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Some Chemical and Functional Characteristics of a Fiber-Free Coconut Protein Extract Obtained by the Enzymic Chemical Process

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The coconut protein extract obtained through the enzymic chemical process contained 32.4% protein and 42.6% of nitrogen-free extract. It also had a high total sugar content (39.2%) and a relatively small amount of reducing sugars (6.2%). Hydrolysis and paper chromatography were used to determine the composition of the total sugars. Column chromatography showed that 66% of the protein had a molecular weight higher than 5000. Only 41.3% of the protein proved to be nondialyzable, suggesting a molecular weight higher than 12 000. Such a protein fraction was electrophoretically homogeneous. The protein showed minimum solubility at pH 7.0 and 34.2% was heat coagulable at 120 °C. The extracted product was highly dispersible with an emulsifying capacity of 24.8 ml/g and did not form a gel at 15 and 30% levels. Preliminary tests showed possible practical applications of the extracted product in drinks and bakery products formulations.

Recently an enzymic chemical technique was developed to effect the protein extraction of coconut meal (Molina and Lachance, 1973). This technique was found to be equally effective for those meals obtained through a solvent or through an expeller oil-extraction process. The fiber-free coconut protein extract obtained has been found to have a higher nutritive value than the original coconut meal (Lachance and Molina, 1974).

More recent work has shown the enzymic chemical technique to be applicable to cottonseed meals which have undergone partial protein denaturation through the oil-extraction process (Childs, 1975). In addition, the same author (Childs, 1975) indicates that the total costs of the extraction, as well as the time to effect it, can be substantially lowered by using ultrasonic energy to activate both the enzymic and the chemical stages. These findings undoubtedly will accelerate the implementation of the enzymic chemical protein extraction technology for a better utilization of protein-rich by-products as coconut and cottonseed meals.

The implementation of an enzymic chemical protein extraction technology in Latin America would be advisable since most oilseed meals (coconut, cottonseed, and the like) are produced through dry oil-extraction processes which generally result in a partial denaturation of their protein.

Based on the above concepts it was thought of interest to define some chemical and functional characteristics of the coconut protein extract obtained through the original enzymic chemical technique as described by Molina and Lachance (1973).

MATERIALS AND METHODS

The coconut protein extract sample used was an aliquot of the freeze-dried extract obtained by Molina and Lachance (1973) and used for the nutritional evaluation studies (Lachance and Molina, 1974).

Moisture, nitrogen, ash, oil, and crude fiber were determined according to AOAC (1970). Protein determinations were carried out by the macro biuret colorimetric test as described by Bailey (1967) using bovine serum albumin fraction V (Sigma Chemicals Co., St. Louis, Mo.) as standard. Total sugars were determined by the phenol–sulfuric method (Dubois et al., 1956). Determinations of total reducing sugars were performed by the neocuproine-HCl method (Dygerts et al., 1965). Both total sugars and total reducing sugars were expressed as glucose. Total free amino acids were determined by the ninhydrin colorimetric method as described by Rosen (1957) using leucine as a standard. Glucose and galactose were determined using the glucostat and galactostat enzymatic reagent (Worthington Biochemicals Corp., Freehold, N.J.), respectively, and following the methods detailed in the Worthington Biochemicals Corp. Publications No. 4-68 and 4-66 for each of the reagents. All determinations were carried out in triplicate.

The dialysis experiments were done with a cellulose dialyzer tubing (1.59 cm diameter) retaining materials with a molecular weight of 12 000 or higher. In all cases the dialysis was carried out for 48 h at room temperature (25 °C) against running water using chloroform as a preservative. The sample was 100 ml of a 10% aqueous suspension of the material.

The identification of simple sugars was performed by descending paper chromatography using Whatman No. 1 chromatographic paper. The chromatogram was developed for 22 h at room temperature (25 °C) using the upper layer of an ethyl acetate–water–pyridine (2:2:1) mixture as solvent, as recommended by Jermyn and Isherwood (1949) for the separation of simple sugars. The sugars were visualized by the method described by Pintauro (1967) using aniline oxalate. Reagent grade samples of glucose, galactose, arabinose, xylose, and mannose were used as standards; the samples utilized for this test were both an aqueous suspension of the protein extract and an aliquot of this aqueous suspension which had been treated with

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a combination of Takamine cellulase 4000 and Takamine hemicellulase Ce-100 (Miles Laboratories Inc., Elkhart, Ind.) at an enzyme concentration of 1 mg of each enzyme per g of the extract. Such enzymatic treatment was carried out at 50 °C and a pH of 4.8 with constant agitation for 24 h, using toluene as a preservative. No R_f values were calculated since the chromatogram was developed for 22 h, and the solvent had dripped considerably.

The column chromatography experiments were conducted using a Sephadex column (Pharmacia type K 25/45) with a diameter of 2.5 cm and a length of 45 cm. The gel used was Sephadex type G-25 fine (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) suspended in a 0.05 M Tris-maleate buffer (pH 8.6). The bed was 32 cm long having a total volume of approximately 170 ml. The column thus prepared was found to have a void volume of 60 ml using blue dextran 2000 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) and a flow rate of approximately 0.5 ml/min. The sample applied was 7 ml of the supernatant from a 5% suspension of the freeze-dried coconut protein extract in 0.05 M Tris-maleate buffer (pH 8.6), centrifuged at 27 000*g* at 10 °C for 30 min. All protein was in the supernatant. The column elution was carried out for 24 h at room temperature (25 °C) using 0.05 M Tris-maleate buffer (pH 8.6) saturated with chloroform as the eluent. All fractions collected (69, with a volume of 10 ml) were subjected to protein and total amino acid determinations.

Two samples were used for the disc gel electrophoresis experiments. Sample 1 was the supernatant resulting from the centrifugation (27 000*g*, for 30 min at 10 °C) of the nondialyzable fraction of a 10% suspension of the material in Tris-glycine buffer (pH 8.9), dialyzed against the same buffer for 18 h at 4 °C using the same cellulose dialyzer tubing as in the dialysis experiments mentioned above. Sample two was the supernatant obtained when centrifuging (27 000*g*, for 30 min at 10 °C) a 10% suspension of the protein extract, in the same Tris-glycine buffer (pH 8.9), diluted to the same volume as that of the nondialyzable fraction used to prepare sample 1. The disc gel electrophoresis apparatus used was purchased from Buchler Instruments. A Beckman Spinco Duostat power supply was used to control a constant current of 4 mA per tube of gel. The techniques applied for the preparation of the polyacrylamide gels and the composition of the gel system were the same as those utilized by Ranadive (1971). The gels, which were about 6 mm in diameter, were prepared with about 1.5 ml of gel solution. The upper buffer used in the gel system was Tris-glycine (pH 8.9). Electrophoresis was carried out for 20 min at a constant current of 4 mA per tube and a gradient of 100–200 V. Bromophenol blue served as the marker dye for the anionic system applied. The protein bands were stained with coomassie blue (Ranadive, 1971).

The protein solubility was determined at different pH values (range 5.0–8.6) using a 0.05 M Tris-maleate buffer system. A 5% suspension was prepared at each pH tested. The suspensions were then subjected to a reciprocal agitation (120 strokes per minute) in a constant temperature water bath (New Brunswick Scientific, Model R76) at 30 °C, for 10 min. After this time, the suspensions were centrifuged at 27 000*g* for 40 min at 10 °C, and protein determinations were carried out in the supernatants.

The heat coagulability of the protein from the extract was studied at two temperatures (100 and 120 °C) for different times (15, 30, 60, and 120 min) using a 5% aqueous suspension of the material (pH 6.9). After the incubation period the samples were centrifuged at 27 000*g*, for 40 min at 10 °C, and protein determinations were carried out in the supernatants.

Table I. Percent Composition of the Freeze-Dried Coconut Protein Extract as Obtained by the Enzymic Chemical Extraction Process (Dry Basis)

Component	Extract
Ether extract	15.8
Crude fiber	0.0
Protein (N × 6.25)[a]	32.4 (29.7 ± 1.2)[b]
Ash	9.2
Nitrogen-free extract	42.6
Total sugars[c]	39.2 ± 1.2
Reducing sugars[c]	6.2 ± 0.8
Glucose	3.3 ± 0.6 (36.0 ± 1.3)[d]
Galactose	2.1 ± 0.4 (2.8 ± 0.6)[d]

[a] The protein conversion factor of 6.25 was used as suggested by Molina and Lachance (1973). [b] Obtained through the biuret colorimetric method. [c] Expressed as glucose. [d] Values obtained after the enzymatic hydrolysis using a combination of cellulase and hemicellulase.

The ability of the extract to form a gel was tested in 15 and 30% aqueous suspensions of the material. The suspensions were heated to approximately 93 °C (boiling water bath) for 15 min, cooled to room temperature, and refrigerated overnight. A 10% aqueous suspension of Promine-D (Central Soya, Chicago, Ill.) was used as a standard. The test samples were compared visually with the standard.

The emulsifying capacity of the extract was determined according to the method described by Swift et al. (1961). The oil used was cottonseed oil (General Biochemicals, Chagrin Falls, Ohio). The test was carried out at 35 ± 1 °C. Carnation skim milk powder was used as a standard.

RESULTS AND DISCUSSION

The percent composition (dry basis) of the freeze-dried coconut protein extract is presented in Table I. In general, the proximate composition data are very similar to those reported previously for the same material (Lachance and Molina, 1974; Molina and Lachance, 1973). As the data reveal, the main constituents of the extract are protein (29.7 or 32.4%, as determined by the biuret method or by multiplying the nitrogen content by 6.25, respectively) and the nitrogen-free extract (42.6%). The amino acid composition and the nutritive quality of the protein have been described elsewhere (Lachance and Molina, 1974). The nitrogen-free extract consists mainly of nonreducing sugars, since only 15.8% of the total sugars were found to be reducing sugars. This finding suggests that the main carbohydrate fraction of the freeze-dried, fiber-free coconut protein extract obtained by Molina and Lachance (1973) exists in a polymerized form. The total sugar fraction represents 92% of the nitrogen-free extract.

Of the total (6.2 ± 0.8%) reducing sugars determined in the extract, 53.2% was glucose and 33.9% was galactose. Furthermore, after the enzymatic treatment of the extract with a combination of cellulase and hemicellulase, 91.8% of the total sugars were found to be glucose, and only 7.1% galactose. Thus, the polysaccharides present in the extract are mainly glucose polymers while galactose seems to occur primarily only in the free form.

The paper chromatography confirmed the identity of the determined sugars, glucose and galactose; no other spot was detected through these analyses.

These findings are in accordance with those of Chandrasekaran and King (1967) who reported glucose and galactose as the major sugar components of a coconut meal extract.

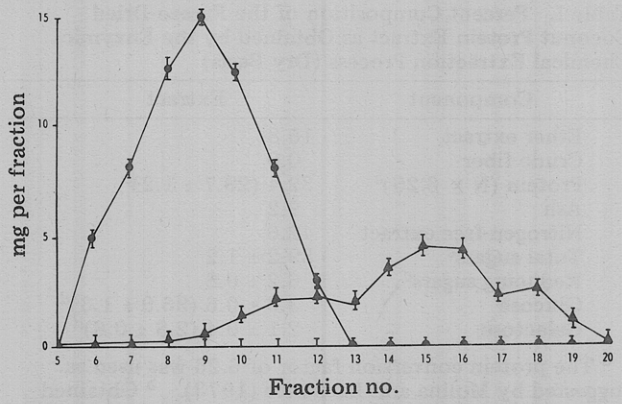

Figure 1. Protein and amino acid (expressed as leucine) content of each fraction (10 ml) collected, as determined by the biuret and ninhydrin colorimetric methods, respectively: (●) protein; (▲) amino acid.

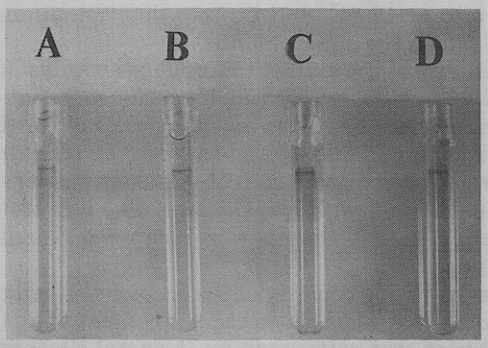

Figure 2. Protein pattern of coconut protein extract (tubes C and D) and its nondialyzable fraction (tubes A and B), separated by polyacrylamide gel electrophoresis. The protein bands were stained with coomassie blue.

Although the oil content appears to be quite high (15.8%), suggesting that the extract is a good caloric source, such a component was not analyzed further since its concentration in the product appears to be a function of the residual oil content of the specific coconut meal used as raw material for the extraction (Molina and Lachance, 1973).

The column chromatography results, using a gel Sephadex type G-25 fine column, which elutes compounds with a molecular weight higher than 5000, are given in Figure 1.

A 66% yield of the crude protein of the extract, calculated as N × 6.25 (Molina and Lachance, 1973), was eluted, indicating a molecular weight of 5000 or more. The crude protein not accounted for by the biuret method appears to consist mainly of free amino acids, as shown by the ninhydrin determinations on fractions 13–20. On such fractions, 27% of the crude protein could be accounted for as free amino acids, expressed as leucine.

The dialysis experiments indicated that 41.3% of the protein was nondialyzable, suggesting that around 25% of the protein eluted through the column chromatography had a molecular weight varying between 5000 and 12 000.

The disc gel electrophoresis results are presented in Figure 2. Three different protein bands were found in the case of the extracted product, while only one could be found in the case of its nondialyzable fraction. It is assumed that the two bands not seen in the nondialyzable fraction of the extract belong to those protein fractions of a molecular weight ranging between 5000 and 12 000 indicated by the column chromatography and the dialysis experiments cited above. Furthermore, the electrophoretical results obtained with the nondialyzable fraction

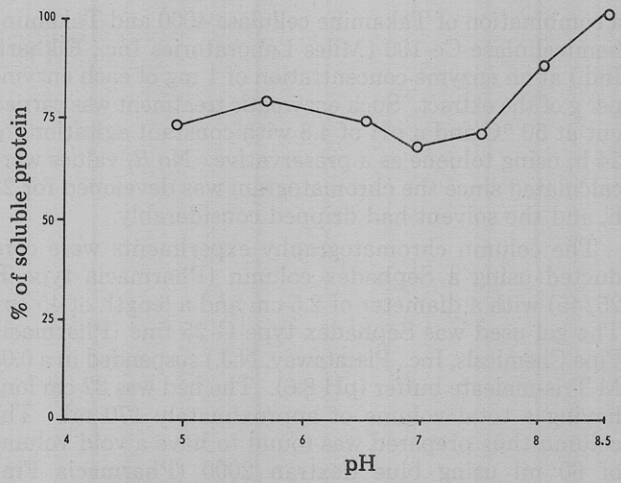

Figure 3. Protein solubility as a function of pH determined for the freeze-dried extract.

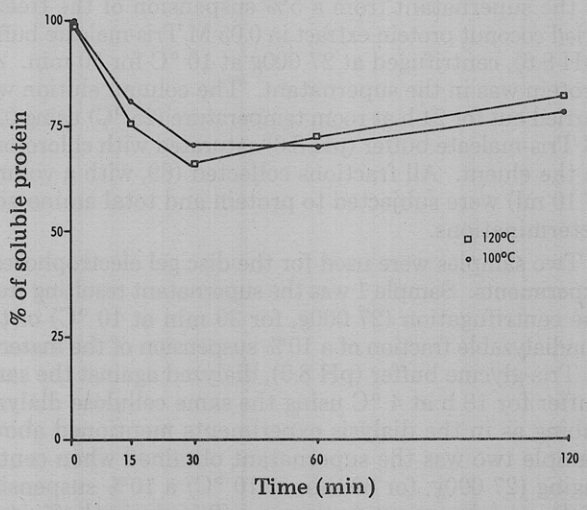

Figure 4. Effect of heating time on the protein solubility of the freeze-dried extract using temperatures of 100 and 120 °C.

indicate that, under the experimental conditions used, the fraction of the extracted protein with a molecular weight of 12 000 or higher is quite homogeneous.

The solubility of the extracted protein at the pH values tested is presented in Figure 3. As the data show, the protein presented its minimum solubility at a pH of 7.0; it began to decrease again at the lower pH tested (4.95), a fact which suggests the probable presence of a second point of minimum solubility at a lower pH as reported by other authors (Samson et al., 1971) for coconut meal proteins.

The heat coagulability patterns obtained at the two temperatures tested (100 and 120 °C) for different periods of time are presented in Figure 4. As the data indicate, a short-term heating (30 min) resulted in more insolubilization of the protein material than a relatively longer heating period (2 h), a characteristic which was observed at the two temperatures tested. Since these findings highly resemble those reported by Fukushima and Van Buren (1970) for soy-milk proteins, it is feasible that a similar explanation as that given by these authors (Fukushima and Van Buren, 1970) to explain the decrease in protein insolubilization through prolonged heating may apply in the present case. It is of interest to note that the maximum protein insolubilization attained after heating at 120 °C for 30 min (34.2% of the total protein) falls within the 41.3% of the total protein content found to be nondialyzable.

The emulsifying capacity of the extract was found to be 24.8 ± 1.1 ml per g of extract, thus indicating a relatively low value for this property. A rise in this value could probably be expected in an extracted product with a lower oil content than the present one. The extract was found not to form a gel either at 15 or 30% concentrations after being heated in boiling water for 10 to 15 min and refrigerated overnight. Through these tests it was found that the extract was highly dispersible in water at room temperature without presenting any sedimentation at all.

Milk drinks prepared with either 6 or 10 g of extract per cup of milk proved to have an acceptable flavor, texture, and general appearance, thus indicating the possibility of using the material. Cookies prepared by substituting 15 or 30% of the regular wheat flour with the extracted product proved as well to have an acceptable flavor, texture, color, and general appearance, indicating also the feasibility of using the extract in bakery product formulations. During these tests it was observed that the extract had some whippability characteristics.

The acceptability tests of the above products were carried out using ten semitrained panelists and the consumer preference test described by Kramer and Twigg (1966).

From the preceding findings and observing some relationships between chemical and functional properties with possible practical applications as recently reported by Wolf (1970), there remains little doubt that the coconut extracted material obtained through the enzymic chemical technique as reported by Molina and Lachance (1973) could be used for the manufacture of conventional food products, while concomitantly enhancing the total content and probably the quality (Lachance and Molina, 1974) of the protein in the product. Nutritionally this would be so, particularly in cereal products where lysine is the most limiting essential amino acid, since Lachance and Molina (1974) have shown the coconut protein extract to be especially rich in this amino acid. Furthermore, the total protein content of the extracted product exceeds by far that of most cereal flours or products.

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Gas Chromatographic Analysis of Oryzalin Residues in Agricultural Crops and Soil

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A method is described for determining residues of oryzalin (trademark Surflan, Elanco Products Co., Indianapolis, Ind.) [3,5-dinitro-N^4,N^4-di(*n*-propyl)sulfanilamide] in crops and soil. The samples were extracted with methanol and cleanup was carried out by liquid–liquid partitioning and column chromatography on alumina using 95:5 benzene–ethyl acetate as eluent. Oryzalin was converted to the N^1,N^1-dimethyl derivative for measurement by gas–liquid chromatography using an electron capture detector. Recoveries of oryzalin from crops fortified at 0.05 ppm averaged 72%. Soil samples fortified at 0.1 ppm showed an average recovery of 92%. Residues as low as 0.01 ppm may be detected.

Oryzalin, 3,5-dinitro-N^4,N^4-di(*n*-propyl)sulfanilamide (I), is a selective, preemergence herbicide for the control of certain annual grasses and broadleaf weeds in soybeans and other selected crops (Burnside, 1970; Elmore, 1972). Unlike the herbicide trifluralin (II), oryzalin cannot be quantitatively estimated directly by gas chromatography. Under a wide range of operating conditions, the gas chromatographic peaks are broad and exhibit considerable tailing, making quantitative assessment uncertain. The reduction in theoretical plate efficiency shown by the gas chromatographic peak is most probably associated with the greater polarity and hydrogen bonding potential of the sulfonamide group compared with the trifluoromethyl substituent of trifluralin. When oryzalin is converted to 3,5-dinitro-N^1,N^1-dimethyl-N^4,N^4-di(*n*-propyl)sulfanilamide (III), the derivative exhibits excellent gas chromatographic characteristics. Sulfonamides which have hydrogen atoms on the nitrogen atom can be alkylated by

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