range of approximately 190–205°C, and the weight loss was mostly associated with the degradation of the glycerol and protein fractions of smaller molecular weights. The degradation of the protein fractions with a larger size was primarily associated with the third stage of weight loss (from 350°C). At 600°C, BRP12 had a higher residual mass (20.25%) compared with the control film (14.85%), possibly caused by a higher fiber content in the BRP12 film. Therefore, the TGA curves show that the addition of BRP has affected in small extent the thermal stability of the films.

Film Surface Characteristics. The micrographs of the upper and lower surfaces of the BRP12 and control films are illustrated in Figure 5. The control film had a homogeneous and smooth surface, regardless of whether the surface was in contact with the plate or air. The structure of the GCR-based film was similar to that of cold water fish skin[40] and tilapia skin[41] gelatin films. Concerning BR12, the surface that was in contact with the plate during drying was more homogeneous and similar to the control film, whereas the upper section (in contact with air) had a visible rougher structure. Heterogeneities in the structure of gelatin films may be explained by the renaturation of collagen during the formation of the film and by the spatially heterogeneous distribution of solutes in the gelatin solution during the evaporation process, which lead to maximum concentration of solutes at the evaporation surface.[42]

The distribution of the insoluble fiber in the film matrix during drying process likely contributed to this phenomenon, which induced the occurrence of higher concentration of fiber particles at the upper surface of the film and created a less homogeneous top surface. Another studies reached similar results. Blended films made of sugar beet pulp and polyvinyl alcohol (75/25) had visible insoluble particles in SEM images, indicating that they could not dissolve each other.[43] Bionanocomposites based on chitosan nanoparticles and fish skin gelatin showed rough surfaces when added of high filler content due to the aggregation of the nanoparticles at the top surface of the film.[40] The same happened to warm-water fish gelatin film added of lignin, which presented disruption of the smooth and homogeneous structure in comparison with the gelatin-based film caused by the incorporation of the fiber.[44]

Indoor Soil Burial Degradation. The films were buried in organic soil with the intention of reproducing a realistic approach to the biodegradation conditions found in natural environments. The mixed microflora present in the soil includes bacteria, actinomycetes, fungi, and protozoa. These microorganisms may act synergistically in the process of biodegradation, along with the action of their protein enzymes.[18] The experiment was conducted up to 15 days; but, from this point, the macroscopic deterioration of the samples hindered their recovery and evaluation. The appearance of the films before and after 15 days left on the ground is shown in Figure 6. Macroscopic examination during soil burring revealed that the films absorbed water and lost their initial aspect. Besides, the periodically addition of water probably contributed to the loss of soluble compounds. At the end of the experiment, the films lost their structural integrity and their degradation was clearly pronounced.

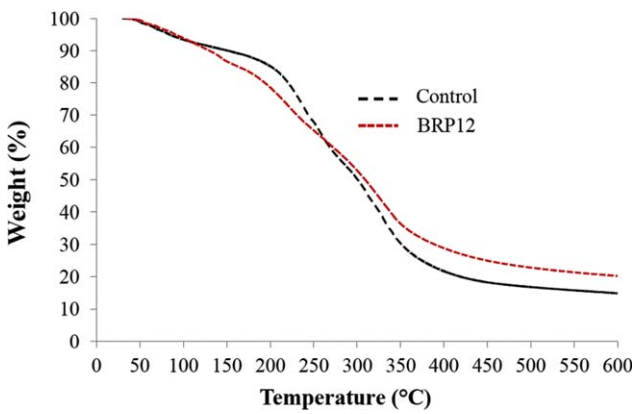

Figure 4. TGA curves of GCR-based films incorporated with 12% BRP. Control: without added BRP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

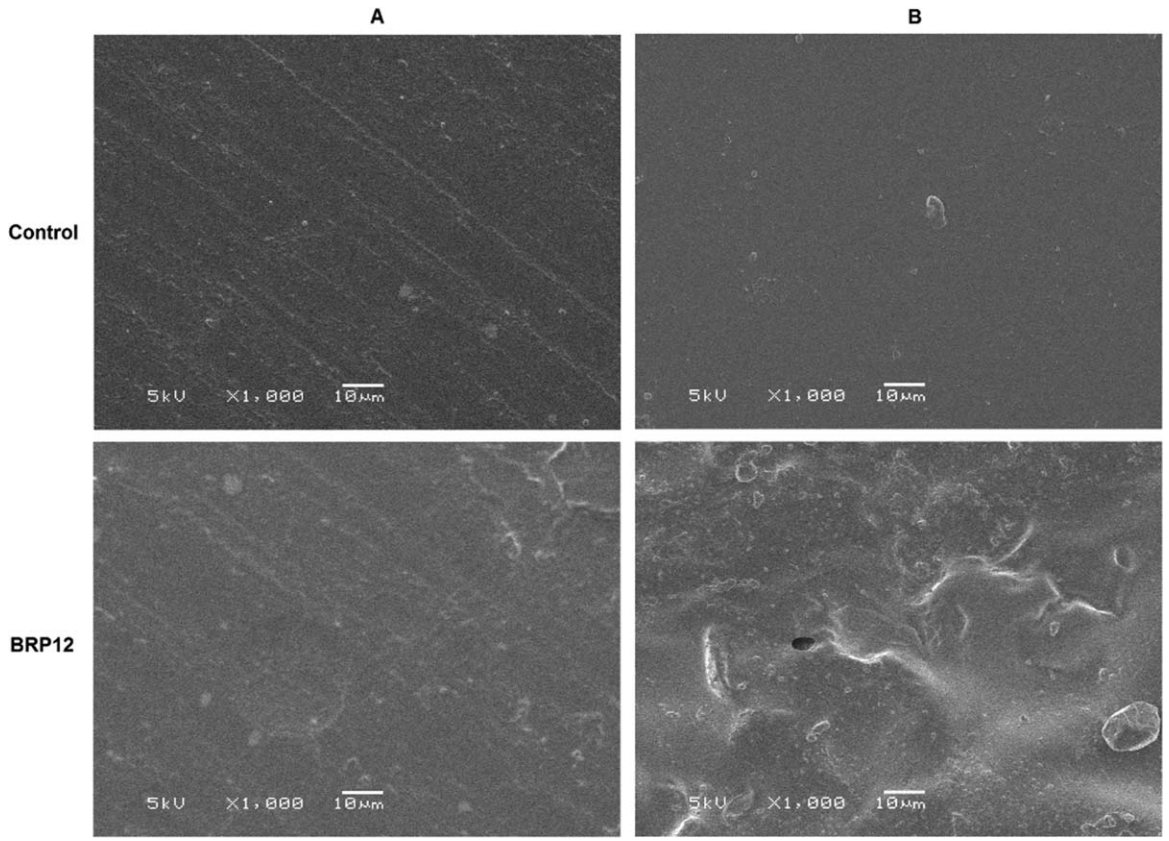

Figure 5. SEM micrographs of the lower (A) and upper (B) surfaces of the GCR-based films incorporated with 12% BRP. Control: without added BRP.

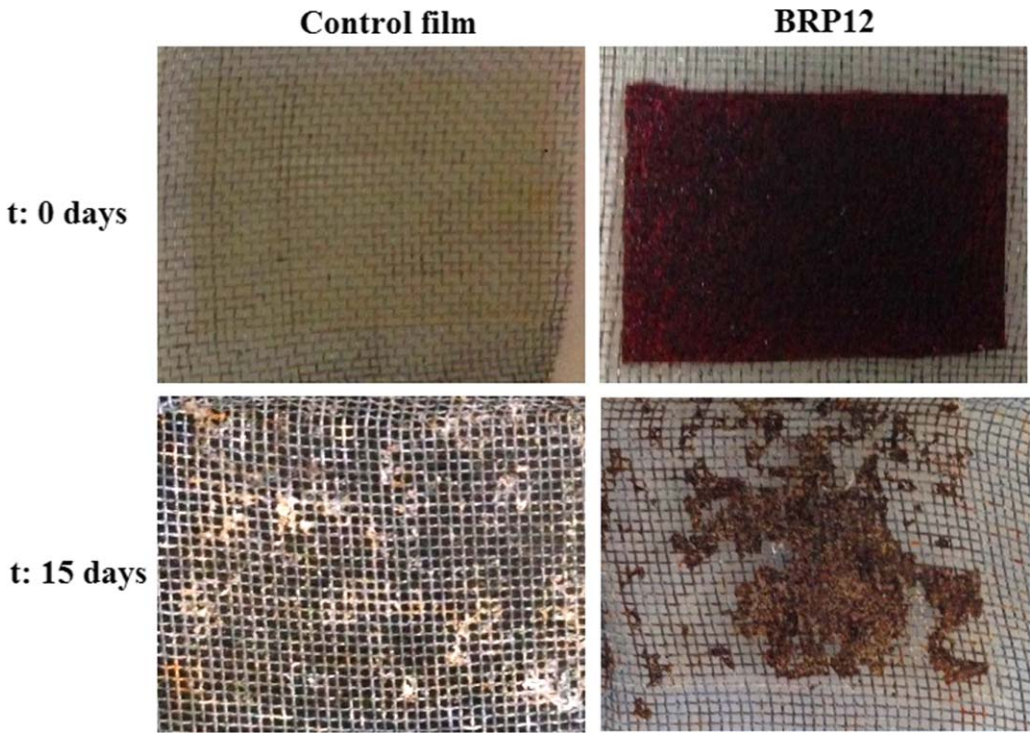

Figure 6. Visual aspect of control and BRP12 films prior and after 15 days of exposure to soil burial degradation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table VI. Peroxide Values (PVs; mEq Kg^{-1}) of Sunflower Oil Packed in BRP12, in Closed Plastic Bottles (PLA) and Placed in Open Glass Petri Dishes (GLA; Control: Film without BRP)

	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35
Control	3.45 ± 0.22^{bC}	4.39 ± 0.63^{aC}	4.37 ± 0.14^{aC}	4.69 ± 0.30^{aC}	4.58 ± 0.55^{aC}	4.67 ± 0.46^{aC}
BRP12	2.40 ± 0.25^{bD}	2.34 ± 0.21^{bD}	2.42 ± 0.12^{bD}	3.20 ± 0.42^{aD}	3.84 ± 0.25^{aC}	3.72 ± 0.15^{aD}
PLA	77.37 ± 7.76^{dA}	88.33 ± 0.68^{cA}	155.23 ± 14.61^{bA}	214.66 ± 20.80^{aA}	219.27 ± 25.12^{aA}	226.37 ± 17.14^{aA}
GLA	37.97 ± 1.68^{eB}	79.46 ± 5.47^{dB}	124.08 ± 1.06^{cB}	140.59 ± 4.79^{Bb}	166.86 ± 6.82^{aB}	170.76 ± 5.25^{aB}

Mean ± SD ($n = 3$). Different superscript lower case letters in the same line indicate statistically significant differences ($P < 0.05$). Different superscript capital letters in the same column indicate statistically significant differences ($P < 0.05$).

Weight loss of the films was taken as an indicator of the degradation of the control and BRP12 films. After 15 days under the degradation conditions, the BRP12 lost around 76% of its initial weight, while control film lost 88%. Another study reported that bovine gelatin films presented 40% weight loss after 10 days under similar conditions. Composites based on natural fibers are susceptible to biodegradation, but this process depends on the degradation of its individual components and the loss of interfacial strength between the fiber and the polymeric matrix.[6] On the basis of the results, the developed films were rapidly disintegrated and can be considered biodegradable materials.

Effect of the Films on the Retardation of Sunflower Oil Oxidation. The oxidation of lipids is one of the main causes of food spoilage and can negatively affect the nutritional and sensory characteristics of food products, especially those with high contents of polyunsaturated fatty acids.[45] In this study, the PV of sunflower oil samples was periodically measured (Table VI) to evaluate the protective action of the films against lipid oxidation. The initial PV value of the sunflower oil was 1.92 mEq kg^{-1}. The oxidation of oil packed in PLA and GLA increased throughout the storage period, and the PV values were over 170 mEq kg^{-1} after 35 days. After day 21, oil oxidation appeared to stabilize, which may indicate the formation of other products derived from secondary lipid oxidation.

The control and BRP12 films had a positive effect on the stability of sunflower oil during the entire storage period, and at the end of the experiment, the packed oil presented PV values under 10 mEq kg^{-1}, which is the recommended limit by Codex Alimentarius[46] for refined oils to be considered fresh. Cassava starch films incorporated with yerba mate extracts were effective in protecting palm oil from oxidation likely because of the content of phenolic compounds and flavonoids in the extract.[47] The remaining contents of phenolic compounds and betalains in the beet root residues likely had the same influence on the antioxidant properties of the film, as was observed in the DPPH analysis.

Table VII. Color Parameters of Sunflower Oil Packed in Control and BRP12 Films after 35 Days under Extreme Conditions

		Day 35	
Parameter	Day 0	Control	BRP12
L^*	90.33 ± 0.09^{a}	89.08 ± 0.05^{b}	89.02 ± 0.08^{b}
a^*	-2.10 ± 0.00^{a}	-2.68 ± 0.03^{b}	-2.71 ± 0.05^{b}
b^*	1.28 ± 0.01^{a}	1.28 ± 0.04^{a}	1.24 ± 0.08^{a}

Mean ± SD ($n = 3$). Different superscript lower case letters in the same line indicate statistically significant differences ($P < 0.05$).

Concerning the control film, the antioxidant properties of gelatin films from different sources have also been reported. Squid skin gelatin films exhibited antioxidant activity when assayed by FRAP and ABTS likely due to the contents of amino acids or peptides that may act as electron donors.[48] Gómez-Guillén *et al.*[29] summarized some antioxidant peptide sequences isolated from fish skin gelatin and other collagenous sources that may explain the antioxidant properties of the GCR-based film.

In addition, the color parameters L^*, a^*, and b^* of sunflower oil packed in the control and BRP films were determined in order to investigate the migration of color compounds from the biopolymer into the oil. As shown in Table VII, after 35 days of experiment, the oil packed in the films showed slight changes in its color, possibly caused by the extreme conditions of storage that occasioned its color degradation. Besides, the color parameters of oil packed in both films presented no statistically significant differences. Thus, the results suggest that there was not migration of pigment compounds from the studied films into the oil.

CONCLUSIONS

This study demonstrated the preparation of biodegradable films based on residues of beet root and gelatin capsules. The incorporation of BRP into the film matrix positively influenced the MC, WS, swelling, WVP, and DPPH radical scavenging activity but occasioned a less smooth and homogeneous surface. The studied films presented good thermal and mechanical properties. The degradation test clearly evidenced that the films are biodegradable and candidates to replace nonbiodegradable materials and can serve as a source of organic matter for composting. The results also showed that the films retarded the primary oxidation of sunflower oil, suggesting that they could be an excellent alternative in antioxidant food packaging. Furthermore, the development of biodegradable materials based on renewable resources, in addition to the use of industrial residues, is important in the advancement of ecofriendly technologies.

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