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Properties of Biodegradable Films Made with Proteins Extracted from Castor Bean (Ricinus communis) Cake: Effect of Protein Extraction pH

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Aiming to increase the value of the castor bean cake through new technological applications, the objective of this study was the development of a biodegradable film using proteins extracted from castor bean cake. Specifically, the target of this work was to study the effect of protein extraction pH (10, 11 and 12) on some physical properties (mechanical properties, water solubility and moisture) of the films. The protein extraction was carried out in a reactor with 20 % castor bean cake dispersed in NaOH solutions (pH=10, 11 and 12), with mixing speed of 400 rpm and at 50 ºC. The separation of the protein extract was made through centrifugation (4,000 rpm). The extracts were freeze-dried and analyzed to determine their chemical compositions. Sorption isotherms of these proteins were determined at 25 ºC. The films were produced by casting, i.e., by dehydration of film-forming solutions prepared with constant concentrations of protein (7.5 g protein/100g film-forming solution); cross linking agent (0.8 g glutaraldehyde/100 g protein), and plasticizer (25 g of glycerol/100 g protein). The mechanical properties were determined at 25 ºC by tensile and puncture tests and solubility in water and moisture were determined gravimetrically. The freeze-dried protein extracts constituted protein-rich (66-69 %) material at all studied pHs, and also mineral-rich (12-24 %) ones, the latter possibly due to the use of NaOH. No effect of the protein extraction pH was observed on the protein hygroscopicity. The films produced in this study had brownish color and homogeneous aspect, independently of the protein extraction pH. The pH influenced the mechanical properties of the films. Higher pH values provoked higher tensile strength and puncture force values: 4.5±0.1 MPa and 6.5±0.2 N, respectively, for the films made using the proteins extracted at pH 12. However, independently of the observed differences, those films were little extensible, with elongation at break and puncture deformation around 1.5 % and 3.2 %, respectively. Also, the film solubility in water was affected by the protein extraction pH. While films produced using the proteins extracted at pH=10 were totally water soluble, films of the proteins extracted at pH=12 had 58.5±0.2 % solubility. The moisture of the films was not affected by the pH, remaining around 13 %. In conclusion, the pH of the castor bean protein extraction has influence on the properties of the resulting films, without necessarily affecting the protein content of the freeze-dried extract.

1. Introduction

Castor bean is highly quoted in biodiesel productive chain, because besides having a high content of oil, is a culture of large social appeal, due to the intensive use of workforce in land and by allowing the consortium with other cultures, such as beans, peanuts or corn (Lacerda, 2013). In Brazil, castor bean is considered as a “cash crop”, allowing the generation of a marketable product, with a liquid market favoring the producer access to agricultural supplies and household consumption goods (Lacerda, 2013).

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Despite the high amount of protein, the castor bean cake has not been used as animal food,due to the presence of highly toxic ricin (Lacerda, 2013). Thus, an interesting alternative to value this by-product, which may become a contribution for the success of biofuel chain, would be to use fractions of those proteins in the development of biodegradable films to be used in agriculture.

The protein of castor bean cake is composed by 60 % of globulins, 16 % of albumins, 4 % of proteases and 20 % of glutelins (Severino, 2005). Thus, it may be considered to be a good source for the production of biodegradable material based on proteins, because many of those materials have been developed with proteins having similar fractions (Tharanathan, 2003).

The amino acid composition of this protein, with arginine (11 %), histidine (11 %), lysine (3.1 %), methionine (1.5 %), among others (Beltrão, 2003), allows previewing some chemical changes, such as cross linking with bifunctional aldehydes, thus enabling, the production of material with better mechanical properties (Marquié, 2001). However, in order to obtain protein from castor bean cake, it is necessary to perform its extraction, which may be made by solubilization in alkaline medium. Evidently, the pH used may affect the extracted protein fractions.

Thus, aiming to valorize this by-product with new technological applications, the goal of this work was the development of a biodegradable film from proteins extracted from castor bean cake. Specifically, the effect of pH (10, 11 and 12) of the protein extraction on some physical properties (mechanical properties, water solubility and moisture) of the produced films was studied.

2. Experimental

2.1 Material and extraction of castor bean cake protein

The castor bean cake was a courtesy of company A. Azevedo Ind. Com. de Óleos Ltda, Itupeva/SP. The reagents, such as glutaraldehyde (Merck), redistilled glycerol (Synth), sodium hydroxide10 (Synth), were acquired in the local market.

The extraction of proteins was performed in a reactor (Tecnal, model Tecbio V 4.5L) with digital controls of temperature, stirring speed and pH, adding 20 % of castor bean cake in solution of NaOH (pH= 10, 11 and 12), with stirring speed of 400 rpm at 50 ºC (Makishi et al., 2013). The separation of proteic extract from the insoluble residue was made by centrifugation at speed of 4,000 rpm (Marquié et al., 1995). The proteic solution (supernatant) was freeze-dried in a freeze-dryer (Heto, model FD 1.0-60), after freezing in liquid nitrogen, for further use. The freeze-dried castor bean cake proteins (FDP) were characterized prior to use.

2.2 Chemical analysis of FDP

The freeze-dried castor bean cake proteins (FDP) were characterized regarding dry matter, mineral matter, brute protein, ether extract and brute fiber, according to Silva and Queiroz (2002).

2.3 Sorption isotherms of FDP

The sorption isotherms of films were gravimetrically determined according to Project COST 90 (Jowitt et al., 1983) at a temperature of 25 ºC. The films were initially placed in desiccators with Phosphorus pentoxide during a period of 10 days. After this period, the samples were packed in airtight containers with saturated salt solutions. Those recipients were kept at 25 ºC in chambers with controlled temperature (BOD Tecnal, Modelo TE-390).

The salt solutions were fixed in order to include a wide range of relative humidity: lithium chloride, magnesium chloride, potassium carbonate, magnesium nitrate, sodium nitrite, sodium chloride and potassium chloride. The equilibrium moisture of samples was determined after drying at 105 ºC, in triplicate.

The GAB model (Guggenheim-Anderson-De Boer), Eq.(1) was used to represent the experimental data of sorption isotherms (Rizvi, 1995).

$$X_w = \frac{C_{GAB}\, k_{GAB}\, X_m\, a_w}{(1 - k_{GAB}\, a_w)(1 - k_{GAB}\, a_w + C_{GAB}\, k_{GAB}\, a_w)} \quad (1)$$

Where: X_w is the moisture of material in dry basis (g water/dry solid), a_w is water activity, X_m is the moisture of monolayer, and C_{GAB} and k_{GAB} are constants of the model, related with the effect of temperature in the monolayer and in the sorption multilayer, respectively.

2.5 Film production

The films were produced by casting, in other words, by dehydration of film-forming solutions (FFS) with constant concentration of extracted FDP in pH = 10, 11 and 12 with the following: 7.5 g of FDP/100 g of

FFS, 25 g of plasticizer glycerol/100 g of protein, 0.8 g of glutaraldehyde cross linker/100 g of protein. Then those films were evaluated, subjectively (manageability, overall appearance) and objectively (mechanical tests of traction and puncture, moisture after packing in relative moisture of 52.9 % and water solubility), whose methodologies are described below.

2.6 Film characterization

2.6.1 Visual aspects and thickness

The visual aspect of films was evaluated subjectively with respect to homogeneity (color, presence of insoluble particles, bubbles, etc) and manageability (ease preparation of samples for performance of characterizations), exudation of plasticizer in the films surface and ease of detachment from support. The thickness was determined with a digital micrometer of 0.001 mm precision (Mitutoyo).

2.6.2 Mechanical properties

The mechanical properties of films were determined by puncture tests (puncture force and deformation) and tensile tests (tensile strength, elastic modulus and elongation at break) using a texturometer TA.XT2i (Stable Micro System).

In puncture tests, the samples were fixed in a cell of 46 mm of diameter and submitted to puncture with cylindrical probe with 3 mm of diameter and speed of 1 mm/s (Sobral et al., 2001). The puncture force (PF, in N) was determined directly from the curves of strength versus displacement of the probe, and the puncture deformation (PD, in %) was calculated with Eq(2).

$$PD = \frac{\sqrt{d^2 + l_0^2} - l_0}{l_0} 100 \quad (2)$$

Where: PD= puncture deformation (%); *d*= displacement of cylindrical probe in the puncture moment (mm) and l_0= initial length of film (23 mm).

The tensile tests were performed according to Thomazine et al. (2005). The film samples (15 x 90 mm) were cut and fixed in a specific probe. The initial separation distance was fixed in 50 mm and the test performed at constant speed of 0.9 $mm.s^{-1}$. The tensile strength (TS, in MPa) and the elongation at break (EB, in %) were obtained directly from the curves of tension versus deformation, and the elastic modulus (EM, in MPa) was determined calculating the angular coefficient of the linear part of the curve tension versus elongation.

2.6.3 Solubility in water

The solubility in water (SW) was determined according the methodology described by Gontard et al. (1992). The films were cut in the shape of discs with 2 cm of diameter and immersed in distilled water (50 mL), and kept at 25 ºC in stove (BOD model TE 390, Tecnal), under mechanical agitation, during a period of 24 hours. After this period, the samples were dried in order to determinate the final mass of the sample. The initial dry mass of samples was determined after drying during a period of 24 h (105 ºC). Solubility in water was expressed, then, in terms of solubilized dry mass.

3. Results and Discussions

3.1 Chemical analyzes of FDP

Generally speaking, the increase of pH of the extraction solutions triggered greater ($p<0.05$) values of dried solids (DS), minerals (M) and total fibers (TF) amounts, but not influencing ($p>0.05$) the results of proteins (P) and lipids (L) contents (Table 1). It may be suggested that the increase of NaOH quantity used to raise the pH may have contributed for the increase of DS and M amounts due to the pH increase. The high amounts of proteins of FDP, between 65 and 69 % (Table 1), allow considering them as protein concentrates, with large potential for development and production of biodegradable films.

Table 1: Results of chemical analysis of freeze-dried castor bean cake proteins extracted in pH = 10, 11 and 12

pH	DS	M	P	TF	L
10	94.0 ± 0.1[b]	10.6±0.1[b]	65.6 ± 0.6[a]	0.2 ± 0.0[b]	1.4 ± 0.1[a]
11	94.3 ± 0.1[b]	18.6±0.4[a]	69.2 ± 2.2[a]	0.1 ± 0.0[b]	1.1 ± 0.1[a]
12	95.6 ± 0.3[a]	18.4±0.2[a]	69.0 ± 0.6[a]	0.2 ± 0.1[a]	1.1 ± 0.5[a]

DS=dried solids; M=minerals; P=proteins; TF=total fibers; L=lipids;

a-b, different characters in the same column indicate that values are significantly different ($p<0.05$).

3.2 Sorption isotherms of FDP

The sorption isotherms of water steam of FDP had sigmoid shape, and may be classified as type II. No differences were observed in isotherms behavior of lyophilized proteins from the castor bean cake due to effect of pH extraction, in other words, they kept the same shapes as a function of pH extraction of proteins (Figure 1).

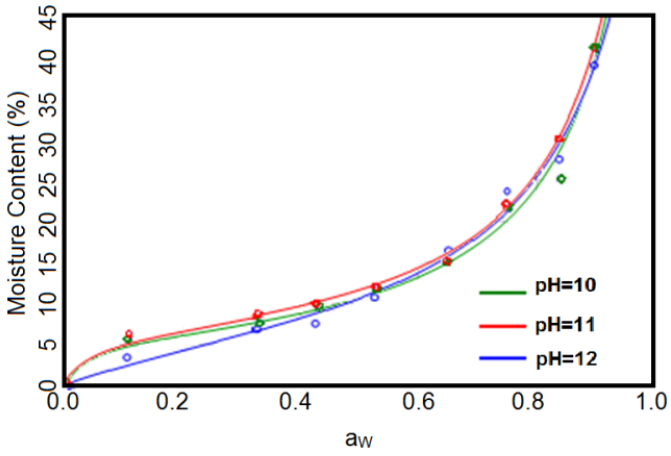

Figure 1: Sorption isotherms of FDP castor obtained in pH 10, 11 and 12

It can be observed in Figure 1 that the GAB model, Eq.(1), represented satisfactorily the experimental data. The parameter values in the GAB equation, obtained by non-linear regression (R2 > 0.99), are presented in Table 2. It is possible to observe in this table that pH 12 affected the hygroscopic properties of proteins, especially those related with the monolayer, such as monolayer moisture content (Xm) and the monolayer constant (CGAB). This behavior indicates a greater sensibility to eventual changes in the relative ambient humidity.

Table 2: GAB equation parameters

Extraction pH	X_m(%)	C_{GAB}	K_{GAB}	R^2
10	6.4	21.0	0.94	0.999
11	6.0	29.9	0.94	0.991
12	7.6	3.7	0.90	0.995

The monolayer moisture content (Xm) of freeze-dried proteins of castor bean cake obtained in pH 10, 11 and 12 (Table 2) oscillated between 6 and 7.6 %. The values of CGAB constants of FDP of castor bean cake obtained in pH 10 and 11 were similar of those observed by Abugoch et al. (2010), for films made of quinoa and chitosan proteins, and to those observed by Sobral and Habitante (2001), for pigskin gelatin, which observed values for CGAB remained between 18 and 20. Thus, the value found in this work for CGAB in pH 12 was quite low than that observed by these same authors. The values of KGAB parameters of these analyzed samples, were similar to those observed by Abugoch et al. (2010) and Sobral and Habitante (2001), which observed values between 0.80 and 0.95.

3.3 Films properties

3.3.1 Thickness and visual aspects

Films produced with FDP showed a brownish coloring and a homogeneous visual aspect, independently from the protein extraction pH. The coloring was a result of the presence of pigments in the raw material, in other words, not necessarily consequence of the reticulation reaction.
The films produced with FDP in pH 10, 11 and 12, showed a thickness around 0.122 mm (Table 3).

3.3.2. Water solubility and moisture

The effect of extraction pH was also clear on the water solubility, which was of 100, 62 and 59 %, for films produced in pH 10, 11 and 12, respectively (Table 3). In other words, the improvement of proteins quality with the increase of pH also resulted in increasingly less soluble films. Marquié et al. (1995), studying films made from glandless dilapidated cottonseed flour, observed 100 % of solubility in films without cross linker

agents, which decreased with the glutaraldehyde addition. Considering that these films were indicated for agricultural use, it is desired that these material have low water solubility, thus, films produced in pH = 12 would be more appropriated.

Table 3: Solubility and moisture of films made of castor protein.

Extraction pH	Thickness (mm)	Solubility (%)	Moisture (%)
10	0.120±0.002[a]	100.0±0.0[a]	12.5±0.4[a]
11	0.123±0.004[a]	61.6±1.5[b]	13.7±0.7[a]
12	0.122±0.002[a]	58.5±0.2[b]	12.9±0.2[a]

a-b, different letters in the same column indicate that the values are significantly different (P<0.05)

On the other hand, the pH did not affect the films moisture, which remained around 13 % (Table 3). This result is quite interesting because of the big amount of NaOH used in the increase of the protein extraction pH, which could make the films more hygroscopic, as observed on the FDP sorption isotherms. However, this was not observed in the films, according to the presented results in Table 4.

3.3.3 Mechanical properties

In general, the proteins extraction pH did not affect the mechanical behaviour of films produced with these proteins, the mechanical curves obtained in the puncture tests, as well as in the traction one, showed the same behaviour. However, it was observed that the proteins extraction pH influenced the mechanical properties of films. The pH increase implied in more resistant films to puncture and tensile (Table 4).

The puncture force increased from 4 to 6.5 N in consequence of the increase of the proteins extraction pH from 10 to 12, respectively, but without effect (p>0.05) in puncture deformation, which stayed around 3 % (Table 4). Marquié et al. (1995), studying films made from glandless dilapidated cottonseed flour and cross-linked with glutaraldehyde, observed higher values for puncture force (6 N).

Observing the tensile tests results (Table 4), there was a similar behavior to the one of puncture tests, where the pH extraction increase implied in more resistant and rigid films (p<0.05), with the increase of tensile strength values from 2.4 to 4.5 MPa and elastic modulus from 0.94 to 2.4 MPa/%, however similarly to the observed in puncture tests, the elongation at break was not influenced (p>0.05) by the protein extraction pH, ranging between 28 and 45 %. Ayhllon-Meixueiro et al. (2000) studied films made from Sunflower Proteins Isolate without cross linking and found similar values of tensile strength (3.9 MPa) and superior values of elongation at break (215-251 %). This same behavior was found by Hernández-Muñozet al. (2005), in films made from gliadin using glutaraldehyde as cross linking agent, observing 2.8 MPa for tensile strength and 245 % to elongation at break. In general, the values of mechanical properties determined in this paper were similar to those found by Makishi et al. (2013), working with identical system.

Table 4: Mechanical properties of films made of castor protein.

pH	PF (N)	PD (%)	TS (MPa)	EB (%)	EM (MPa/%)
10	4.0±0.9[b]	3.6±0.3[a]	2.4±1.0[b]	27.8±7.3[a]	0.9±0.2[b]
11	5.2±0.3[ab]	3.2±0.4[a]	3.9±0.3[ab]	28.4±8.4[a]	1.2±0.2[b]
12	6.5±0.2[a]	2.9±0.1[a]	4.5±0.1[a]	44.7±11.0[a]	2.4±0.5[a]

PF=puncture force; PD=puncture deformation; TS=tensile strength; EB=elongation at break; EM=elastic modulus
a-b, different letters in the same column indicate that the values are significantly different (P<0.05).

Despite the fact that extraction pH did not affect the protein content in extraction (Table 1), the quality of proteins might have been affected, in a way that this effect was felt in the mechanical properties related to the films resistance. This quality may have been in different fractions of peptides, but also in amino acids residues more available for the reticulation reaction with the glutaraldehyde, such as lysine (Makishi et al., 2013).

4. Conclusions

Proteins extracted from castor bean cake have good film-forming properties. The pH extraction of proteins from castor bean cake has influence on the films properties, not necessarily having influenced the content of protein in the freeze-dried extract. The films physical properties, such as water solubility and mechanical properties, were better in films produced with proteins extracted in pH = 12.

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