Preparation and Characterization of Coconut Protein Isolates[1]

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ABSTRACT

The following protein fractions have been separated from dried, defatted fresh coconut meats: 1) soluble at pH 2, precipitated at pH 3.9; 2) soluble at pH 8, precipitated at pH 3.9; 3) soluble at pH 10.5, precipitated at pH 3.9; 4) extracted sequentially at pH 10.5 from pH 8 residue and subsequently precipitated at pH 3.9; 5) extracted at pH 7 by 1.0M NaCl and precipitated at pH 2; 6) soluble in water at pH 7.0; and 7) proteins remaining in solution at pH 3.9. The recoveries were 1) 45 to 57%; 2) 39 to 53%; 3) 31 to 35%; 4) 14 to 19%; 5) 48 to 54%; 6) 9 to 11%; and 7) 3 to 6%. Hydrochloric was the most efficient precipitating acid of those tested, including sulfuric, phosphoric, acetic, and nitric. Each of the precipitated isolates was dried by lyophilization. While all of the isolates were quite soluble at pH 2, there were substantial differences in solubilities at pH 7, 8, and 10. All isolates were quite insoluble from pH 4 to 6. The amino acid composition of most of the isolates did not vary markedly from that of the original meal. The water-solubles were somewhat higher in lysine, arginine, and glutamic acid and lower in the remaining amino acids. The isolate extracted sequentially at pH 10.5 from pH 8 residue was lower in lysine and glutamic acid.

Although fresh coconut meats contain only about 4% protein, they are nevertheless a potentially important source of protein because of the great world production of coconuts, primarily in regions deficient in high protein foods. The world's coconut yield has been estimated from copra data to be 5.6 million metric tons annually (1). These data refer only to coconuts used for copra and do not include those used directly for food. This amount of copra is roughly equivalent to 224 million kg. of protein (basis nitrogen X 6.25), probably none of which is being used for human food consumption.

A 20% protein flour of good quality can be obtained from coconuts by removal of the oil and water. Studies have indicated that the protein efficiency ratio of coconut flour is comparable to that of casein (2). Protein efficiency ratio, biological value, net protein utilization, and digestibility values for coconut products suggest good nutritional quality for coconut proteins (3). Yet, present world practice of handling coconuts through the intermediacy of copra produces a protein meal, after oil removal, which is generally not fit for human consumption (4). Furthermore, the high fiber content of coconut flour (9 to 11%) limits effectively its use as a protein supplement (5).

Because of the low protein content of coconut flour and its high fiber content, even when prepared under conditions which render it suitable for human food use, protein isolates from coconuts take on added significance in attempts to make use of this untapped source of food protein. Isolates, with over 80% protein and very

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low fiber content, could be used more easily as food supplements. Furthermore, like soy proteins or casein, coconut protein isolates may also find added value as the result of any favorable functional properties they may possess.

Previous studies in this laboratory have shown that coconut meal (flour), containing about 20% protein and prepared without excessive heat treatment, displayed suitable solubility for efficient protein extraction in aqueous or salt solutions (6). These investigations showed that it was possible to obtain 80 to 90% protein solubility without resorting to enzymatic extraction conditions, as suggested by Chandrasekaran and King (7).

Peters (8, p. 6) has prepared coconut isolates from a centrifugal wet-process. The National Institute of Science and Technology in The Philippines has operated a pilot plant in which coconut protein concentrates and isolates have been prepared from granulated coconut (9).

Guided by data obtained from preliminary work on the solubility or extractability of coconut proteins, these present studies were undertaken to characterize coconut protein isolates prepared under a variety of extraction conditions.

MATERIALS AND METHODS

Preparation of Coconut Meal

In the production of coconut meal used for the preparation of isolates, care was taken to use steps not expected to denature proteins. Fresh coconuts were cracked and the meats removed from the shells. After shredding through an Urschel mill, the meats were dehydrated in a freeze-dryer, then solvent extracted with hexane. Meal so obtained, after further milling, contained 22.2% protein, 9.9% moisture (after equilibration at room temperature and humidity), 1.1% oil, 5.2% ash, and 7.5% crude fiber (6).

Preparation of Coconut Protein Isolates

Coconut meal was slurried in water or salt solution adjusted to the predetermined pH of extraction, at a 20:1 solvent-to-meal ratio. The mixture was stirred for at least 30 min. on a magnetic stirrer and the pH readjusted, if necessary. This was followed by centrifugation at 5,900 X g for 20 min. (Sorvall RC2-B refrigerated centrifuge) and suction filtration (Whatman filter paper No. 4) to remove flocculent materials from the supernatant extract. The extract was then adjusted to pH 3.9 and centrifuged. After decanting the supernatant layer, the precipitated proteins were washed once with water. Meanwhile, the residue from the initial extraction was re-extracted at the appropriate pH, and the protein separation procedure repeated. The protein isolates were lyophilized and combined. Figure 1 presents a schematic outline of the extraction procedure.

Solubility of Protein Isolates

The protein isolate (400 mg.) was slurried in 40 ml. water (total volume after adjusting pH with HCl or NaOH) and stirred for at least 30 min. at room temperature. After centrifugation at 7,710 X g for 20 min., the extract was filtered to remove flocculent materials. An aliquot of the supernatant was taken for Kjeldahl nitrogen analysis. Percent nigrogen extracted was computed from the total volume of solvent used for the extraction.

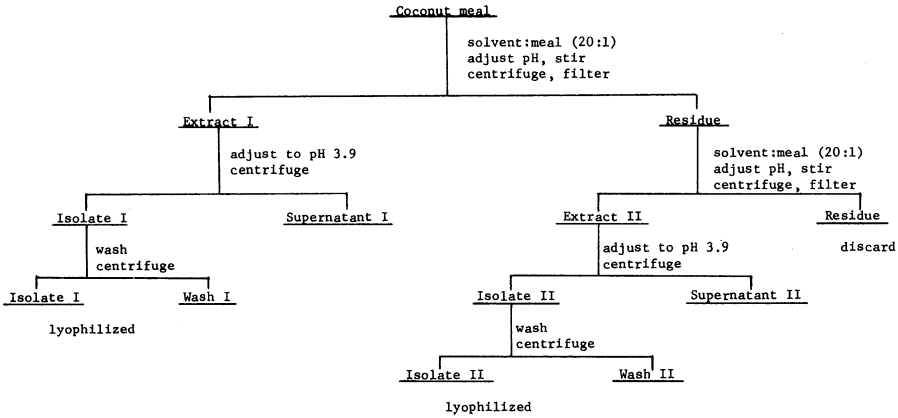

Fig. 1. Extraction procedure for coconut protein isolates.

Analytical Methods

Moisture, oil, ash, and crude fiber were determined by standard methods (10). Nitrogen (protein estimated as nitrogen X 6.25) was determined by standard macro-Kjeldahl (10,11) or micro-Kjeldahl procedures (12,13). Amino acid analyses were obtained on Beckman Model 120C amino acid analyzer, following the procedure of Spackman et al. (14); unavailable lysine was determined according to an unpublished procedure by M. C. Thomas and C. M. Lyman at Texas A&M University.

RESULTS AND DISCUSSION

The nitrogen solubility of the starting coconut meal over the aqueous pH range is given in Fig. 2. From a study of the pH-solubility profile of coconut meal, a

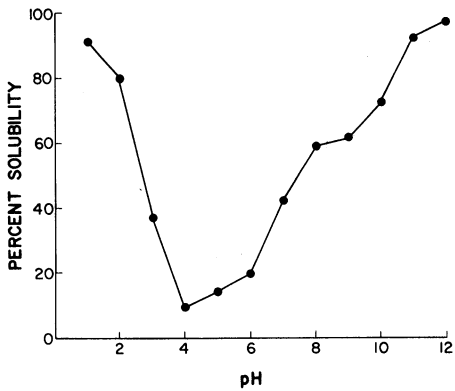

Fig. 2. Solubility profile of the proteins of coconut meal.

determination was made as to the pH conditions both for extracting coconut proteins and for precipitating the proteins from the extracts.

Previous workers have used different pH values for precipitating coconut proteins: pH 3.9 (8, p. 20); 3.4 to 3.5 (15); 4.5 (16). To resolve the differences in the literature values, extractions of coconut meal were made at a series of pH points between pH 3.0 and 5.0. From these it was evident that solubility was minimal from pH 3.6 to 3.9. It was decided to precipitate proteins at pH 3.9.

Guided by the high solubility of coconut proteins on both the acid and alkaline sides (Fig. 2), protein fractions were prepared by extraction under acid (pH 2) and alkaline (pH 10.5) conditions. For the present investigation, stronger acid and alkaline conditions were not employed as a precaution against possible protein denaturation. The small plateau in the solubility profile at about pH 8.0 to 8.5 suggested another set of conditions for preparing protein isolates: extraction at pH 8, followed by a sequential extraction of the residue at pH 10.5. These isolates were made by precipitating the extracts at pH 3.9.

Because of the large percentage of globulins in coconut proteins (62% by the Osborne classification) (6), a salt extract was prepared using 1M NaCl at pH 7, at which pH coconut protein displayed maximum solubility with 1M NaCl solution. Since pH-solubility profiles of coconut meal proteins under salt conditions indicated minimum solubility at acid conditions (6), the salt extract was precipitated at pH 2 by the addition of HCl. To minimize residual NaCl in the isolate, the precipitated extract was dialyzed against water prior to lyophilization.

Two other protein fractions suggested themselves: proteins extracted by water alone (albumins, 31% by Osborne classification tests) and the proteins remaining in solution at the point of minimum solubility, pH 3.9. Both these protein isolate preparations involved dialysis of the extracts against water, prior to air-concentration in the bags, and lyophilization.

Table I gives typical yields of protein isolates, calculated from the nitrogen content of both isolates and starting meal. Table II summarizes nitrogen material balances for the extraction procedure employed in the laboratory.

Table I shows the typical yields of the protein isolates. Each of the isolates was prepared a number of times. The efficiency of protein recovery from meal to isolate was about 50% for preparations under acid (pH 2) and salt (1M NaCl, pH 7) conditions. The total recovery for the step-wise extraction at pH 8 followed by

TABLE I. PREPARATION OF PROTEIN ISOLATES: YIELDS[a]

pH	Isolate I	Isolate II	Total	Protein Content
	% N recovered from meal			%
2	43	10	53	94.6
8[b]	26	13	39	91.6
10.5[c]	19	...	19	90.7
10.5	21	12	33	88.2
7 (salt)	40	13	53	97.6
3.9	3	...	3	50.5
Water	8	...	8	74.8

[a]To be read in conjunction with Fig. 1.

[b]From freshly prepared meal, yield of isolate: 59.7%; protein content: 97.4%.

[c]Sequential from meal extracted at pH 8.

TABLE II. PREPARATION OF PROTEIN ISOLATES: NITROGEN MATERIAL BALANCE[a]

pH	Extract I	Extract II	Total	Supernatant I	Wash I	Isolate I	Supernatant II	Wash II	Isolate II
	% N recovered from meal			% N of extract I			% N of extract II		
2	60.9	14.1	75.0	22.8	5.6	71.2	24.2	2.6	68.9
8	50.8	15.7	66.5	46.1	15.2	51.7	10.5	1.1	82.0
10.5[b]	21.3	...	21.3	4.4	1.0	88.4	...	...	...
10.5	80.6	15.2	95.8	72.1	3.3	25.9	18.0	0.7	76.1
7 (salt)	58.6	23.2	81.8	17.9	...[c]	67.5	19.9	...[c]	55.7
3.9	13.0	2.1	15.1	...	...	...	...	...	...

[a]To be read in conjunction with Fig. 1.

[b]Sequential from meal extracted at pH 8.

[c]Data not taken.

TABLE III. EFFECT OF ACID PRECIPITANT ON PROTEIN YIELD
(proteins extracted at pH 8)

Acid	Protein Content %	Yield of Isolate[a] %
1N HNO_3	93.4	47.5
1N H_2SO_4	93.0	41.8
1M H_3PO_4	89.3	47.6
1N HOAc	91.7	36.2
1N HCl	91.5	52.9

[a]On the basis of protein content of meal and isolate.

TABLE IV. AMINO ACID COMPOSITION OF COCONUT PROTEIN ISOLATES AND FRACTIONS[a]
(expressed as % of total protein)

Amino Acid	Coconut Meal	Extracted at pH 2, Precipitated at pH 3.9	Extracted at pH 7, (1M NaCl) Precipitated at pH 2	Extracted at pH 8, Precipitated at pH 3.9	Extracted at pH 10.5, Precipitated at pH 3.9	Extracted at pH 10.5, Sequented from pH 8, Precipitated at pH 3.9	Water-Solubles
Lysine	3.3	3.4	3.5	3.8	3.5	2.8	5.5
Unavailable lysine	0.3	0.3	0.2	0.3	0.3	0.2	0.3
Available lysine	3.0	3.1	3.3	3.5	3.2	2.6	5.2
Histidine	1.7	2.0	2.0	2.2	2.0	1.4	1.6
Arginine	12.6	12.8	14.3	15.1	14.3	14.2	16.8
Aspartic acid	8.9	9.0	8.6	8.2	9.0	9.1	5.1
Threonine	2.9	3.4	3.2	2.9	3.4	3.8	2.1
Serine	4.5	4.9	4.5	4.2	4.8	5.3	2.5
Glutamic acid	20.2	18.3	16.4	20.1	19.2	16.5	25.4
Proline	3.6	3.5	3.4	3.4	3.7	3.4	2.6
Glycine	4.2	4.4	4.1	4.3	4.5	4.5	3.7
Alanine	4.5	4.3	4.1	3.9	4.3	4.6	2.6
Valine	5.4	5.8	5.6	5.4	5.8	6.1	3.1
Methionine	1.8	2.1	2.2	1.8	2.2	2.4	0.9
Isoleucine	3.3	4.0	3.7	3.4	3.9	4.3	1.9
Leucine	6.2	7.3	6.9	6.9	7.2	7.6	4.5
Tyrosine	3.0	3.1	2.7	2.6	3.0	3.5	2.5
Phenylalanine	4.3	5.4	4.8	4.8	5.2	4.9	2.6

[a]No assays made for tryptophan.

extraction at pH 10.5 was about 60%. For the isolates prepared under alkaline (pH 10.5) and near neutral (pH 8) conditions, the protein yields were under 40%.

These data represent preparations made after the coconut meal had been stored in the laboratory for about 5 months. These protein recoveries were basically the same as those obtained from freshly prepared coconut meal, except in the case of the isolate at pH 8. In Table I, the recovery of proteins extracted at pH 8 was 39%; earlier recoveries, with freshly prepared meal, ranged from 60 to 67%. This suggests that the amount of proteins recoverable from coconut meal changed with age. Material balance data (Table II) indicate that the lower recovery with the aged meal is due to the fact that about half of the extracted proteins failed to precipitate at pH 3.9.

Nitrogen material balances indicated that the nitrogen was extracted at about the levels that were anticipated from the solubility profile. However, some recoveries were lower than anticipated, inasmuch as the protein did not precipitate in the quantities that would be predicted from the solubility profile. This was especially true of the protein which was extracted directly at pH 10.5. While 96% of the available nitrogen was found in the extract, over 70% of the dissolved nitrogen remained in solution at pH 3.9. On the other hand, the protein extracted sequentially at pH 10.5 from the meal previously extracted at pH 8 was 88% insoluble (precipitated) at pH 3.9. These phenomena are under further investigation.

An attempt to maximize the recovery of protein by the use of acids other than hydrochloric for precipitating proteins showed that HCl was the most efficient among the acids tried (Table III).

A further attempt to determine the reason for the increased solubility at pH 3.9 of the extracted proteins (and the yield of precipitated protein isolate much lower than expected) also failed to give a satisfactory answer. From Table II, it should be noted that efficiency of precipitation was usually (except for the pH 2 isolate) much higher in the second and third (for the pH 10.5 sequential isolates) extracts than for the first extract. It appeared possible that the carbohydrates which were soluble under the extraction conditions affected the solubility of the proteins. In the pH-solubility studies, though the carbohydrates were also in solution, the fibrous material and other insoluble solids could still interact with the rest of the mixture; in the case of the protein extracts, however, all insoluble materials have been removed by centrifugation and filtration.

An extraction was performed at pH 8 conditions, and the extracts from the first and second extractions combined. The extract was divided into two portions: the first was precipitated at pH 3.9 and lyophilized; the second was dialyzed against water for 3 days, air-concentrated, and lyophilized. The first portion gave about 10% more protein isolate than the second, showing that carbohydrates and other dissolved substances in the extract did not hinder protein precipitation at pH 3.9.

Characteristics of Protein Isolates

Protein isolates prepared under different pH conditions varied in physical appearance. The isolates obtained at pH 10.5 were brownish in color, those at pH 2 and 8 slightly grayish, whereas the salt isolate was white. The salt isolate was fluffy whereas the other major isolates were more granular.

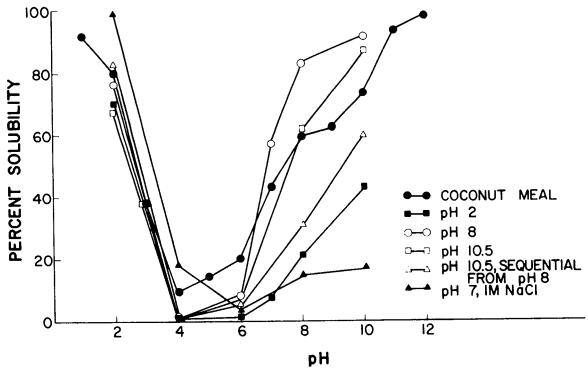

Fig. 3. Solubility profiles of coconut protein isolates extracted under the indicated conditions and precipitated at pH 3.9 (except the salt extract, which was precipitated at pH 2.0).

An important functional property of protein isolates is their solubility at various pH values. In addition to serving as a useful indicator of protein denaturation, the solubility profile may also suggest possible uses. The solubility characteristics of the several coconut protein isolates varied (Fig. 3). All were quite soluble at pH 2, including the salt isolate. The latter is particularly noteworthy, inasmuch as it had been prepared by precipitation at pH 2, although at that time it was in 1M NaCl solution. All the isolates were relatively insoluble from pH 4.0 to 6.0. At pH 8.0 and 10.0, wide differences became apparent. The isolate prepared by salt extraction was surprisingly insoluble under alkaline conditions. Those extracted initially at pH 8 and 10.5 retained good solubility at pH 8 and 10.

Table IV summarizes amino acid analyses for coconut meal and the coconut protein isolates. Except for the water-soluble proteins and the isolate obtained by a sequential extraction at pH 10.5 of the pH 8 residue, the amino acid composition of the isolates does not differ markedly from that of the original meal. The water-solubles were high in lysine, arginine, and glutamic acid and lower in the other amino acids; the pH 10.5 isolate from the sequential extraction was low in lysine and glutamic acid. Available lysine figures were also comparable to the starting meal, indicating that the proteins had not been denatured during their extraction from the meal and preparation into protein isolates.

Further studies are in progress on the other functional properties of the coconut protein isolates.

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