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Castor Bean Cake Protein-based Biodegradable Films: Gallic Acid Effect

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HIGHLIGHTS

- The proteins modification by gallic acid (GA) keeps films dark and opaque.
- Films solubility, tensile strength and elasticity modulus were dependent on the GA.
- The gallic acid stabilize interactions between proteins by non-covalent bonds.
- The films based on castor bean cake proteins modified by gallic acid are biodegradable.

Abstract: The objective of this work was to evaluate the effect of gallic acid (GA) concentration on some physical properties and biodegradability of films produced with proteins extracted from the castor bean cake. The films, prepared by the casting technique, showed homogeneous and brownish appearance. As the GA concentration increased (from 0 to 10 g/100 g protein), the films gradually became darker and more opaque; while the gloss had few significant differences. Solubility, tensile strength and elasticity modulus values of films varied due to changing concentrations of gallic acid. Elongation at break and water vapor permeability values did not have significant changes. A 60 % mineralization value of the film containing GA was obtained at 21 days, evidencing its biodegradability. These dark and opaque films could be used in agriculture, specifically in seedling bags as the dark color decrease the incidence of light, preventing root weakening, and the seedlings can be transplanted directly without removal of the film.

Keywords: *Ricinus communis L.*; physical properties; biodegradability; seedling bags

INTRODUCTION

The development of biodegradable films is part of attempts to overcome environmental problems caused by the accumulation of non-biodegradable synthetic packaging. In agriculture, films are used in the manufacture of greenhouses, low tunnels, soil cover, seedling bags, waterproofing of reservoirs and irrigation canals, among others. In the production of seedlings, the biodegradable films can be a good alternative in the improvement of the management, since the seedlings can be transplanted directly without removal of the packaging.

Biodegradable films can be produced from proteins, polysaccharides and lipids obtained from a variety of crop sources, especially from waste streams produced by the agro food industry [1]. Proteins are heteropolymers composed of many amino acids linked together through peptide bonds and with strong intermolecular interactions by sulfhydryl bonds, hydrogen bonds and van der Waals forces. This unique structure confers to proteins a wide range of functional properties, including significant intermolecular binding potential allowing protein-based films to exceed mechanical properties of polysaccharide and lipid-based films [1–3]. The proteins-based films have physical properties which could be considered as restrictive to practical applications. Some strategies for improving the physical and/or functional properties of these materials involve enzymatic, physical or chemical treatments of proteins [2,3].

Castor plant is an important non-edible oilseed crop used for biodiesel production. The castor bean cake, produced during oil extraction in Brazilian industries, contains approximately 40 % of proteins; whereas the freeze-dried protein extracted by dispersing milled cake in alkaline medium contains 66 – 69 % [4–7]. The valorization of these proteins aims to contribute with the biodiesel production chain. The proteins extracted from castor bean cake showed good filmogenic properties, allowing the production of films with good plasticity and elasticity [5,6,8]. Films have been produced with proteins extracted at different pHs, different concentrations, with the addition of tannic acid [5], glutaraldehyde and glyoxal [6] and cellulose fibers [8]. The films produced with the proteins extracted at pH 12 had a more cohesive structure, lower water vapor permeability, high elongation at break and lower hydrophilicity when compared to films produced with proteins extracted at pH 10 or 11 [5]. The films produced with proteins modified with glyoxal presented the best mechanical properties and the less solubility in relation to the films made with proteins modified with glutaraldehyde [6]. The mechanical properties of the films produced with glyoxal-modified proteins can be improved with the addition of cellulose fibers [8].

Gallic acid is the main structural unit of tannins, is a non-toxic vegetable product, and has an excellent protein-modifying capacity, and not yet used in castor cake protein film technology. Gallic acid has been used in the modification of proteins extracted from sunflower cake for film production [9]. These films had lower solubility and higher tensile strength and elongation at break when compared to the films produced with the same proteins modified with tara and chestnut tannins [9]. Films of zein and chitosan prepared with gallic acid had antimicrobial and antioxidant activity [10–12]. Gallic acid acts through hydrogen and hydrophobic interactions rather than covalent bonds as in the case of aldehydes [9,13]. The interactions stabilizing the protein network can be weakened at concentrations of gallic acid above optimum, damaging the film's coherence [9].

The objective of this work was to evaluate the effect of gallic acid (GA) concentration on some physical properties and biodegradability of films produced with proteins extracted from the castor bean cake.

MATERIAL AND METHODS

Film production

The films were produced with proteins extracted from castor bean cake, obtained by donation (A. Azevedo Ind. Com. Oils Ltd., Itupeva, SP, Brazil). The protein extraction was performed by solubilization in alkaline medium (pH = 12) [5]. The protein content and amino acid profile were determined previously and the results were published elsewhere [6,7,14].

The films were produced by casting technique at room temperature (22 - 25 °C) [5]. The freeze-dried protein (7 g/100 g solution) was slowly mixed with distilled water under continuous stirring for 30 minutes, to ensure complete dispersion. Then, gallic acid (0, 1, 3, 6, and 10 g/100 g of protein) purchased from Sigma-Aldrich (Munich, Germany), previously dispersed in ethanol (8 %, w/v) was added, and the mixture was maintained under stirring for more 30 minutes for allowing the reaction between the gallic acid and proteins. Finally, glycerol was added (30 g/100 g protein) and the mixture was kept stirring for more 5 minutes, thus obtaining a film-forming solution (FFS). No agent was added to control the pH of FFS, which was 11.0 ± 0.3

at the end of the FFS production process. FFS was spread onto 15 cm in diameter polystyrene plates and dried in an oven with forced air circulation at 30 °C for 15 - 18 h. The films were stored in desiccators containing saturated solution of $Mg(NO_3)2.6H_2O$ at 23 °C, at 54 % relative humidity, for 5 days, prior to characterization. This film production process was repeated three times for each experiment.

Film characterization

Film thickness and moisture content

Film thickness was measured using a digital micrometer (MITUTOYO, Japan) with 0 - 25 mm range and 0.001 mm graduation. The final thickness was the average of ten measurements made randomly throughout each determination. Moisture content was determined by drying the film sample (~ 2 g), at 105 °C, for 18 - 24 h. These results were expressed on a wet basis.

Color, opacity and gloss

Color, opacity, and gloss were measured on the top surface of the films, i.e. on the drying surface, using a colorimeter MiniScan XE – HunterLab (Hunterlab Associates Laboratory, Virginia, USA) controlled by the software program Universal 3.2 [15]. The films were placed on the surface of a standard white plate and the parameters L^*, a^*, and b^* were measured using CIELab color scale. The color difference (ΔE^*) was calculated using the standard black plate ($L_s^* = 0.02$; $a_s^* = 0.08$; $b_s^* = 0$) according to the Equation 1:

$$\Delta E^* = \sqrt{(L^* - L_s^*)^2 + (a^* - a_s^*)^2 + (b^* - b_s^*)^2} \quad (1)$$

Opacity was calculated directly using the program Universal Software 3.2, as the ratio of the opacity of the films overlapping the black pattern (*Yb*) and the white standard (*Yw*). For gloss determination [16,17], the Rhopoint NGL 20/60 glossmeter, at an angle of 60°, was used.

Film solubility

Film solubility was measured in water [18]. Films were cut into discs (2 cm in diameter), immersed in distilled water (50 mL), and kept under mechanical stirring (Marconi-MA 141 stirring table, SP, Brazil) for 24 h at room temperature (22 – 23 °C). After this period, samples were filtered through 80 g/m^2 and 3 µm porosity filter paper (Nalgon, Itupeva, SP, Brazil). Filter paper containing the film without solubilization was dried (105 °C, 24 h) and weighed. The final dry mass was determined by discounting the weight of the filter paper. Initial dry mass was calculated knowing the initial moisture of the samples. Solubility was expressed in terms of dissolved dry mass.

Mechanical properties

The mechanical properties were determined by a tensile test using a texture analyzer TA.XT2i (TA Instruments, Godalming, UK), according to the American Standard Testing Method (ASTM) D882-95a [19]. The conditions of these tests were: film size = 15 mm x 100 mm; initial distance between the grips = 50 mm and crosshead speed = 0.9 mm/s. Values of tensile strength (TS) and elongation at break (EB) were obtained directly from the stress versus elongation curves, and the elasticity modulus was calculated from the slope of the linear region of the stress versus elongation curves, using the software of equipment (Exponent Lite Express v. 4.0).

Biodegradability

The biodegradability tests were performed only with films produced with 6 g gallic acid/100 g protein, using respirometric method NBR 14283 [20], which determines the aerobic biodegradability of residues in soil by measuring the amount of carbon dioxide released. The tests were carried out at the Sanitation Laboratory of the State University of Campinas (Unicamp, SP, Brazil) for 93 days. The soil used for the tests were collected at the Campus and presented the following characteristics: 33.7 % clay, 14.1 % silt, 52.9 % sand, 1.23 % organic matter, and pH = 5.

The films were finely cut with scissors and mixed with the soil, using an application rate of 0.38 g of film per 50 g of soil (20 Ton/hectare of soil). The film mixture plus soil was transferred to the respirometer and stored in BOD (Tecnal 390, SP, Brazil), under controlled temperature of 25 °C. The aeration system was

performed with an aerator with a flow rate of 2.5 L/min of air. During the experiment, soil moisture was maintained constant at 50 % of its water retention capacity (≈ 211 g/kg of soil). The carbon dioxide released from the film biodegradation was determined by conductivity measurements for 93 days [21]. The biodegradability (B) of the films [22], expressed as a percentage, was calculated according the Equation 2:

$$B = \frac{C_f - C_c}{C_t} \times 100 \quad (2)$$

Where C_f is the amount of CO_2 released in the respirometer containing the film, between the beginning of the test and the time t (mg); C_c is the amount of CO_2 released in the control respirometer, between the beginning of the test and the time t (mg); C_t is the theoretical amount of CO_2 in the film (mg).

The theoretical amount of CO_2 (C_t) produced by the films [22] was calculated according the Equation 3:

$$C_t = w \times x_c \times \frac{44}{12} \quad (3)$$

Where w is the mass of film (mg) introduced into the respirometer; x_c is the carbon content of the film (without gallic acid = 40.8 %, with gallic acid = 36.4 %) determined by elemental analysis and expressed as a mass fraction.

Statistical analysis

Data were analyzed by ANOVA and Tukey's multiple tests at 95 % confidence level, using the statistical program "Statistical Analysis Systems" (SAS).

RESULTS AND DISCUSSION

Film thickness and moisture content

The thickness of the castor bean cake proteins (CBCP) films remained ($p > 0.05$) between 91 and 100 μm (Table 1). This result was probably due to the control of the dry mass of the film-forming solutions per support area, in addition to the modification of the protein by gallic acid, which did not affect the density of the protein matrix. The thickness of these films was greater than the thickness of low-density polyethylene films (48 ± 3 μm) usually used in the management of plant seedlings [5,23].

Table 1. Thickness, moisture content, color, and gloss of films made with castor bean cake protein modified by gallic acid.

Gallic Acid (g/100 g protein)	Thickness (μm)	Moisture content (%)[1]	Color parameters			Gloss (60°)
			*L**	*a**	*b**	
0	91 ± 7^{a}	15.3 ± 0.6^{a}	19.7 ± 2.1^{ab}	27.0 ± 0.7^{a}	31.0 ± 3.5^{a}	66.2 ± 0.1^{a}
1	98 ± 18^{a}	15.8 ± 0.2^{a}	15.6 ± 0.9^{b}	24.4 ± 1.5^{a}	21.5 ± 1.7^{b}	63.6 ± 1.8^{a}
3	95 ± 3^{a}	15.3 ± 0.7^{a}	5.5 ± 1.5^{c}	13.6 ± 3.5^{b}	5.6 ± 1.9^{c}	68.3 ± 2.5^{a}
6	93 ± 1^{a}	15.7 ± 0.1^{a}	1.5 ± 0.6^{c}	3.1 ± 0.5^{c}	0.8 ± 0.4^{c}	63.9 ± 3.8^{a}
10	100 ± 4^{a}	13.3 ± 0.4^{b}	0.9 ± 0.3^{c}	1.7 ± 0.9^{c}	-0.3 ± 0.2^{c}	55.5 ± 1.2^{b}

Different letters on the same column indicate significantly different values ($p < 0.05$). [1] on a wet basis.

The films conditioned at 53 % RH presented moisture values ranging from 13.3 to 15.8 %, which decreased ($p < 0.05$) with higher gallic acid concentrations (10 %), probably due to the hygroscopic effect of GA in high concentration. These moisture values (Table 1) were similar to those obtained in films from CBCP modified with tannic acid, glutaraldehyde, and glyoxal [5,6]

Color, opacity and gloss

The color of the CBCP films is an important characteristic for application in agriculture, specifically in the management of plant seedlings. These films should preferably have a dark color to avoid the incidence of light, preventing root weakening. The CBCP films without gallic acid presented a dark brown color, evidenced by the luminosity ($L^* = 20$), and chrome values ($a^* = 27$ and $b^* = 31$) (Table 1). Similar results were observed for films produced with this protein modified with tannic acid, glutaraldehyde, and glyoxal [5,6]. The addition of gallic acid in the CBCP film formulation led to a decrease in the L^*, a^*, and b^* parameters ($p < 0.05$) (Table

1), as well as an exponential decrease in the total color difference values (*ΔE**), calculated in relation to the black standard (Figure 1). The decrease in *ΔE** values means that the color of the films is close to black, that is, the modification of the proteins by gallic acid has produced darker films. The addition of gallic acid led to the major color changes since this agent is not stable to high pH [24], thus oxidize yielding darkened films. Similar results were observed for films produced from chitosan; tannic acid addition changed the film color, resulting in brownish films [25].

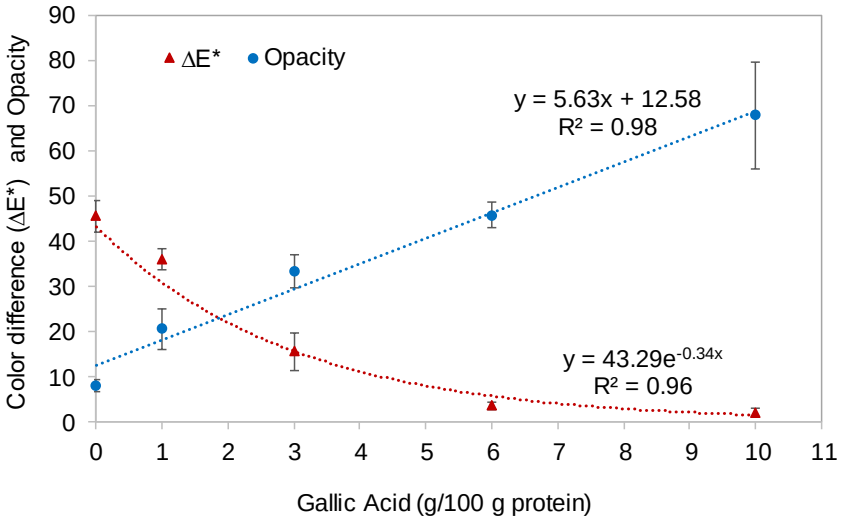

Figure 1. Color difference (Δ*E**) and Opacity of films made with castor bean cake protein modified by gallic acid.

On the other hand, it was also observed that the CBCP film opacity increased linearly with increasing the gallic acid concentration in the formulation (Figure 1). Opaque films are also interesting for application in agriculture that requires protection from sunlight. Changes in color and film opacity as a function of protein modification by tannins were also observed in sunflower cake protein films [26].

The gloss of the CBCP films were not significantly ($p > 0.05$) affected by the addition of gallic acid at concentrations of 1, 3, and 6 g/100 g protein, which remained around 65, a value similar to that of the film without gallic acid (Table 1). A decrease ($p < 0.05$) in film gloss was observed with increasing acid concentration to 10 g/100 g, indicating an increase in film surface irregularities, thus affecting light reflectance. Castor protein-based films modified by gallic acid (0 - 10 g/100 g protein) present an intermediate gloss level [27]. The gloss values of these films were lower than those of glutaraldehyde and glyoxal modified castor bean protein films (gloss 60°= 88 - 96) [6] and those of montmorillonite gelatin films (gloss 60° = 70 - 160) [28].

Film solubility

The modification of CBCP by gallic acid (1 - 6 g/100 g protein) led to a reduction ($p < 0.05$) of the films solubility from 87 to 76 % (Table 2). However, films prepared with proteins modified with 10 g of gallic acid/100 g were more soluble ($p < 0.05$) in water than those containing 1 - 6 g of gallic acid/100 g of protein. Increasing the percentage of gallic acid beyond the maximum amount that can be bound to the network would therefore load the film with additional gallic acid, damaging its coherence. Therefore, at concentrations above a certain limit, gallic acid make the films more soluble.

The relative high solubility of these films can be due to both the protein composition, with several polar amino acids with good affinity for water, such as aspartic acid and arginine present in high concentrations [6] and a large amount of glycerol (30%) in the formulation. The solubility values obtained in this study were similar to those of glutaraldehyde modified castor cake protein (solubility = 65 - 76 %) but higher than those produced with glyoxal-modified proteins (solubility = 43 - 50 %) [6]. The difference in behavior between these protein modifiers may be due to the type of bond formed during modification of the proteins. In the case of glutaraldehyde and glyoxal, the chemical bonds are covalent, while in the particular case of gallic acid, which has three hydroxyl groups in the molecule, the bonds may be hydrogen with carboxyl groups of aspartic and glutamic acids present in high proportion in castor cake proteins [6]

Table 2. Solubility in water and mechanical properties of films made with castor bean cake protein modified by gallic acid.

Gallic Acid (g/100 g protein)	Solubility (%)	Tensile strength (MPa)	Elongation at break (%)	Elasticity modulus (MPa/%)
0	87.0 ± 3.5^{ab}	2.8 ± 0.4^{ab}	67.5 ± 10.2^{a}	0.63 ± 0.17^{ab}
1	82.9 ± 3.1^{bc}	2.2 ± 0.1^{b}	81.9 ± 4.2^{a}	0.45 ± 0.05^{b}
3	76.1 ± 1.9^{d}	2.5 ± 0.1^{ab}	91.1 ± 6.2^{a}	0.55 ± 0.04^{ab}
6	76.7 ± 2.8^{cd}	3.4 ± 0.5^{a}	70.7 ± 10.7^{a}	0.86 ± 0.14^{a}
10	91.3 ± 0.1^{a}	2.7 ± 0.1^{ab}	66.8 ± 0.5^{a}	0.71 ± 0.01^{ab}

Different letters on the same column indicate significantly different values ($p < 0.05$).

Mechanical properties

Variation in the concentration of gallic acid changed tensile strength and elasticity modulus of CBCP films, but elongation values were not changed significantly (Table 2). A high tensile strength was observed for film prepared with 6 g gallic acid/100 g protein. These results showed that the new bonds formed strengthened the biopolymeric matrix. Similar results were observed in films of sunflower protein isolate modified by gallic acid [9]. These authors reported that in concentration of gallic acid above 2 %, the films had their resistance and flexibility diminished significantly. This demonstrates the importance of evaluating the effect of gallic acid concentration for each protein in particular. Gallic acid, in excess had not a plasticizer effect in the CBCP films, as observed in films made with zein and gluten [11,29].

Considering that the CBCP films produced with 6 g of gallic acid/100 g protein presented lower solubility, higher tensile strength, higher elasticity, lower opacity and dark color; they were chosen for the study of biodegradability in soil. For comparison purposes, films with proteins from castor-cake modified with tannic acid (carbon content of the films = 36.8 %) were also produced, whose preparation methodologies and properties are described by Chambi and coauthors [5], besides films prepared only with these proteins.

Biodegradability

The biodegradability of the films was determined by measuring the carbon dioxide released during the breakdown of the organic components of the castor protein films by natural microorganisms present in the soil in the presence of oxygen. All films exhibited an analogous degradation behavior, which can be represented in 3 steps (Figure 2). In the initial phase, the degradation rate of the films increased sharply, reaching 45-62 % in the first 10 days of the test. The second phase lasted 60 days and was characterized by a lower degradation rate. During this phase, the biodegradation gradually increased until reaching 78 to 92 %. Finally, a plateau phase was observed during the remaining 23 days of the test. At the end of the test (93 days), all available carbon in the films without tannins was depleted (100 % biodegradability), while the films prepared with tannic and gallic acids had a degradation rate of 88 and 89 %, respectively.

The films prepared with proteins modified with both gallic and tannic acid showed a decrease in the degradation rate when compared to the film without these acids (Figure 2). These results indicate that the increase in the number of intermolecular bonds, leading to the formation of protein-tannin complexes in the film matrix, delayed the biodegradation.

The biodegradation kinetics were satisfactorily represented ($R^2 \geq 0.98$) by logarithmic models (Table 3), from which it was possible to calculate the average degradation time ($t_{1/2}$) of these materials. This time corresponds to the period (days) of degradation of 50 % of the material. The films produced with castor bean cake proteins had $t_{1/2}$ between 8 and 13 days, similar to the degradation time of the cellulose films ($t_{1/2}$ = 6 - 10 days), and higher than the gluten films ($t_{1/2}$ = 4 days) [30]. However, the biodegradability of the cellulose and gluten films assessed by the CO_2 release was determined in liquid medium (activated sludge from a wastewater treatment plant) rather than the medium (soil) used in the present study. The biodegradability of films based on poly (lactic acid) and its copolymers had 69 - 72 % and 33 - 69 % biodegradability, respectively, after 110 days [31].

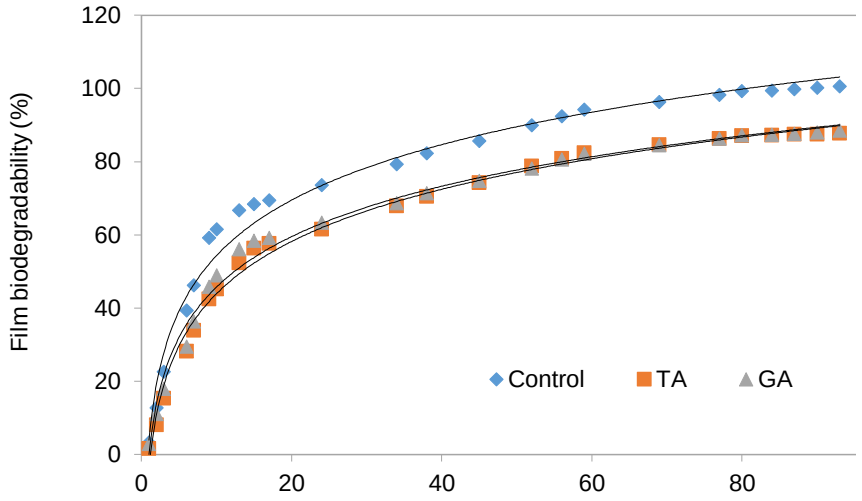

Figure 2. Biodegradability of films made with castor bean cake protein modified by gallic acid (GA) or tannic acid (TA). Control films were produced without addition of GA and TA. Points represent experimental data, and lines represent the results of fitting of equations in Table 3.

According to the biodegradability assessment standards [32], 60 % of the carbon dioxide should be mineralized to CO_2 within 45 days, so that the polymer can be called biodegradable. Thus, castor bean protein-based films with no addition of tannins and addition of gallic and tannic acids can be considered biodegradable, since 60 % of the materials were mineralized, i.e. were biodegraded at 13, 21, and 22 days, respectively. These values were calculated using the equations in Table 3.

Table 3. Biodegradability (B) equations of films made with castor bean cake protein modified by gallic acid as a function of time (t).

Films	Equations	R^2	$t_{1/2}$ (days)
Control[1]	B = 21.87 ln (t) + 3.96	0.98	8
Gallic Acid	B = 19.86 ln (t) + 0.01	0.99	12
Tannic Acid	B = 20.58 ln (t) – 3.47	0.99	13

1 Without addition of gallic or tannic acid; t1/2, mean degradation time.

CONCLUSION

The CBCP film properties were affected by the gallic acid concentration: color parameters, gloss, solubility in water, tensile strength, elasticity and biodegradability. These effects can be due to an increase in the number of non-covalent bonds between adjacent peptides. The CBCP films were biodegraded in a slow manner than similar films without chemical modification.

Gallic acid influenced positively on the films color and opacity, producing films more dark and opaque in comparison with films without chemical modification. Due to these characteristics, these films can be interesting for application in agriculture, specifically in the management of plant seedlings. Moreover, films containing 6 g gallic acid/100 g protein presented better mechanical properties and low solubility in water than the other concentrations used in this work.

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