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Castor Bean (*Ricinus communis*) Cake Protein Extraction by Alkaline Solubilization: Definition of Process Parameters

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The objective of this paper was to study the parameters of an extraction process of protein from castor bean cake by solubilisation in alkaline medium. Initially, the castor bean cake was ground, sieved and submitted to chemical analyses in order to determine its composition. The protein extraction was carried out in laboratory scale, under the following conditions: stirring speed of 400 or 600 rpm, temperature of 30 or 50 ºC and cake/NaOH solution-ratio of 10 or 20 %, at constant pH = 9. The separation of the supernatant (extract) from the solid insoluble residue was done by centrifugation (4,000 rpm). The extracted protein yields were calculated and the chemical composition of both the freeze-dried extracts and dehydrated (40 ºC/24 h) residues were determined. The composition of the castor bean cake was: 39.3 ± 0.6 % protein; 9.2 ± 0.1 % moisture; 11.9 ± 0.0 % ash and 2.8 ± 0.5 % lipids. Higher protein yields (25.3 %) during the protein extraction process were observed when using lower cake-solution ratio (10 % at 400 rpm and 50 ºC). That yield increased to 27.3 % when the solid insoluble residue was washed out with pure water and centrifuged again. The protein content of the freeze-dried extracts varied between 56.9 ± 0.7 (400 rpm, 20 % cake and 30 ºC) and 64.8 ± 0.7 % (400 rpm, 20 % cake and 50 ºC, with residues washing), allowing to consider this product as a protein concentrate. Besides, the protein content of the dehydrated residues ranged from 32.4 ± 0.2 % (400 rpm, 20 % cake and 50 ºC, with residue washing) to 35.3 ± 1.3 % (600 rpm, 20 % cake and 50 ºC). The electrophoresis analyses of the extracted protein with bands between 37 and 51 kDa and between 19 and 29 kDa suggested that this can be a good raw material in the biodegradable film technology.

1. Introduction

Castor bean oil is being widely used for biodiesel production. This production chain has glycerol and castor bean cake as its main by-products. The use of this cake for the production of biodegradable materials and for animal feed may be interesting for the success of the biodiesel agro industry. Each ton of oil produced from the castor bean seed implies the production of 1.13 t of cake (Lima et al., 2011). The castor bean cake is rich in proteins and fiber, but it cannot be used in animal feed because it presents high toxicity, due to the presence of ricin, ricinin, agglutinin and allergen CB-1A (Madeira et al., 2011).
An interesting alternative to add even more value to the castor bean cake would be to extract the proteins and use them at the production of biodegradable films that can be used in agriculture, specifically in bags for seeds plantation. Proteins can be extracted from oil seed cakes by solubilisation in alkaline medium. The production of films made of proteins implies the use of a plasticizer, such as glycerol, that is the main by-product of the production process of transterification for biodiesel production, whose production corresponds to approximately 10 % of the produced biodiesel volume (Singhabhandhu and Tezuka, 2010). Thus, the objective of this paper was to study the parameters of an extraction process of protein from castor bean cake by solubilisation in alkaline medium.

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2. Experimental

2.1 Sample preparation and proteins extraction from castor bean cake

Castor bean cake, obtained by donation (A. Azevedo Ind. Com Oils Ltd., Itupeva, SP), was ground in a mill rotor, with temperature control in a bath at 10 °C, and sieved, using ABNT 20 sieve (mesh 20), for separation of larger particles. For particle size characterization of the ground cake, granulometric sieves of 12, 16, 20, 28, 35 and 48 mesh were used, coupled to an electromagnetic shaker.

The conditions of the proteins extraction process were the following: stirring speed of 400 or 600 rpm, held with the help of a mechanical mixer (Model TE-039); temperature of 30 or 50 ºC, held with a thermostatically bath (Model MA 184/BX), and cake/NaOH solution-ratio of 10 or 20 %, at constant pH = 9, using NaOH solutions. These processes were performed at lab scale, in beckers of 1,000 mL. The solubilized proteins separation (supernatant) was performed by centrifugation at 4,000 rpm, for 20 minutes, using a refrigerated centrifuge (20 °C) (Model Centra GP&R 31250525, USA). Moreover, for just one treatment, the residue of protein extraction process was again suspended in distilled water, and submitted to centrifugation.

To evaluate the yield of proteins extraction the contents of protein in the castor bean cake and protein solutions (supernatant) obtained at the extraction pH were determined.

To test the effect of the content of residual lipids in the castor bean cake, a sample was drained of lipids by extraction with petrol ether, in a Soxhlet instrument.

2.2 Characterizations of castor bean cake and extracted proteins

The castor bean cake and the extracted and freeze-dried proteins were analysed to determine the levels of dry matter (DM), ash, fat, protein (P) and total fiber (TF), according to AOAC Official Methods 925.10, 923.03, 920.87, 962.09, respectively (AOAC, 2005). Protein was calculated as nitrogen content multiplied by factor 6.25. The electrophoresis analysis was performed according to the SDS-PAGE methodology (Laemmli, 1970). All determinations were made in triplicates.

2.3 Statistical analysis

Experimental results were analyzed by ANOVA and Tukey's multiple tests at 95 % confidence level using the statistical program "Statistical Analysis Systems" (SAS, 2003).

3. Results and Discussion

After sieving process, an amount of ground product remained retained in the sieves of 12, 16 and 20 mesh. These products were mixed to be characterized and were named as coarse fraction. The product which remained retained in the sieves of 28, 35 and 48 mesh, called intermediate fractions, also were mixed for characterizations. And, the product which stopped in the bottom of the sieve was considered as the thinner fraction. This thinner fraction was the most abundant one (Figure 1).

For proteins extraction, only intermediate and thin fractions were used. Besides the relatively high protein content, the coarse fraction was difficult to handle in the extraction process, with a tendency to float, probably because of the high total fiber content (Table 1). Besides, it represented a small amount of the total (Figure 1).

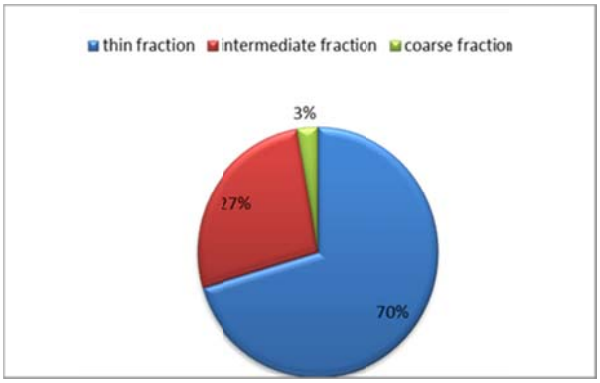

Figure 1: Proteins distribution in granulometric fractions of castor bean cake

The content of proteins increased ($p<0.05$) with reduction of particles dimension, differently from the observed to the total fiber, which increased ($p<0.05$) with the increase of particles dimension (Table 1).

Thus, it can be suggested that coarser particles were rich in materials from the shell, with high amount of total fiber and proteins, with the possibility to be used as organic fertilizer.

Table 1: Chemical composition of castor bean cake fractions

Components	TF	IF	CF
Dry Matter	92.8±0.0[a]	92.8±0.0[a]	92.8±0.0[a]
Ashes	14.8±0.2[a]	7.2±0.1[b]	7.4±0.4[b]
Fat	3.1±0.1[a]	2.5±0.3[b]	2.6±0.5[b]
Protein	49.3±0.8[a]	22.9±0.4[b]	19.4±0.5[c]
Total Fiber	11.6±0.9[c]	28.4±0.3[b]	33.1±1.6[a]

a-e different characters in the same column indicate that the values are different ($p<0.05$)
TF - thinner Fraction; IF - Intermediate Fraction (Mesh 28, 35, 48); CF - Coarse Fraction (Mesh 12, 16, 20).

3.1 Castor bean cake characterization

After fractionating the castor bean cake, only the intermediate and the light fractions were used for this research. Table 2 shows the composition of the cake using both intermediate and thinner fractions. It was verified that a level of 8.7 % moisture for the castor bean cake was considered good for storage. According to Custodio et al. (2005), the recommended limit of moisture for storage of oilseeds bran is 11 %, with the possibility of conservation for a long period of time, since the low level of humidity reduces the microbial activity. The castor bean cake had a protein level of 39.7 % being adequate both for use in soil (as fertilizer) or in animal food, if detoxified. Several works about the nutritional quality of cakes and bran have shown a potential to use this material in animal feed (Abdalla et al., 2008). Oilseed cakes have high protein content, with values up to 36 % for peanut cake and almost 40 % for castor bean cake (MOTA; PESTANA, 2011).

Table 2: Castor bean percentage compostion

Components (%DM)	CB
Moisture	8.76±0.13
Protein	39.77±1.07
Fat	3.17±0.12
Ashes	10.33±0.08
Total Fiber	19.69±1.15

DM = dry matter; CB = castor bean

The content of ashes was 10.3 %, similar to those observed by Severino (2005). The value of lipids in the cake was 3.1 % indicating that it was a good quality product. The lipid mean value of the castor bean cake may vary depending on the extraction method, for example, if the extraction method is mechanical, an oil residue of 6 to 13 % may remain in the castor bean cake, if pressing followed by solvent treatment is used, the oil residue in the castor bean cake may vary between 1 to1.5 % (SEVERINO, 2005).

3.2 Extraction parameters of the castor bean cake protein

According to results of the yield of the extraction process of proteins from castor bean cake without washing of residues (Table 3), the largest yield (25 %) was determined in the process (T3) for 10 % of cake in the extracting solution, 400 rpm and 50 ^{o}C. But, it was observed that the protein yield increased when the insoluble residue (decanted) was submitted to washing with distillate water and submitted to a new centrifugation (T5). This result allows us to suggest that, despite the proteins solubilisation, a certain amount of protein remained adsorbed in dry residue particles, due to the large specific surface of the material, since the majority of these particles were very thin (Figure 1). Thus, washing residues with distilled water enabled the extraction of this adsorbed fraction of protein in a second moment. On the other hand, the lowest yield (11 %) was observed in the process with higher concentration of castor bean cake (20 %) and in the lowest temperature (10 ºC), at 400 rpm (T4).

Table 3: Protein extraction yield obtained at pH9 in different processing conditions

Treatment	Conditions of the extraction process					Protein extraction yield (%)
	Stirring speed (rpm)	Cake concentration in extraction solution (%)	Extraction temperature (ºC)	Residue wash after extraction	Extraction using defatted cake	
T1	400	20	50	-	-	20.6 ± 0.6^{bc}
T2	600	20	50	-	-	19.2 ± 0.5^{c}
T3	400	10	50	-	-	25.3 ± 0.2^{ab}
T4	400	20	30	-	-	11.4 ± 4.2^{d}
T5	400	20	50	X	-	27.3 ± 0.7^{a}
T6	400	20	50	-	X	19.3 ± 0.6^{c}

Different characters in the same column indicate that the values are different ($p<0.05$)

The complete elimination of lipids in the castor bean cake did not necessarily implied an improved performance in terms of protein extraction, which yield stayed around 19 %, for the process with 20 % of cake in solution, at 50 ^{o}C and 400 rpm (T6).
The insoluble residues (decanted) were dehydrated and subjected to chemical analysis, being noticed that the protein levels ranged from 32 % (400 rpm, 20 % of cake and 50 ^{o}C, with residues washing) and 35 % (600 rpm, 20 % of cake and 50 ^{o}C). In other words, they continue to be a product rich in proteins, interesting for animal feed.

3.3 Proteins and ash contents in protein extracts

The solutions containing solubilized protein and separated by centrifugation had low concentration of dry matter, ranging from 2 to 4 % (Table 4). This result was a consequence of the high quantity of alkaline solution needed in the extraction process. However, the dry matter of these extracts constituted in concentrated protein, because it protein content (on dry basis) ranged between 57 and 65 % (Table 4). The lowest protein content was observed for the treatment (T4) providing the lowest yield (Table 2).

Table 4: Composition of protein extracts obtained by extraction at pH 9 in different processing conditions

Treatment	Protein solutions composition		
	Dry matter (%)	Protein (d.b %)	Ash (d.b. %)
T1	4.4 ± 0.0^{a}	61.1 ± 0.8^{ab}	9.8 ± 2.2^{a}
T2	4.3 ± 0.2^{a}	62.4 ± 4.3^{ab}	10.1 ± 0.5^{a}
T3	1.9 ± 0.0^{c}	64.0 ± 0.6^{a}	11.2 ± 0.1^{a}
T4	3.2 ± 0.0^{b}	57.0 ± 0.7^{b}	7.8 ± 0.1^{a}
T5	3.0 ± 0.3^{b}	63.6 ± 2.4^{ab}	9.9 ± 0.5^{a}
T6	4.1 ± 0.4^{a}	64.8 ± 0.6^{a}	10.7 ± 1.0^{a}

Different characters in the same column indicate that the values are different ($p<0.05$) d.b. - dry basis

Similarly, the ash content ranged between 8 and 11 %. Lee et al. (2007) working on protein and starch extraction in two varieties of lentil employing alkaline treatment (NaOH), found that the effect of pH (8 to 9.5) and temperature (22 to 40 ^{o}C), promoted a higher yield on the protein extraction, that ranged from 51.9 to 63.4 %, similar to the observed in this paper.

3.4 Electrophoresis

Electrophoresis analyses (SDS-PAGE) of protein extracts (T3) can suggest that they were composed by albumin and globulins, corresponding to fractions of 39-40 kDa, ~ 20 kDa and 6-7 kDa (Figure 2). Higher molecular weight fractions (50-95 kDa) were also observed. The bands with molecular mass between 20 and 40 kDa represent mainly the 11S globulins and their dissociated subunits, and albumins 4-6S (lectins) as observed by Youle and Huang (1978).

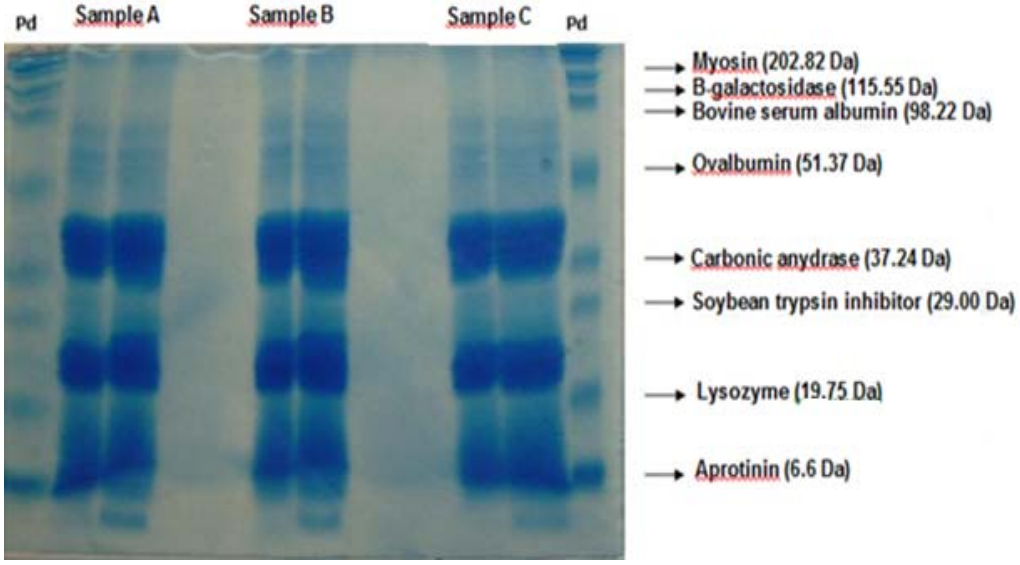

Figure 2: electrophoresis gel through 12 % polyacrylamide

4. Conclusions

The experiments of extraction of proteins from castor bean cake allowed choosing the best extraction parameters as temperature = 50 ºC, stirring speed = 400 rpm, cake concentration in extracting solution = 20 %; for pH = 9 and NaOH as alkalizing agent.

The freeze-dried protein extracts of castor bean constitute protein concentrates. These proteins can be interesting for the production of biodegradable materials. The residues obtained in the extraction process continued to have high protein levels.

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