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Functional Properties of Coconut Protein Isolate Obtained by Ultrafiltration*

PRATAP CHAKRABORTY

Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta-700 032, India

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Ultrafiltration yielded 15% more coconut protein than acid coagulation. The protein content of the cream separated from coconut milk was 88% which was slightly lower than that obtained from acid precipitation. There was only 3.3% solid loss in the permeate during ultrafiltration. The protein obtained by ultrafiltration had superior functional properties and had higher chemical score due to better amino acid profile.

With the increase in population and wide prevalence of protein malnutrition, attempts are being made to utilise protein from several unconventional sources and their functional properties worked out[1]. Coconut cake contains 23 per cent nutritionally balanced protein. Because of its high fibre content its potential use in food industry as such is limited[2]. The coconut would be a valuable source of high grade protein if a suitable method of oil extraction could be devised. The traditional method of oil extration is to dry the kernel of the coconut to give *copra*, which is pressed in expeller at a high temperature. The residue containing the protein is not fit for human consumption and the oil is of poor quality. It is possible to extract oil and protein from fresh coconuts without subjecting it to long periods of drying or high temperature. Considerable work has been carried out on wet processing of coconuts for the simultaneous recovery of oil and proteins[3-5].

Ultrafiltration (UF) and reverse osmosis (RO) have been tried in a majority of food processing operations relating to dairy industry[6-9]. Food Protein Research and Development Centre, Texas A and M University, USA has tried to isolate protein from glandless cottonseed, peanut and soybean using membrane systems and various factors like flow rate, temperature and pressure for protein isolation have been reported. Most of the work reported so far was performed on cellulose acetate membranes, which have limited operating temperatures, in most cases below 50°C and pH between 3 and 8. The membrane technology has been developing rapidly and membranes tolerating high temperature, different pH and remarkably high permeation rate have been developed. The initial rates of feed solutions greater than 50 gal/ft^2/day (gfd) are not uncommon.

The objective of the present work is to evaluate the functional characteristics of coconut protein obtained by the combined aqueous extraction and membrane concentration processes. The various parameters like the effects of pressure, feed velocity, temperature and feed composition on permeate flux using different membranes and membrane systems, were also investigated.

Materials and Methods

Preparation of protein concentrate by ultrafiltration process: Nuts were broken and meat removed from the shell along with testa, washed with coconut water and cut into small bits using the Urschell mill. (Fig 1.) The shredded meat was ground in a hammer mill (The Fitzpatrick Co) fitted with 80 mm screen, followed by grinding in a disc attrition mill (Baur Bross Co) with minimum clearance of the discs. The ground coconut meat was placed in a jacketed stainless steel tank with a mixer attachment and heated to 60°C. The thick paste was pressed in a pulp press at 40 psig. The extraction procedure was repeated for higher yield of oil and protein. The coconut milk obtained from pulp press was passed through a vibrating screen (120 mesh) to remove the residue, and the pH of the filtered milk was adjusted to 7.0 with IN sodium hydroxide. The coconut milk which is an emulsion of water, oil and protein, was centrifuged in a 3 phase Westfelia separator (Model LWA 205-1) to collect the cream (emulsion containing 63-66 per cent oil). The emulsion was chilled

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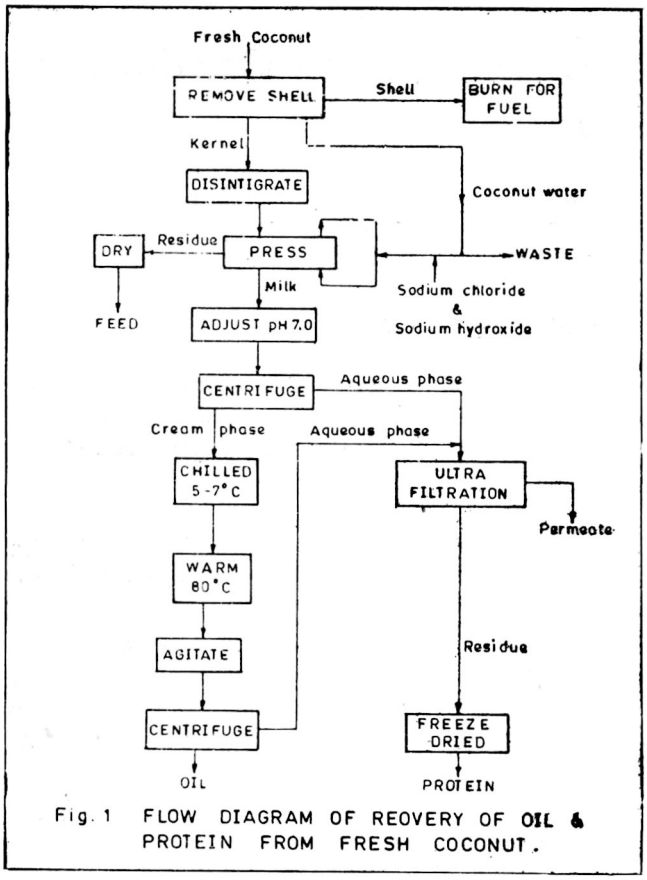

Fig. 1 FLOW DIAGRAM OF REOVERY OF OIL & PROTEIN FROM FRESH COCONUT.

(5–7°) for 2 hr, heated to 80°C and agitated with a high velocity pump to invert the emulsion[10]. The broken emulsion was centrifuged (5000 ×g) to separate the oil.

Pooled aqueous phase (from two centrifugation steps) was utilized to separate the protein concentrate through ultrafiltration. Two types of modules from the DDS generation have been examined, first the DDS–40–3–GR6–type with a 4 m^2 membrane area and the latter the DDS–30–GR6–type with a 4.5 m^2 membrane area. The feed solutions were pumped to each membrane system at various temperatures and pH. Feed solutions were processed in volumes of 20–50 gallons. The aqueous phase was passed through membrane and recirculated until the final concentrate contained more than 25 per cent dry matter. The concentrate was then freeze dried.

Preparation of coconut protein isolates by acid coagulation: The ground coconut meat was dried under vacuum at 40°C to a moisture content of 3 per cent. The dried material was subject to solvent extraction (hexane) for oil recovery. The protein was recovered from defatted coconut meal by the following three different methods:

(*i*) Extraction at pH 10.5 and precipitation at 4.0 (P_1).

(*ii*) Extraction at pH 7.0 by 1 M NaCl and precipitation at pH 2.0 (P_2).

(*iii*) Proteins extracted at pH 2.0 and precipitated at pH 4.0 (P_3). The precipitates were separated by centrifugation, washed, neutralized to pH 7.0 and freeze dried.

The protein samples were ground to pass through 100 mesh sieve in a micro analytical mill. Moisture, total solids, oil, ash and nitrogen solubility index were determined according to standard AOCS[10] method. The nitrogen solubility profile was determined by AOCS[11] method.

Gel Electrophoresis: Polyacrylamide gel electrophoresis was carried out by the method of Weber and Osborne[12] with slight modifications in the preparation of gel, buffer and preparation of sample for electrophoresis. The buffer was prepared by dissolving 7.8g of $NaH_2PO_4.H_2O$, 38.6g $Na_2HPO_4.7H_2O$, 2.0g SDS per liter of distilled water. The incubating and dialysis buffers were both 10 mM sodium phosphate, pH 7.0; the former contained 1 per cent β-mercaptoethanol and 1 per cent SDS and the latter contained 0.1 per cent of each of these reagents. Acrylamide solution was prepared by dissolving 22.2 g of acrylamide and 0.3g BIS in 100 ml of distilled water. The gels were stained in Coommassie Brilliant Blue prepared by dissolving 1.25g of the dye in 500 ml of a solution composed of 454 ml of 50 per cent methanol, and 46 ml of glacial acetic acid. The destaining solution was prepared by mixing 75 ml of glacial acetic acid, 50 ml methanol and 875 ml of distilled water.

Amino acid analysis: Amino acid analyses were conducted on a Beckman Model 120C analyser using the two column procedure of Spackman *et al.*[13] after hydrolysis of 35 mg of sample with 7 ml 6N HCl under vacuum in a sealed ampule for 24 hr at 110°C. Cystine and methionine were measured as cysteic acid and methionine sulfone by performic acid oxidation of the samples followed by hydrolysis with 6N HCI as above. For tryptophan analysis, 35 mg of sample were hydrolyzed with 14 per cent barium hydroxide under vacuum at 110°C for 18 hr. After neutralization and Ba^{++} precipitation, aliquots of the buffered hydrolysate were analyzed for tryptophan content on a 10 cm column of Type PA–35 resin. The standard deviation for the determination of each amino acid was calculated. The calculations of chemical scores were based on first-limiting amino acid relative to the amino acid requirements of the rats as recommended by Carpenter and Bjarnason[14].

Water absorption: The water absorption characteristic was determined by mixing protein samples with water and centrifuging the protein dispersion. The amount of water retained in the protein pellet after

centrifugation was reported as water absorption. The protein concentration was 10 per cent and samples were centrifuged at 1600×g for 25 minute.

Fat absorption: Fat absorption characteristics were measured by adding 4g of sample to 24 ml of corn oil in a 50 ml centrifuge tube. The contents were stirred for 30 sec every 5 min and after 30 min the tubes were centrifuged at 1600×g for 25 min. The free oil was decanted and percentage of absorbed oil determined by differences.

Emulsification: The experiments were carried out with a constant amount of water, salt and protein (100g) in a 400 ml flask filled to an omnimixer. The emulsification was performed with a stirrer at a rate of 10,000 rpm. The salt concentration was 0.75 per cent in water phase. Before adding fat, the protein was dispersed for 30 min. The refined soybean oil was added at a rate of 1 ml/sec until the formed emulsion collapsed is determined by change of viscosity. The amount of oil was determined by weighing.

Whipping properties: Foaming capacity or whipping of the samples was studied according to the method of Coffmann and Garcia[15]. One gram of sample was whipped with 100 ml distilled water for 5 min in a Waring Blender of speed setting "HI" and was poured into a 250 ml cylinder and the total volume at time intervals of 0.0, 0.5, 1.0, 2.0, 2.5, 3.0, 6.0, and 24 hr was noted. The volume increase (%) was calculated according to following equation:

$$\text{Volume increase}(\%) = \frac{\text{Vol. after whipping} - \text{Vol. before whipping}}{\text{Vol. before whipping/ml} \times 100}$$

All the experiment were conducted at room temperature (28°C).

Gelation properties: Dried isolate 10 per cent was suspended in distilled water, mixed well and heated in boiling water bath for 10 min and cooled in a refrigerator. The gel strength was measured at 23°C immediately after gelation, using a Brookfield Viscometer (Model LVF) and Helipath Stand (Model C) equipped with a T-B spindle and operated at 2.5 rpm. Results were reported in centipoise (cps). Average of a number of reading was recorded.

Enzymic hydrolysis: Protein hydrolysate was prepared by use of Alkalase (Novo Industri A/S Denmark). The following hydrolysis parameter as reported by Chakraborty *et al.*[17] were used. Substrate concentration 10 per cent protein, enzyme concentration 2 per cent alkalase 0.6L, temperature 50°C and pH 8.0. The protein suspension was heated to 90°C in a water bath for 12 minute and immediately cooled to 50°C before addition of enzyme (Fig 2). The pH was maintained with 4N NaOH during hydrolysis by means of Radiometer pH-stat equipment. The hydrolysis was terminated after desired reaction time by heating to 85°C for 5 min and cooled immediately to room temperature. The suspension was adjusted to pH 7.0, centrifuged and freeze dried.

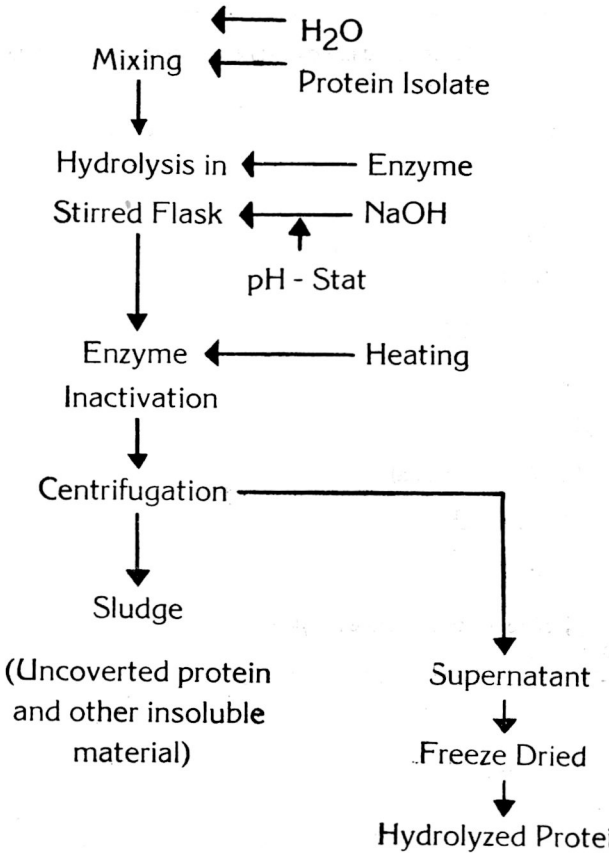

Fig 2. Flow diagram for the preparation of enzymic hydrolyzed coconut protein isolate.

Results and Discussion

Fig 1 shows the recovery of oil by aqueous processing and protein by ultrafiltration method. The relative chemical composition, yield and some physicochemical characteristics of coconut kernel, coconut protein isolates obtained by various methods are presented in Table 1. The protein content of the isolates prepared by isoelectric precipitation were above 90 per cent. The relatively high proportion of other constituents decreased the protein content in the concentrate obtained by ultrafiltration. The protein yields on the basis of total protein present in the coconut kernel were not identical in various isolates prepared. The protein yield by acid extraction at pH 2.0 was minimum (isolate P_3, Table 1). The yield of protein was higher by NaCl extraction method (P_2). The ultrafiltration process demonstrated highest protein yield which is 15 per cent more compared to other extraction pro-

TABLE 1. CHEMICAL COMPOSITION* AND PHYSICO CHEMICAL CHARACTERISTICS OF COCONUT PROTEIN[a]

Constituent	Coconut kernel	Coconut protein isolate			
		Isoelectric			Ultrafiltered
		P_1	P_2	P_3	
Moisture (%)	36	2.0	1.8	2.2	2.5
Protein (%) (Nx 6.25)	4.5	91.2	93.2	92.0	88.5
Ether extractives (%)	41.5	0.9	9.8	1.1	1.2
Fibre (%)	3.5	0.2	0.3	0.2	0.6
Ash (%)	1.1	1.5	1.8	1.5	2.3
Yield (% of total protein)	—	46	49	28	64.5
Salt soluble protein[1]	97	91	15	13	95
Molecular wt	—	13000 to 57000	13000 to 57000	26000 to 57000	13000 to 57000

[1]As % of total kernel protein (μ =0.5, pH 7.2)
*Dry basis
a—The standard deviation for result is approximately 2% of the value in the table.

cedures. Protein obtained through ultrafiltration showed maximum solubility at the measured pH due to less denaturation. The low yield of protein isolate (P_3) showed lack of low molecular weight protein sub units as evidenced by polyacrylamide gel electrophoresis pattern. All other protein isolates showed the same molecular weight having seven sub units ranging from 13,000 to 57,000 MW.

The continuous ultrafiltration method consists of number of steps which may be expanded according to bulk capacity of the plant within an economical limit. Two types of modules from the DDS generation had been examined; first the DDS–40–3–GR6 type with a 4m² membrane area and afterwards the DDS–30–GR6 type with 4.5m² membrane area. Fig 3 demonstrated the membrane capacities as a function of average pressure. The capacity reached at its maximum at around 5 kg/cm² at 20°C where 25 per cent protein from coconut extract may be obtained. The pressure applied in the ultrafiltration system was inversely proportional to per cent protein in the extract (Fig 4). The

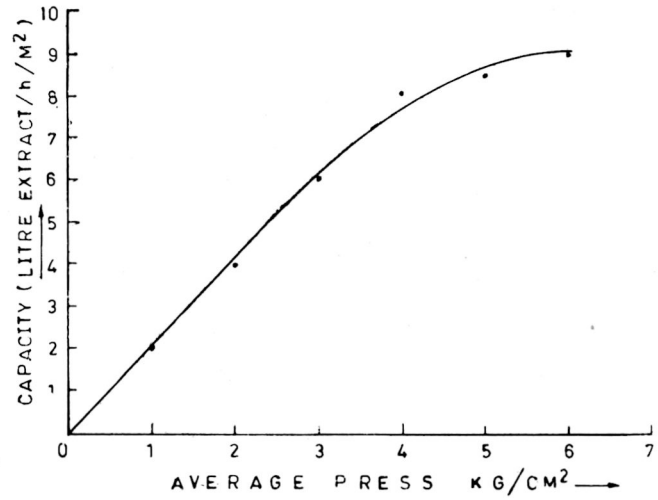

Fig. 3. Membrane capacity as function of average pressure for ultra filtration of coconut extract to 25% protein at 20°C.

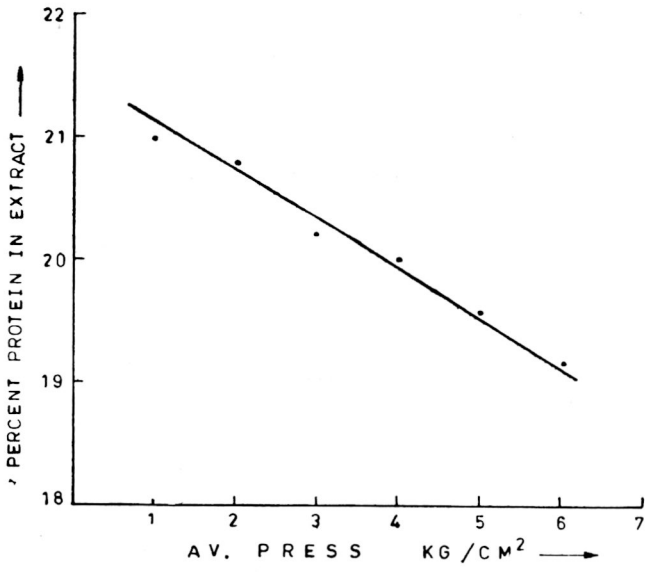

Fig. 4. Coconut protein ratio as functions of average pressure for ultrafiltration of coconut extract at 20°C.

TABLE 2. PERFORMANCE OF A CONTINUOUS ULTRAFILTRATION SYSTEM

Performance characteristics	First step DDS-40-3-GR6 with 4m² membrane area	Second step DDS-30-GR6P with 4.5m² membrane area
Mean flux (1/3hr/m²)	23.5	15.1
pH	7.5	7.4
Temperature (°C)	50	47
Protein conc (%)	10.7	25.2
Permeate	0.9	1.1
Solid conc (%)	12.5	28.4
Permeate	2.7	3.3

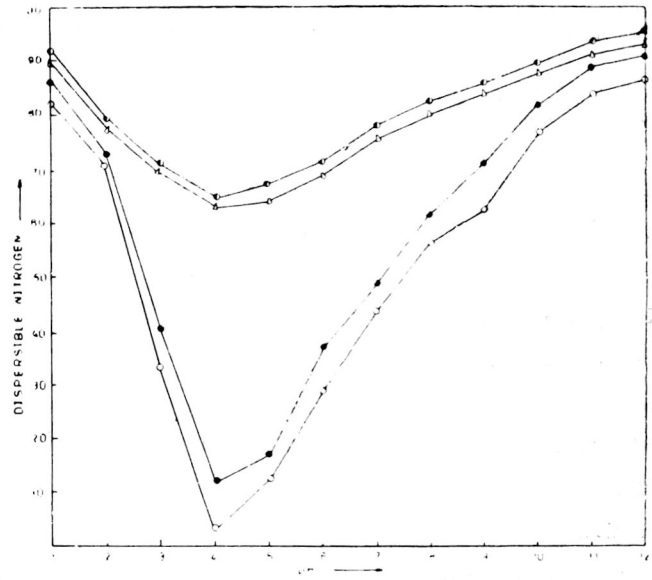

Fig 5. SOLUBILITY PROFILE OF COCONUT PROTEIN
ACID COAGULATED ULTRAFILTERED
HYDROLYSED ACID COAGULATED
HYDROLYSED ULTRAFILTERED

results of Fig 3 and 4 together were considered for optimum membrane capacity for the production of coconut protein isolate by ultrafiltration. Table 2 demonstrates the performance of a continuous ultrafiltration system where the flux decreased more in the second step probably due to higher protein concentration in the membrane surface. The total solids lost in the second step of ultrafiltration as permeates was only 3.3 per cent which was appreciably lower than isoelectric precipitation method. The nitrogen solubility profile of ultrafiltered protein showed higher solubility compared to acid coagulated isolate (P_1) (Fig 5). This may be due to less denaturation of proteins. The solubility of protein hydrolysate increased appreciably in the entire pH region. The change of solubility around pH 3.6 was remarkable due to hydrolysis of proteins.

The 10 per cent aqueous dispersion of the protein isolate and hydrolysed product demonstrated slight variation in pH (Table 3). The functional characteristics of ultrafiltered protein showed highest values (Table 3). However, the protein isolate (P_1) was very close to ultrafiltered sample in all the functional properties studied. The other two protein isolates (P_2 and P_3) were particularly low in all the physico-chemical characteristics as would be expected from their low nitrogen solubilities in this pH range (Table 1). Both the hydrolysed products demonstrated almost same functional properties which were appreciably below the values for the isolates.

TABLE 3. FUNCTIONAL PROPERTIES OF COCONUT PROTEIN ISOLATES AND HYDROLYSED PRODUCTS

Protein product	pH of dispersion	Water absorption (%)	Fat absorption (%)	Oil emulsification (%)	Vol. increase on whipping (%)	Brookfield viscosity of heated 10% slurries (cps)
Isolate (P_1)	7.0	310	395	38	329	6200–41600
Isolate (P_2)	6.9	186	199	21	30	133–3300
Isolate (P_3)	6.9	177	117	23	81	1500
Isolate (Ultrafiltered)	7.3	343	416	39	378	33200
Hydrolysed product Isolate (P_1)	7.1	84	121	11	68	—
Hydrolysed product (ultrafiltered)	7.2	79	117	9	59	—

TABLE 4. ESSENTIAL AMINO ACID CONTENTS (G/100G PROTEIN) AND INDICES OF COCONUT PROTEIN AND HYDROLYSED PRODUCTS

Essential amino acid	FAO provisional pattern[1]	Coconut kernel	Protein isolate (isoelectric P_1)	Protein isolate (ultrafiltered)	Hydrolysed protein (isoelectric P_1)	Hydrolysed protein (ultrafiltered)
Isoleucine	4.0	3.7	3.5	3.6	3.3	3.5
Leucine	7.0	7.0	7.0	8.9	7.2	9.1
Lysine	5.5	3.8*	3.3*	4.1*	3.5*	4.2*
Methionine+cystine	3.5	4.2	4.4	4.6	4.3	4.4
Phenylalanine+tyrosine	6.0	7.7	7.2	7.8	7.5	8.0
Threonine	4.0	3.1	3.1	4.0	3.3	4.1
Tryptophan	1.0	1.3	0.9	1.0	0.9	1.0
Valine	5.0	5.5	5.2	5.8	5.1	5.6
Chemical score		69	60	75	64	76

*First limiting amino acid as determined by chemical analysis.
[1] WHO, 1973.

The essential amino acid distribution of coconut kernel protein isolates and hydrolysed protein isolates as compared to FAO provisional pattern is shown in Table 4. Amino acid analysis was used to predict the protein quality of the isolate and hydrolysed product by calculating chemical score based on 1973 FAO provisional pattern. The first limiting amino acid of coconut protein was lysine (Table 4). Comparing the values of lysine in kernel, isolates and hydrolysed protein isolates, it was apparent that water soluble fraction (whey) discarded in the preparation of isolate (P_1) contains more of this essential amino acid. Further, the lysine content of ultrafiltrated protein was higher (4. lg/16gN) when compared to acid coagulated protein (3.3g/16gN). This indicates that it is possible to prevent loss of lysine by ultrafiltration procedure. The coconut protein unlike other plant proteins is high in sulfur containing amino acid, an important criteria to be considered as food protein

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