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EXTRACTABILITY OF COCONUT PROTEINS

SUMMARY—Studies were made on nitrogen (protein) solubility of coconut meal in aqueous media over a range of pH's. The point of minimum solubility was found at pH 3.9 and nitrogen solubility increased toward the acidic and basic sides. Coconut proteins displayed a markedly different pH-nitrogen solubility profile in salt solutions, showing minimal solubility at acidic pH's (pH ≤ 3), a sharp rise of solubility and a maximum at neutrality. Coconut meal prepared from fresh coconuts with and without testa removed, from parings and from a sample of sun-dried copra showed comparable solubility characteristics. However, meal prepared from commercial desiccated coconut showed low solubility, even under acid and alkaline conditions (pH 2 and 10). The most efficient solvent-to-meal ratio was found at 20:1 (v:w) for a single extraction, and a two-step 15:1 process for multiple extractions. Extractions using salt solutions show that the efficiency of extraction is greatly dependent upon pH; concentrations ≥ 0.25M effect maximum extraction. Osborne classification studies of coconut meal indicate that 90% of the proteins would be classified as albumins and globulins.

INTRODUCTION

COCONUTS are an indigenous product and a major article of commerce in many countries of the world which are suffering from protein deficiency. A conservative estimate, probably low, of world coconut production in the early 1960's gives 5.6 million tons a year. This is equivalent to more than 200,000 tons of crude protein, only a minor proportion of which is presently being utilized for human food consumption (Orr and Adair, 1967; PAG, 1965, Teply, 1967).

The major proportion of coconuts is converted to coconut oil and coconut meal via copra. Commercial processes for extracting coconut oil commonly utilize expellers at temperatures which denature proteins. Furthermore, prevailing world practice in handling coconuts for the production of copra are not conducive to further utilization of the coconut meal for human consumption.

These studies were undertaken as a necessary preliminary step to the study of basic properties of coconut proteins in connection with the larger objective of finding methods for the better utilization of coconuts as protein-food sources. To insure that the present work was being conducted on good quality coconut protein, care was taken to prepare coconut meal by processes not expected to denature proteins.

Previous studies on coconut meal have commonly used meal prepared from commercial coconut oil processing (Chellian and Baptist, 1969) or from sun-dried copra (Butterworth and Fox, 1963), or have made use of meats dehydrated by the application of heat (Sison et al., 1968). Studies by Chandrasekaran and King (1967) suggest that enzymatic processes may be required for the efficient extraction of coconut protein (from a control value of 50–60% to 85%); however, they do not indicate how their starting coconut flour has been prepared, except that it was supplied by the Central Food Technological Research Institute, Mysore, India.

EXPERIMENTAL

Preparation of coconut meal

The coconuts used were food-grade fresh coconuts obtained from the wholesale market in Houston, Texas. Indications are the coconuts came from Central America. As is common with commercial coconuts, these were received dehusked. The meats (kernel) are enclosed in a hard shell (endocarp). Between the shell and the kernel is a thin, brown seed coat (testa) which adheres firmly to the kernel. In the manufacture of desiccated coconut, the testa is removed by paring.

Coconut meal was prepared from the fresh coconuts by procedures that would not be expected to denature the proteins. The nuts were cracked and the coconut water discarded. The meats were removed from the shell, washed with water and then sliced into smaller fragments using an Urschel mill (cutting head 2-J-030510B or 2-K-020060B). The shredded meats (which included the testa) were dried under vacuum at 40°C for 24 hr to a moisture content of less than 2%. The dried meats were further comminuted by means of the Urschel mill, this time using a finer cutting head (2-K-020035C or 2-K-010030B), prior to defatting by several extractions with hexane. Residual solvent was removed with dry air.

Typical analyses of coconut meal prepared in this fashion are given in Table 1. Moisture, ash, oil and crude fiber analyses were made by standard AOCS (1969) procedures. Table 1 also gives typical analyses of fresh coconut meats and desiccated coconut meats involved in the preparation of coconut meal. It is to be noted that the moisture content of the dried meats equilibrated to a level of 7–10% on standing at ambient temperature and humidity.

Extraction of protein

Extraction of coconut proteins from coconut meal was carried out at room temperature, for at least 30 min, using a solvent:meal ratio of 20:1 (v:w), unless otherwise specified. The predetermined pH of extraction was obtained by addition of 0.5N NaOH or 0.5N HCl solution; the pH was rechecked and readjusted after 30 min of stirring. Sample size was 2.0g and the volume of solution was brought to 40 ml after the final pH adjustment. After centrifugation at 4300 × G (Sorvall RC2-B Refrigerated Centrifuge) for 20 min, the supernatant extracts were filtered through Whatman Filter Paper No. 1 to remove flocculent materials. A 20 ml aliquot of each extract was taken for nitrogen analysis. Nitrogen was determined either by macro-Kjel-

Table 1—Coconut meal: Proximate analyses

Sample	Moisture and volatile material %	Oil %	N %	Protein (%N × 6.25) %	Ash %	Crude fiber %
Fresh meats	47.0					
Desiccated meats[a]	1.8	73.0				
	1.0	71.1				
	1.5	69.3				
Coconut meal A[b]	9.9	1.1	3.6	22.2	5.2	7.5
Coconut meal B[b]	7.2	4.8	3.8	23.9	3.9	5.9
Coconut meal C[b]	5.1	0.4	3.8	23.8	4.4	7.6

[a] These are analyses of different lots dehydrated in the freeze dryer.
[b] Coconut meals A, B and C are different samples, prepared from three lots of fresh coconuts obtained at different times.

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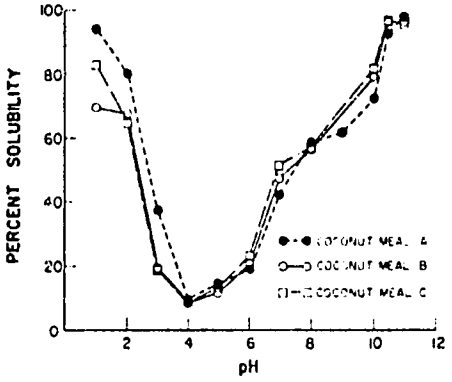

Fig. 1–Protein solubility profiles of coconut meals prepared from three lots of fresh coconuts obtained at different times (see Table 1).

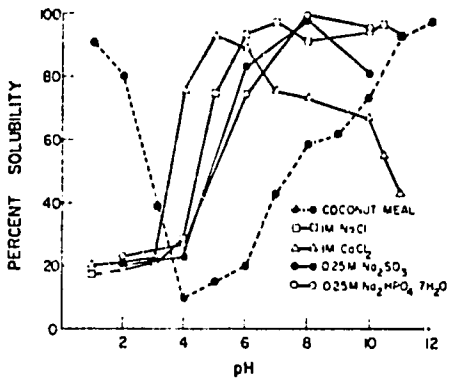

Fig. 2–Protein solubility profiles of coconut meal in several different salt solutions at varied pH's adjusted by the addition of 0.5N NaOH or 0.5N HCl.

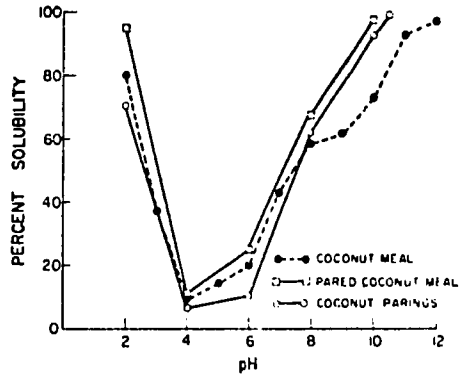

Fig. 3–Protein solubility profiles of a desiccated coconut and a specially prepared copra.

dahl (AOAC, 1965; AOCS, 1969) or micro-Kjeldahl methods (Aminco, 1959; Miller and Houghton, 1945). In most cases, the data shown represent duplicate analys using two samples of meal.

RESULTS & DISCUSSION

pH-Nitrogen solubility profiles

Studies were made on nitrogen (calculated as protein using the customary 6.25 factor) solubility or extractability of three coconut meals in aqueous media over a range of pH's. The data shown in Figure 1 are for extracted meals prepared from three different lots of coconuts. The solubility profile for coconut meal C represents the average of four replications; the 95% confidence limits for a single mean is ± 2.95%.

The point of least solubility was further refined by extractions at pH's 3.0, 3.3, 3.6, 3.9, 4.2, 4.5, 4.8 and 5.0. Minimum solubility was obtained at pH 3.9. [Peters (1960) found maximum precipitation at pH 3.9. Sison et al. (1968) reported the best pH for protein precipitation in a citrate-phosphate buffer at pH 3.4–3.5. Chelliah and Baptist (1969) precipitate coconut protein at pH 4.5.]

Nitrogen solubility increased with increasing acidity and alkalinity. The inflection in the curves near pH 8 suggests that there may be two main protein fractions: one soluble at pH 8–8.5 and the other at pH 10.5–11.

The solubility profile of coconut meal is strikingly different in salt solutions (Fig. 2). In 1M NaCl solution, there is rather low solubility on the acid side, up to pH 4. This is followed by a steep rise in solubility to over 90% at pH 6 and more alkaline conditions. Solubility in 1M $CaCl_2$ displays a similar low solubility at acidic pH's, followed by high solubility near pH 5–6. An unexplained decrease in solubility at more alkaline pH's is also observed.

Similarly, coconut proteins show low solubility at lower pH's for 0.25M Na_2SO_3 and 0.25M dibasic sodium phosphate. The pH-solubility profile rises steeply beginning at pH 4 and reaches a maximum of almost total solubility at pH 8. A decrease of solubility at more alkaline conditions follows.

The unique solubility characteristics of coconut proteins in salt solution have been used to prepare an interesting isolate by extraction in 1M NaCl at pH 7 followed by precipitation at pH 2. The preparation and characteristics of this and other isolates prepared by extraction at pH 2, 8, and 10.5 are the subject of another manuscript now in preparation.

The classical Osborne classification of proteins (Lund and Sandstrom, 1943), as applied to coconut meal, confirms the solubility data obtained in aqueous and salt media. Table 2 indicates that over 90% of the proteins in coconut meal would be classified as albumins and globulins. The sodium tungstate test indicates

Table 2–Classification of the proteins of coconut meal according to solubility[a]

Fractions	Extraction conditions	% of Total N
Albumin	CO_2-free water, pH 6.7	30.6
Globulin	1M NaCl solution, pH 7.0	61.9
Prolamine	70% aqueous ethanol	1.1
Glutelin	0.2% NaOH solution	4.7
Insoluble residue	– – – –	1.8
Nonprotein nitrogen	12% sodium tungstate to precipitate proteins	0.1

[a]Osborne classification.

Table 3–Pared coconut meal and coconut parings: Proximate analyses

Sample	Moisture and volatile material %	Oil %	N %	Protein (%N × 6.25) %	Ash %	Crude fiber %
Fresh pared meats	47					
Fresh testa	35					
Desiccated pared meats	1.1	73.5				
Desiccated testa	2.2	54.7				
Pared meal	10.6	5.2	3.6	22.5	4.2	5.6
Defatted testa (meal)	8.9	3.6	3.2	20.0	2.9	12.1

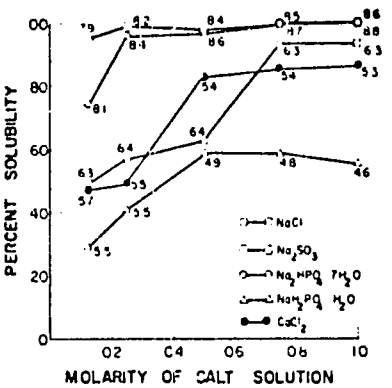

Fig. 4–Solubility of the proteins of coconut meal in different concentrations of several salts. (Data next to solubility points indicate resulting pH's.)

that there is very little nonprotein nitrogen in coconut meal.

The solubility curves (Fig. 1 and 2) were made on coconut meal which included the testa. In addition, some coconut meal was prepared with the testa removed by manual paring. The parings were also dried and defatted and the solubility profile of the proteins determined. Table 3 summarizes some properties of coconut meal without the testa, and also of the coconut parings. The pared coconut meal gave a slightly higher protein analysis than the parings (22.5% protein compared to 20.0%; 22.8% protein compared to 17.7% in another determination). The solubility curves of pared coconut meal and of the parings are comparable to those of coconut meal with the parings.

Solubility profiles of the proteins in meals prepared from commercial coconut products have been obtained. Figure 3 gives the solubility profile for coconut meal obtained by defatting commercial desiccated coconut and sun-dried copra prepared under a "heat tent." The meal from sun-dried copra gave a pH-solubility profile comparable to the laboratory-prepared coconut meal. That from desiccated coconut, however, displayed rather low solubility at both acidic and basic pH's. The very low solubility (26%, compared to about 70% in the laboratory-prepared meal) at pH 8 is also striking.

The behavior of commercial desiccated coconut is very puzzling, inasmuch as the product is perfectly white. Preliminary work done at Texas A&M University (to be reported elsewhere) has indicated that browning might be a useful indication of incipient denaturation, as shown by loss of solubility. This, however, does not seem to be the case with commercial desiccated coconut, which, though pure white, has rather poor solubility.

Efficiency of protein recovery

Protein solubility or extractability is not the only factor governing efficiency of protein extraction. Other variables such as length of time of extraction meal:solvent ratio, temperature of extraction and the effect of added salts are also very important.

Table 4 gives data obtained by varying the meal:solvent ratios, while extracting at pH 8 in aqueous solution and pH 7 in 1M NaCl (conditions chosen as representative for aqueous and salt extraction). The sample size for these extractions was 10g. After centrifugation and gravity filtration, the supernatant volume was measured. Under these conditions, three to five times the weight of coconut meal (i.e., 30–50g) was absorbed by the meal, and was not recovered as part of the separable solution. The amount not separable under practical conditions would vary depending upon the efficiency of the separation techniques used. The data in Table 4 indicate that a solvent:meal ratio of 20:1 (v:w) would be the most efficient, for single extraction.

Table 5 gives efficiency of protein

Table 4 Effect of solvent:meal ratio on extraction

Solvent:meal ratio (ml:g)	At pH 8		At pH 7, 1M NaCl	
	% N recovered[a]	% N extracted[a]	% N recovered[a]	% N extracted[a]
5:1	11.9	60.9	17.1	95.1
7.5:1	25.8	62.3	38.3	101.0
10:1	32.2	60.1	53.6	97.4
15:1	40.8	61.2	63.3	94.0
20:1	47.1	63.9	75.0	97.4
25:1	46.6	59.2	77.9	91.7
30:1	49.4	62.4	83.6	100.3

[a]The percent nitrogen extracted (or soluble) is based on the total nitrogen present in the coconut meal sample and the total volume of solvent used for extraction. The percent nitrogen recovered refers to that nitrogen in the separable supernatant extract, excluding the amount of extract (solvent) absorbed by the meal. The difference between the percent nitrogen extracted (dissolved) and recovered represents the nitrogen in solution in the absorbed solvent. Percent nitrogen recovered is the more useful data for process development purposes.

Table 5 Effect of successive extractions with different solvent:meal ratios

Solvent:meal ratio (ml:g)	Extraction	At pH 2 % N recovered[a]	At pH 8 % N recovered[a]
10:1	First	45.8	33.7
	Second	17.7	12.5
	Third	7.1	8.2
	TOTAL	70.6	54.4
15:1	First	65.5	40.7
	Second	17.9	13.3
	TOTAL	83.4	54.0
	(Third)	5.9	9.5
20:1	First	61.3	43.6
	Second	14.1	14.8
	TOTAL	75.4	58.4

[a]Based on actual recovery of extract.

Table 6 Effect of time on extraction

Time (min)	At pH 8 % N extracted[a]	At pH 7, 1M NaCl % N extracted[a]
5	58.6	96.9
15	59.6	99.5
30	58.4	99.0
45	59.3	98.6
60	60.5	103.0
90	65.3	104.0

[a]Based on total solvent used.

Table 7–Effect of temperature on extraction[a]

Temperature (°C)	% N extracted[b]
4	49.0
10	57.7
15	61.5
29	71.6
52	96.1
75	92.3

[a]Solvent: 0.2M phosphate buffer, pH 7.0.
[b]Based on total solvent used.

extraction and recovery with multiple extractions, using 10:1, 15:1 and 20:1 ratios. From these data it appears that a two-step 15:1 extraction may be most efficient. Though using one step less than a triple 10:1 extraction, the overall yield of the two-step 15:1 is almost as high. Compared to the two-step 20:1 extraction, it uses almost a third less solvent, with only a small loss of efficiency of protein recovery. The most practical extraction conditions would be selected on the basis of total process economics.

Other factors affecting protein solubility

In determining the effect of length of time of extraction on nitrogen solubility, it usually took at least 5 min to attain the desired pH, and then 15 min for centrifugation. Thus, the "time" variable employed was artificially set as actual stirring time after the desired pH had been attained. Table 6 indicates that maximum nitrogen solubility is attained in 15 min or less.

Table 7 summarizes the effect of temperature on nitrogen solubility. A temperature increase to about 50°C increases nitrogen solubility. The decrease observed at 75° may be due to partial coagulation of protein at the higher temperature. A similar decrease in solubility at higher temperatures has also been reported in studies on sunflower seed proteins in the same laboratory (Gheyasuddin et al., 1970).

The effect of various added salts on nitrogen solubility has also been studied. From Figure 2, giving the solubility curves of coconut proteins under varying pH's for different salt solutions, it is evident that these salt solutions, except toward the acid end of the pH scale, are rather effective solvents for coconut meal.

Figure 4 summarizes the effect on nitrogen solubility of several salts at various concentrations. The tests were run using the salts without adjusting the pH. In Figure 4, data next to the solubility points indicate the resulting pH at each salt concentration.

Concentrations at 0.25M or higher of dibasic sodium phosphate or sodium sulfite extract most of the available nitrogen in coconut meal. NaCl is most efficient at concentrations of 0.75M or higher. $CaCl_2$ solutions, at 0.5M or higher concentrations, effect over 80% extraction. Monobasic sodium phosphate, however, reaches a maximum extraction of a little less than 60% of the available nitrogen, at concentrations of 0.5M or higher. This difference in solubility could probably be explained in part by noting the resulting pH's: both Na_2SO_3 and Na_2HPO_4 attain pH's on the basic side of neutral, in which coconut protein is very soluble (as seen in Figure 2) while NaH_2PO_4 results in pH's between 4.6–5.5. Perhaps by coincidence, $CaCl_2$ and NaCl effect pH's at or near the highest points in their pH-solubility profiles (see Fig. 2).

It should be noted that the very high solubility of coconut proteins in dibasic sodium phosphate and sodium sulfite is in marked contrast to the rather poor solubility of sunflower seed proteins under similar conditions, in work done in the same laboratory (Gheyasuddin et al., 1970). This confirms the need to study each plant seed protein system individually and makes generalizations impossible prior to actual experimental work.

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