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Some Physicochemical and Nutritional Properties of Castor Bean (*Ricinus communis*) Protein

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Defatted castor bean pomace (CBP) prepared by two different procedures was used for extraction and characterization of the proteins. Extractions were performed at pH 11 and at higher pH with 0.5% NaOH using a solid/liquid ratio of 1:5 (w/v) at 45 °C. The proteins of the two extractions at pH 11 and in 0.5% NaOH were precipitated at pH 5.8 and 4.5, respectively, and were isolated by filtration after 5 min of heating at 100 °C. These treatments eliminated the toxicity completely and apparently all of the allergens. Amino acid analysis of the isolates showed deficiencies in lysine and sulfur-containing amino acids. Feeding tests with weanling rats gave net protein utilization (NPU) ranging from 34 to 46%. Supplementation with 3% L-lysine plus 1.54% DL-methionine improved the NPU value from 37.5 to 49.2%.

The world production of castor bean seeds is 899 000 metric tons annually (F.A.O., 1974). After the extraction of castor oil the remaining material is the castor bean pomace (CBP), which contains 36% protein representing a source of 150 000 tons of protein per year.

The CBP contains a highly toxic albumin, ricin (Osborne et al., 1905), which can easily be inactivated by humid heat treatment (Jones, 1947). It also contains a powerful allergenic protein fraction which is more heat resistant (Coulson et al., 1960). Considerable effort has been made by many investigators to obtain a detoxified and deallergenized CBP for use in animal feedstuffs. Gardner et al. (1960) described the following treatments as promising: dry heating the CBP at 205 °C for 125 min or cooking under various conditions with alkali or acid, with or without added formaldehyde. Mottola et al. (1971, 1972a,b) described several procedures and proposed the following treatments: vapor cooking at 10 psi for 60 min; cooking at 80 °C for 45 min with ammonia; and cooking with lime at 120 °C for 15 min. Vilhjalmsdottir and Fisher (1971) described a hot water extraction to remove the growth-depressing factors of heat detoxified CBP.

Castor bean pomace was tested in animal feeding studies and was found to be an acceptable protein source for ruminants (Fuller et al., 1971; SANBRA, 1960; Weiss, 1971) and for chicks (Vilhjalmsdottir and Fisher, 1971), although its biological value is not very high. Other factors, such as the mildly toxic alkaloid ricinine and the residual oil, did not have adverse physiological effects on animals when administered in moderate doses (Fuller et al., 1971). Lowering the crude fiber content also increased the biological value (Vilhjalmsdottir and Fisher, 1971).

Isolation of the protein from CBP would permit the thermal detoxification of ricin as well as the elimination of most of the allergens because these compounds do not precipitate with the majority of the proteins. It also permits the elimination of fiber, thus improving the biological value of the proteins.

MATERIALS AND METHODS

Castor Beans. Dehulled castor bean seeds of the "Guarani" variety were used for all studies. They were obtained from the Oil Seed Collection of the Agronomic

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Table I. Composition of the Diets for NPU Determinations

Components	Rel amounts, %
Protein (N × 6.25)[a]	10
Soybean oil	8
Mineral mix[b]	5
Vitamin mix[c]	2
Sucrose	25
Corn starch	to 100

[a] From the protein isolates (I) or casein. [b] According to Rogers and Harper (1965). [c] Vitamin Diet Fortification Mixture from Nutritional Biochemicals Corporation (NBC).

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Preparation of Castor Bean Pomace (CBP). Two types of CBP were prepared: (a) E-CBP, by crushing the seeds and extracting the oil with ethyl ether; and (b) H-CBP, by crushing the seeds and cooking the resulting paste at 100 °C under controlled water content followed by hot hexane extraction that included suspension, agitation, and filtration, using the method described by D'Aquin et al. (1960). The two types of CBP were desolventized by a current of air.

Extractability of the Proteins. The influence of pH, NaOH concentrations, different salt concentrations, temperature, and time of extraction on the extractability of proteins from CBP was studied in aqueous solutions using a solid/liquid ratio of 1:5 (w/v). The suspensions were stirred for 30 min and the insoluble solids separated by centrifuging at 12000*g* for 10 min in a Sorvall RC-2 refrigerated centrifuge.

Isolation of Proteins from Alkaline Extracts. The proteins of E-CBP and H-CBP were extracted at pH 11 and in 0.5% NaOH solutions yielding four protein isolates (I). The final extract was obtained by combining the supernatants of four consecutive extractions after separation of the insolubles by centrifugation. The solid/liquid ratio was maintained at 1:5 (w/v) throughout all extractions. The proteins were isolated from the alkaline solutions by acidifying to the pH of minimum solubility (p*I*) and subsequently boiling for 5 min to detoxify the ricin and form a curd which was filtered off and vacuum dried.

Preparation of a Crude Protein Extract (CPE). The proteins of E-CBP were extracted in aqueous alkaline solution (pH 11) as in the case of the isolates. The pH of the extract was adjusted to 7 with concentrated HCl. The solution was then frozen and freeze-dried. The freeze-dried product was ground sufficiently to pass a 40 mesh screen and kept in the freezer until used in the feeding experiment.

Amino Acid Determinations on the Isolates (I). The amino acid determinations were performed on the acid hydrolysates using the Beckman Model 120C Auto-Analyzer and the procedure recommended by the manufacturer.

Nutritional Evaluation of the Proteins. Net protein utilization (NPU) was determined using the method of Miller and Bender (1955) and a casein-containing diet as a control. Groups of five weanling male rats (25 days old, weighing about 54 g) were allowed water and diet ad libitum for 10 days. Food intake and body weight were recorded every 2 days. The composition of the diets can be seen in Table I.

RESULTS AND DISCUSSION

Proximate compositions of the dehulled seeds and the two types of CBP prepared in the laboratory are shown in Table II. As expected, removal of the oil almost doubles the ash, crude fiber, and protein contents in both types of CBP compared to the dehulled seeds. The only appreciable difference in the compositions of the two types of CBP is the higher content of fat in H-CBP.

Table II. Proximate Composition of Dehulled Castor Bean Seeds and Pomaces (CBP)

Components	%		
	Seed	E-CBP	H-CBP
Moisture	6.36	8.10	7.72
Oil	47.91	0.95	2.57
Ash	2.41	5.15	5.05
Crude fiber	6.65	13.35	11.30
Protein (N × 6.25)	17.85	35.20	34.60

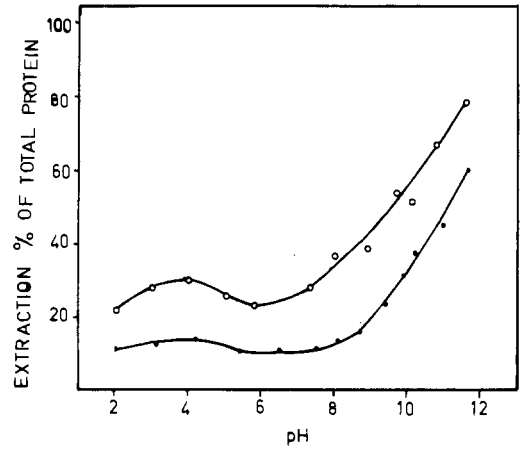

Figure 1. Extraction of the proteins from CBP at different pH values and room temperature (25–28 °C). The two types of CBP were suspended in aqueous medium, solid/liquid ratio of 1:5 (w/v), the pH adjusted with a 2 N solution of either NaOH or HCl, stirred 30 min, and then centrifuged at 12000*g* for 10 min. Protein (N × 6.25) was determined in the supernatants: E-CBP (○); H-CBP (●).

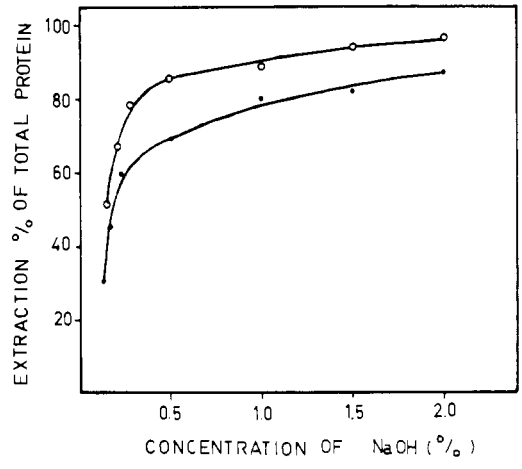

Figure 2. Extraction of the proteins from CBP at different NaOH concentrations. The two types of CBP were suspended in NaOH solutions of different concentrations, solid/liquid ratio 1:5 (w/v), centrifuged at 12000*g* for 10 min. Protein (N × 6.25) was determined in the supernatants: E-CBP (○); H-CBP (●).

Examining Figure 1 it is apparent that increasing the pH of the aqueous solvent starting with pH 2.0 resulted in increased extraction reaching a maximum at pH 3.8, decreasing to a minimum at pH 5.8, and then increasing rapidly and significantly as the pH increased. The extractability of proteins from H-CBP was considerably lower than from E-CBP. The extraction was practically maximum with 0.5% NaOH, as shown in Figure 2. Higher

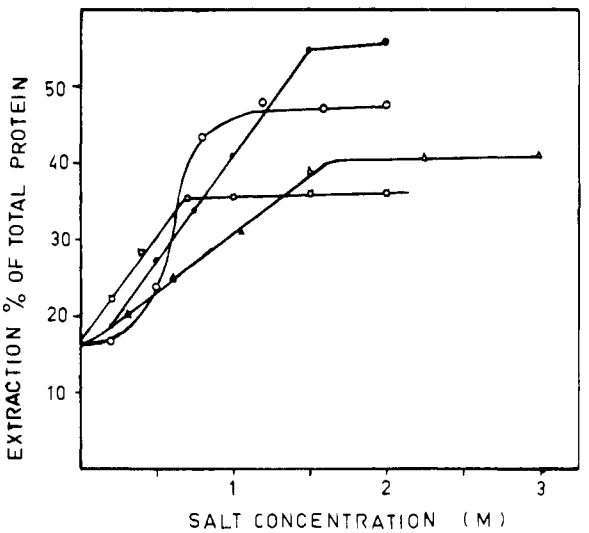

Figure 3. Extraction of the proteins of the H-CBP with different concentrations of salts, solid/liquid ratio 1:5 (w/v), centrifuged at 12000*g* for 10 min. Protein (N × 6.25) was determined in the supernatants: NaCl (•); $CaCl_2$ (○); Na_2SO_4 (□); Na_3PO_4 (△).

Table III. Yields of Extractions and Recoveries of Proteins from CBP

Pomace	Aq soln	Extraction, % of total protein	Recovery, % of extracted protein	Isolate
E-CBP	pH 11	86.0	63.8	EI (pH 11)
E-CBP	NaOH 0.5%	88.7	73.2	EI (NaOH 0.5%)
H-CBP	pH 11	58.2	38.7	HI (pH 11)
H-CBP	NaOH 0.5%	74.7	57.9	HI (NaOH 0.5%)

concentrations of NaOH increased the viscosity of the suspension affecting the separation of insoluble solids.

Using an alkaline solution (pH 11) as the extracting medium, better extractions were obtained at 45 °C and no further solubilization of the proteins occurred after 10 min of extraction for all temperatures tested, when a solid/liquid ratio of 1:5 (w/v) was used.

The addition of salts increased protein extractability compared to aqueous extraction (Figure 3). Maximum extraction for each salt solution was reached with 1.5 M NaCl, 1.25 M $CaCl_2$, 1.5 M Na_3PO_4, and 0.7 M Na_2SO_4. The range of extractability was 55% for NaCl and 35% for Na_2SO_4, the efficiency decreasing in the same order as above. The addition of salt did not increase the extraction when used at pH 11 or above.

Precipitation of the proteins from alkaline extracts (pH 11 or 0.5% NaOH) took place at the pH of minimum solubility (p*I*), 5.8 and 4.5, respectively. Heating at boiling temperature for 5 min under these conditions did not increase the amount of proteins obtained but it coagulated the precipitate, destroying the toxic protein ricin and facilitating its isolation by filtration.

Values for extraction and recovery of protein from the two types of CBP under the conditions used are shown in Table III. It is evident that both extractability and recovery of proteins are higher for E-CBP than for H-CBP. This may reflect the effect of heating on the CBP during extraction of the oil with hexane. This hypothesis is confirmed by the improvement in solubility and recovery when H-CBP was extracted with 0.5% NaOH solution compared to pH 11.

Table IV. Proximate Composition of CBP, Crude Extract, Protein Isolates, and Casein Used in the Biological Assays

Products	Moisture, %	Ash, %	Protein,[a] %
EI (pH 11)	6.52	1.65	92.2
EI (NaOH 0.5%)	4.97	1.91	90.3
HI (pH 11)	5.92	0.90	97.7
HI (NaOH 0.5%)	5.77	1.09	89.5
Crude extract	4.77	5.62	83.1
Casein	9.33	2.74	82.0

[a] Protein (N × 6.25).

Table V. Amino Acid Composition of the Protein Isolates (g of Amino Acid/16 g of N)

Amino acid	EI (pH 11)	EI (NaOH 0.5%)	HI (pH 11)	HI (NaOH 0.5%)
Lys	2.68	2.55	1.89	2.05
His	2.19	2.02	2.23	2.11
NH_3	2.27	2.18	2.38	2.29
Arg	12.97	12.88	15.07	13.33
Asp	14.20	13.47	12.94	13.50
Thr	4.23	4.08	3.20	3.53
Ser	6.81	6.74	6.58	6.79
Glu	25.33	26.04	27.58	29.68
Pro	4.58	4.24	4.29	4.22
Gly	4.97	4.84	4.53	5.00
Ala	5.36	5.18	5.06	5.06
Half-Cys	1.43	0.85	1.03	
Val	4.67	4.55	5.26	4.59
Met	1.50	1.37	1.20	1.33
Ile	3.73	3.61	3.86	3.53
Leu	7.71	7.38	6.26	7.02
Tyr	3.55	3.42	2.46	2.94
Phe	4.39	4.27	3.94	4.27

Table VI. Parameters Used for Determination of the NPU[a]

Dietary protein source	Initial body wt, g/rat	Wt change, g/rat	Protein intake, g/rat	Body protein g/rat	NPU_{op}, %
None	54.3	−10.6	0	7.2	
Casein	54.2	+29.5	8.8	13.0	67.0
EI (pH 11)	54.2	+6.8	7.0	9.7	37.0
EI (NaOH 0.5%)	54.0	+8.0	6.4	10.1	46.0
HI (pH 11)	54.5	+3.7	6.2	9.5	37.6
HI (NaOH 0.5%)	54.5	+5.5	7.0	9.5	34.0
HI (pH 11) Supplemented[b]	54.3	+17.5	8.6	11.4	49.0

[a] Mean values of groups of 5 rats fed the experimental diets for 10 days. [b] EI (pH 11) supplemented with 3% L-lysine and 1.54% DL-methionine.

The partial compositions of the four isolates, crude extract, and casein used in the feeding tests are shown in Table IV. The crude extract did not receive any heat treatment and it was used as a control to confirm the high toxicity of undenatured ricin.

Amino acid compositions of the four different isolates prepared, shown in Table V, revealed that heat treatment and increased alkalinity caused a greater destruction of certain amino acids, particularly lysine, threonine, tyrosine, cystine, and methionine. This destruction becomes critical principally in the cases of lysine and the sulfur-containing amino acids which are limiting amino acids in the castor

bean proteins. However, in the case of E-CBP, the alkalinity alone did not decrease the nutritive values (Table VI).

Feeding tests with rats showed the absence of toxicity in the isolates, proving the efficiency of the heat treatment. On the other hand, the rats that received crude extract protein in the diet showed 100% mortality within the first 5 days of the experiment. The average intake of food by these animals was very low (1.5 g/rat). Examination of the organs after death did not show any visible lesions, probably due to the potency of the toxin, which killed the animals at very low intake.

Protein intake, body weight gain, and NPU are shown in Table VI for the different treatments. One can see that the consumption of diets which contained the isolates and casein was similar, indicating good acceptability of the castor bean protein. Satisfactory food intake and healthy appearance of the animals at the end of the experiment testified to the complete detoxification of ricin.

The average of all NPU values of the isolates was 36% (similar to wheat gluten). The NPU of EI (pH 11) was 37% and that of EI (NaOH 0.5%) was 46%. The addition of 3% L-lysine and 1.54% DL-methionine elevated the NPU value of EI (pH 11) to 49 or 73% that of casein.

In relation to the presence of allergens no objective measurements were carried out on the different materials. However, during the work with CBP the senior author became sensitized by the allergens manifesting sneezing, respiration difficulties, and general discomfort. These manifestations were completely absent when working with the isolates, suggesting that the allergens must have been eliminated to a great extent, if not completely, by the isolation procedure. This indirect observation is confirmed by Coulson et al. (1960) who could not detect any allergenic or allergic reaction due to castor bean proteins precipitated by heat coagulation.

The results of this investigation suggest that castor bean pomace could be considered as a source for large scale production of protein isolates. Further studies should be carried out on the technological, economical, toxicological, and nutritional aspects of these products both as animal and possibly as human foods. One should also look for some functional properties and applications of these proteins other than as food ingredients.

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Studies on Factors of Solubilization of Insoluble Ovomucin during Thick White Thinning

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Insoluble ovomucin prepared in this experiment was successfully incubated under aseptic conditions for 15 days at 30 °C in various solutions. Half-amounts of insoluble ovomucin were gradually solubilized when they were incubated in a buffer solution of pH ~9.5 for 15 days. When the ionic strength of the buffer solution was 0.1, the solubilized parts consisted of a lot of the carbohydrate poor component and only a little of the carbohydrate rich component, but much carbohydrate rich component was solubilized when insoluble ovomucin was incubated in a 5% ovalbumin solution with an ionic strength of 0.1 at pH 9.6 or in a buffer solution with ionic strengths of 0.0001 and 0.001 at pH 10 without other components. From the presumption that a high concentration of ovalbumin may reduce ionic activity in solution, it was suggested that an increase of pH during storage, lowered ionic strength by egg white proteins, and raised storage temperature may be the main causal factors in the solubilization of the carbohydrate rich component from insoluble ovomucin.

Many workers have hitherto been concerned with clarifying the mechanism of thick white thinning and it has been accepted that the main factors causing the thinning are not microbiological but are inherent in the chemical properties of the egg white itself (Feeney et al., 1951; Baliga et al., 1964). A number of workers (Hawthorne, 1950; Cotterill and Winter, 1955; Brooks and Hale, 1959, 1961; Robinson, 1972) have proposed that interaction

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