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ROBERT HAGENMAIER, C. M. CATER and K. F. MATTIL
Food Protein Research & Development Center, Texas A & M University, College Station, TX 77843

A CHARACTERIZATION OF TWO CHROMATOGRAPHICALLY SEPARATED FRACTIONS OF COCONUT PROTEIN

INTRODUCTION

THE TWO CHIEF coconut producing countries are the Philippines and India, both of which suffer from a shortage of edible protein (Abbott, 1966). In the Philippines, almost all (Nyberg, 1968) coconuts are dried to make copra, which is subsequently pressed to recover the oil. The resulting protein is generally unfit for human consumption.

Although the protein content of the coconut is low (about 4%), the quantity of coconuts grown makes recovery of the protein desirable. In 1965 the Philippines produced 7.0 billion grams of coconut protein, which is estimated to be enough for 10g per Filipino per day. According to the data of Abbott (1966) concerning the protein food supply of the Philippines, those 10g would increase the protein supply of the Philippines by 22%.

However, utilization of coconut protein for food is dependent upon the development of a process whereby both oil and food-grade protein are recovered from the coconut. One of the alternate processes often considered is a process whereby the coconut oil and protein are removed from the solids as an emulsion: the emulsion is then centrifuged to yield oil and proteins (Sreenivasan and Rajasekharan, 1967). Some additional knowledge of the solution properties of the coconut proteins are important toward the development of an aqueous process.

Previous investigations have emphasized classification of the coconut protein by solubility behavior (Samson et al., 1971a; Strength, 1970; Butterworth and Fox, 1963), by amino acid analysis (Strength, 1970; Samson et al., 1971b; Rajasekharan, 1964; Peters, 1960) and heat denaturation by heating of the dry meal (Samson, 1971; Butterworth and Fox, 1963).

The purpose of this research is to better characterize the coconut proteins, principally with respect to some of the parameters of interest in the aqueous processing and use of coconut proteins for food, i.e., solubility, heat denaturation, viscosity and amino acid analysis of the individual protein components.

MATERIALS & METHODS

THE ENDOSPERM (with testa) of fresh coconuts was freeze dried; the oil was then extracted with hexane at ambient temperature. The protein was dissolved by extracting the meal with 1M NaCl at pH 7.0 for about ½ hr. The residue was removed by filtration, the pH raised to 8.0 and the solutions clarified by centrifugation. The resulting solution was concentrated by ultrafiltration against a membrane designed to pass molecules with molecular weight below 10,000 (UM-10 membrane, Amicon Corp.). The concentrated solution was then filtered through a 0.22 μm pore size filter (Millipore Corp.) and dialyzed against 1M NaCl. After this procedure the solution contained 90 ± 2% of the total nitrogen in the coconut. Approximately 5% was not separated from the solids and 3% was lost in ultrafiltration.

Analyses of protein concentrations were made by ultraviolet absorption or by the Lowry method (Lowry et al., 1951). Both methods were calibrated with solutions of coconut protein of known nitrogen content, as determined by Micro-Kjeldahl analysis. The average error in determining nitrogen content was 4% by the Lowry method, and about .2% by ultraviolet absorption.

In heat coagulation experiments the protein solutions (aliquots of a stock solution) were heated for 10 min, then cooled to room temperature and centrifuged. The amount of protein remaining in solutions was taken as a measure of undenatured protein.

Protein solubility was determined by preparing approximately 1% suspensions of the protein in solvent, adjusting the pH of aliquots to the desired value and then agitating for about ½ hr at room temperature. The final pH was read and the suspension centrifuged; then the dissolved protein content of the aqueous phase was measured.

Viscosity was measured in an Ostwald Viscometer, with the time recorded to the nearest tenth of a second on a stopwatch. The average of at least three measurements was used. The standard deviation in measurement of viscosity was 0.1%. The temperature was 30°C.

Amino acid analyses were performed with the Beckman Model 120C analyzer. Samples were hydrolyzed in constant-boiling HCl for 24 hr, under a nitrogen flush. Cysteine was analyzed after oxidation of cysteine to cysteic acid with performic acid. Tryptophan was analyzed by the method of Kohler and Palter (1967). Methionine values were corrected for the ob-

Table 1—Some properties of the protein components

	Unfractionated	Large mol wt components	Small mol wt components
Approx mol wt (at pH 6.8)	–	1.5×10^5	2.4×10^4
Intrinsic viscosity[a]	4.3 cc/gm	4.7 cm/gm	–
Relative abundance	100	84 ± 3	14 ± 3
Percent nitrogen of dried, salt-free protein	19.2 ± 0.4	18.8 ± 0.4	19.4 ± 0.4
Extinction coefficient[b]	3.76 (at 280nm)	3.58 (at 280nm)	2.43 (at 276nm)
Amount sol in 0.1M NaCl, at pH 7.0	–	53 ± 7%	100%

[a] The value of $(\eta-\eta_o)/\eta_o$, extracted to zero protein concentration by least-square analysis of data, where η is viscosity and η_o is viscosity of the solvent (1M NaCl); pH was 7.0; temperature, 30°C

[b] The slope of a graph of absorbance vs. concentration, obtained by least-square analysis of data. Concentration in mg N/cc

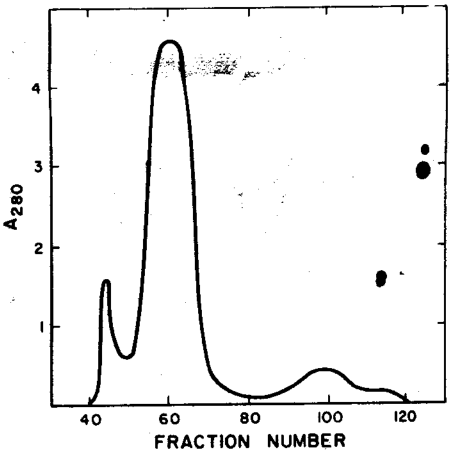

Fig. 1—Sephadex G-200 separation of coconut proteins on a 77 × 5 cm column in 0.95M NaCl buffered at pH 8.2 with 0.01M Na_2HPO_4. Peaks contain 1.6%, 84%, 13.5% and 0.7% of total protein eluted from the column.

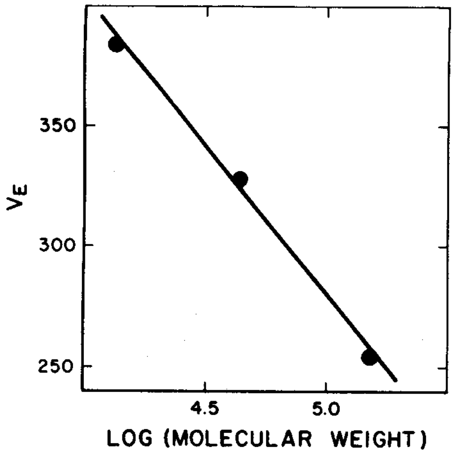

Fig. 2—Calibration curve for determination of molecular weight, calibrated with aldolase (mol wt 158,000), ovalbumin (mol wt 45,000) and ribonuclease (mol wt 13,700) on a 2.5 × 90 cm column of Sephadex G-200, with phosphate buffer (0.4 ionic strength) at pH 6.8. V_0 was 188 cc.

Table 2—Amino acid analyses of protein fractions[a]

Amino acid	Large mol wt component	Small mol wt component	95% Confidence interval
Lysine	3.44	5.01	0.14
Histidine	2.20	1.05	0.13
Arginine	14.70	22.03	0.74
Tryptophan	0.89	1.02	0.10
Aspartic acid	9.47	2.60	0.21
Threonine	3.29	1.13	0.11
Serine	4.89	1.28	0.09
Glutamic acid	17.94	35.51	0.47
Proline	3.60	2.17	0.33
Glycine	4.41	3.13	0.13
Alanine	4.30	0.59	0.15
Cystine	0.98	3.45	0.21
Cysteine	0.16	0.00	0.03
Valine	6.06	0.80	0.24
Methionine	2.84	0.37	0.16
Isoleucine	4.12	0.28	0.33
Leucine	8.15	1.94	0.72
Tyrosine	2.87	2.61	0.13
Phenylalanine	5.10	0.66	0.18
Total	99.41	85.63	2.32

[a] In grams amino acid/16g N

served destruction during hydrolysis of methionine in amino acid calibration mixtures. Two completely independently prepared sets of fractions were analyzed and the amino acid analyses of each were done in duplicate.

RESULTS & DISCUSSION

A TYPICAL chromatographic separation of the coconut protein by Sephadex G-200 is shown in Figure 1. The second and third peaks contain all but 2% of the protein eluted from the column. The second peak is the major protein component, containing 84% of the total nitrogen (by Kjeldahl analysis) and the third peak 14% (average of several separations). The first peak is caused by light scattering and contains a rather small amount of dark-colored protein. The eluant was 0.95M NaCl, buffered at pH 8.2 with 0.01M Na_2HPO_4.

An estimate of the molecular weight of the protein components was made by Sephadex chromatography. A calibration curve of volume of elution vs. log of molecular weight is shown in Figure 2. The volumes of elution of the two major protein components were 258 cc and 365 cc, indicating approximate molecular weights of 1.5×10^5 and 2.4×10^4, respectively. V_0 was 188 cc. More accurate molecular weight determinations were not undertaken in view of the assumed heterogeneity of the fractions. Spychalski (1930) reported a molecular weight of 1.04×10^5 for the main component of coconut globulin.

The chromatography of the coconut protein on Sephadex G-200 had been undertaken as a means of purifying the coconut protein. However, the neat separation into different molecular weight components prompted us to consider the properties of the components separately.

Some of the properties of the components are summarized in Table 1. The amino acid analyses, given in Table 2, are the averages of duplicate analyses on each of two independently prepared sets of samples.

The solubility data presented in Figure

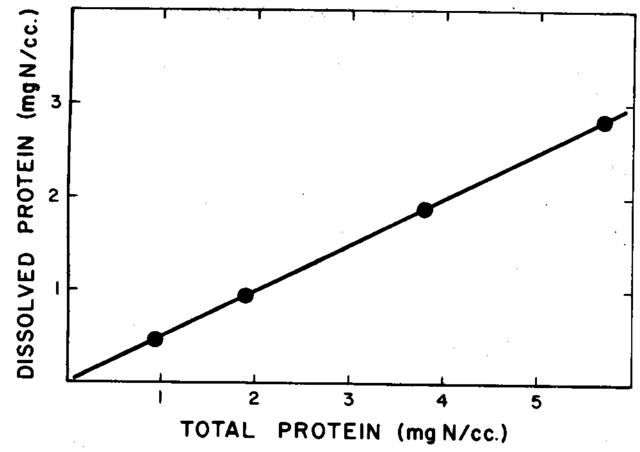

Fig. 3—Dependence of amount of protein dissolved on total amount of large molecular weight component, at pH 6.8, in 0.1M NaCl.

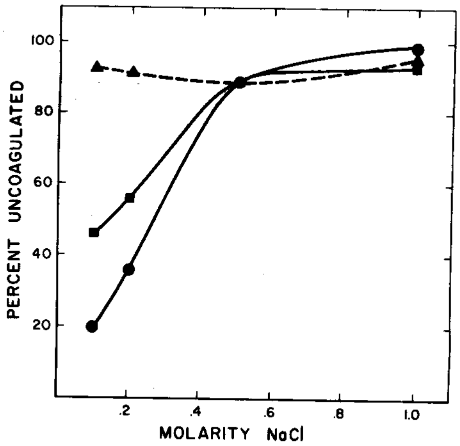

Fig. 4—Dependence of heat coagulation on salt concentration, for the large molecular weight component, at pH 7.4: (●) 100°C; (■) 85°C; (▲) 70°C. The amount coagulated at 0.5M NaCl was the same for all samples.

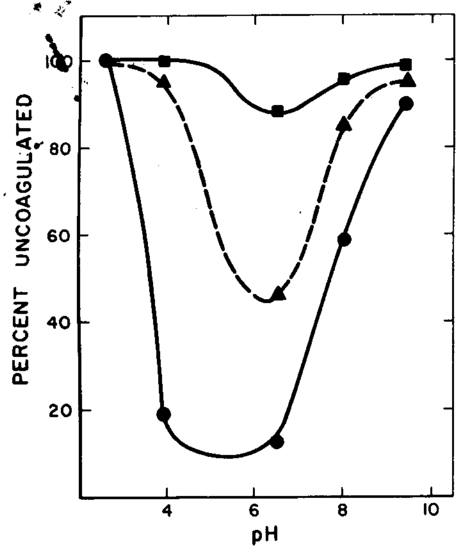

Fig. 5—Dependence of heat coagulation on pH, for the large molecular weight component, in 0.1M NaCl: (●) 100°C; (▲) 80°C; (■) 70°C.

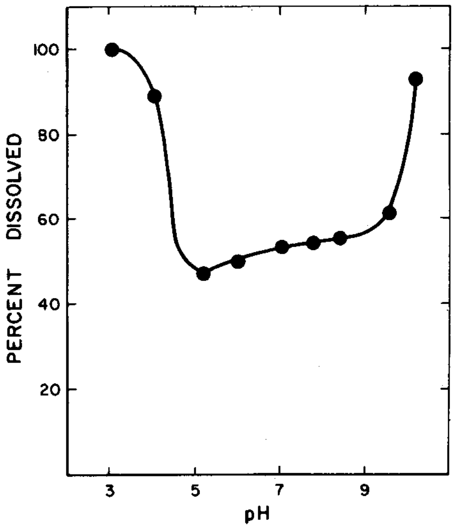

Fig. 6—Dependence of solubility on pH, for the large molecular weight component, in 0.10M NaCl.

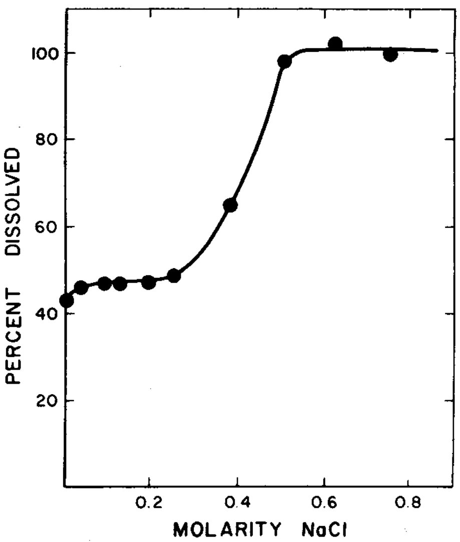

Fig. 7—Dependence of solubility on salt concentration, for the high molecular weight component, at pH 7.0. The lowest salt concentration shown is 0.01M NaCl.

3 indicate that the high molecular weight component is not homogeneous. If it were homogeneous, then the protein should all dissolve until the maximum solubility of the protein is reached—and then no more should dissolve (Taylor, 1953). The straight line dependence of dissolved protein upon total amount of protein present indicates that fraction dissolved in a meaningful parameter of coconut protein solubility. Within the concentration range of 1–5.5 mg N/cc, the solubility of the large molecular weight components seems to be adequately stated by reporting the fraction dissolved, as was done in Figures 6 and 7.

Heat coagulation of the large molecular weight components was at a maximum at neutral pH, low salt concentration and high temperature, as indicated by Figures 4 and 5. Figure 4 contains isotherms at a constant pH (7.4), at different salt concentrations. Figure 5 contains isotherms at a constant salt concentration (0.1M), at different pH values. As might be expected, more protein is coagulated at low salt concentrations and higher temperatures. Also, more coagulation occurs in the pH range 4–7, where it is assumed there is minimum charge on the protein. Peters (1960) reported that the coconut proteins were only heat coagulated in the range pH 3.6–4.1; however he did not specify the solvent used.

The effect of pH and salt on the solubility of the high molecular weight components is shown in Figures 6 and 7. The data show that the point of minimum solubility occurs near pH 5 in 0.1M NaCl. We assume that the isoelectric point is therefore near pH 5. The addition of salt increases the solubility as shown in Figure 7. However, the effect of salt is complicated and varies considerably with pH. At low pH values we have observed a decrease in solubility with addition of salts.

The low molecular weight components were considerably different in their heat coagulation and solubility properties. Under the range of conditions investigated, no significant precipitation or heat coagulation of the small molecular weight components was observed.

The greater heat stability of the low molecular weight proteins could be due to the relatively large amounts of dicarboxylic acids (38.1g/16g N), and basic amino acids (28.1g/16g N), allowing the possibility of many intramolecular salt bridges. Also, the cystine content is higher for the low molecular weight components (3.45 vs. 0.98g/16g N) indicating a larger number of stabilizing disulfide bonds. On the other hand, the greater solubility of the low molecular weight proteins could reflect the usual inverse dependence of solubility on molecular weight of protein (Cohn and Edsall, 1943). The difference in both solubility and heat coagulation of the components might also be attributed to the large differences in aliphatic amino acids, which are present in very small amounts in the low molecular weight components.

The dependence of heat stability on pH and salt concentration means that all of these variables must be considered in finding the maximum safe temperature in the wet processing. However, the data in Figures 4 and 5 indicate that 70°C is safe at nearly all values of pH and salt concentration.

The values for intrinsic viscosity indicate that the coconut proteins have the low viscosity expected of globular proteins. Viscosities were measured in 1.0M NaCl at pH 7, where the protein is all dissolved. Under these conditions the viscosity is very low; however, at lower values of ionic strength, electrostatic factors should cause a somewhat higher viscosity (Tanford and Buzzel, 1956).

For some food uses it is desirable that a protein be soluble at nearly neutral pH values in a solution of low salt concentration. Only half of the high molecular weight proteins are soluble under such conditions. This restricted solubility could have significant implications in considering potential food uses.

In summary, the coconut proteins can be conveniently classified as either high or low molecular weight with the bulk of the proteins being of high molecular weight. The low molecular weight proteins are quite soluble and heat stable. The high molecular weight proteins are heat coagulated at neutral pH values and at low salt concentrations; however, by addition of salt and/or adjustment of pH they may be rendered nonprecipitable by temperatures as high as 100°C.

REFERENCES

Abbott, J.C. 1966. "World Protein Resources." ed. Gould, R.F. American Chemical Society, Washington, D.C.

Butterworth, M.H. and Fox, H.C. 1963. The effect of heat treatment on the nutritive value of coconut meal, and the prediction of nutritive value by chemical methods. Brit. J. Nutr. 17: 445.

Cohn, E.J. and Edsall, J.T. 1943. "Proteins, Amino Acids and Peptides." Reinhold Publishing Corp., New York.

Kohler, G.O. and Palter, R. 1967. Studies on methods of amino acid analysis of wheat products. Cereal Chem. 44: 512.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, J.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265.

Nyberg, J.N. 1968. The Philippine coconut industry. Ph.D. thesis, Cornell University, Ithaca, N.Y.

Peters, F.E. 1960. Preparation and amino acid composition of selected seed protein fractions. Ph.D. thesis, Purdue University, Lafayette, Ind.

Rajasekharan, J. 1964. Chemical and technological investigations on coconut products. Ph.D. thesis, Banaras Hindu University, Banaras, UP.

Samson, A.S. 1971. Heat treatment of coconut meats and coconut meal. J. Sci. Fd. Agric. 22: 312.

Samson, A.S., Cater, C.M. and Mattil, K.F. 1971a. Preparation and characterization of coconut proteins. Cereal Chem. 48: 182.

Samson, A.S., Khaund, R.N., Cater, C.M. and Mattil, K.F. 1971b. Extractability of coconut proteins. J. Food Sci. 36: 725.

Spychalski, R. 1930. Determination of the molecular weight of coconut globulin. Roczniki Chem. 10: 630.

Sreenivasan, A. and Rajasekharan, N. 1967. The use of coconut preparations as a protein supplement in child feeding. J. Food Sci. and Technol. 4: 59.

Strength, D.R. 1970. Preparation, characterization and evaluation of coconut proteins. Third International Congress of Food Sciences and Technology, Washington, D.C.

Tanford, C. and Buzzel, J.C. 1956. The viscosity of aqueous solutions of bovine serum albumin between pH 4.3 and 10.5. J. Phys. Chem. 60: 225.

Taylor, J.F. 1953. "The Isolation of Proteins." ed. Neurath, H. and Bailey, K. Academic Press, Inc., New York.

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