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NUTRITIVE VALUE OF A FIBER-FREE COCONUT PROTEIN EXTRACT OBTAINED BY AN ENZYMIC-CHEMICAL METHOD

INTRODUCTION

COCONUT PROTEINS have been shown to have a relatively favorable amino acid profile (Tasker et al., 1962; Srinivasan et al., 1964) and a fairly high nutritive value (Krishnamurthy et al., 1958; Loo, 1968). However, the protein quality of coconut meal (defatted coconut flour or copra meal) has been shown to be compromised by the relatively high crude fiber content of the meal itself (Rama Rao et al., 1965) and by the heat treatment which the copra is commonly subjected to either before or after the oil extraction (Better and Davidsohn, 1958; Butterworth and Fox, 1963). Such factors also limit considerably the use of coconut meal in diet formulations, especially when such diets are intended for children (Teply and Gyorgy, 1962; Daniel et al., 1968).

Although several methods have been devised for the protein extraction of coconut meal (Chandrasekaran and King, 1967; Chelliah and Baptist, 1969; Rama Rao, 1969; Samson et al., 1971) almost no data are found in the literature concerning the nutritive quality of the proteinaceous material extracted by the different techniques.

The proteins of the coconut meal obtained through the azeotropic oil extraction process have been reported to be 80% water extractable (Rajasekharan, 1967). Rama Rao et al. (1964) reported a protein efficiency ratio (PER) of 1.88 for these water soluble proteins when the extract was dried at 60°C. A PER of 1.86 was found by these authors for the proteins of the extract that precipitated at pH 4.0.

Chandrasekaran and King (1967) reported a PER of 1.81 for their enzymatically extracted protein and a PER of 2.27 for the protein extracted without enzyme. Statistically the difference was reported to be not significant; however, the authors stated that those results were based only on the performance of the animals between the 8th and 15th days of the test.

The aim of the work described in the present paper was to determine the nutritive value of the fiber-free protein extracted from a commercial coconut meal by a simple, enzymic-chemical method devised in our laboratories (Molina and Lachance, 1973) and to establish to which extent the fiber content compromises the nutritive value of the proteins in the original coconut meal, and when added to the extracted material.

EXPERIMENTAL

Nutritive value

Chemical evaluation. Both, amino acid analyses and protein score calculations, were carried out for a commercial food grade coconut meal from Heyman Process Corp. (Long Island City, N.Y.) and for the extract obtained at the pilot plant scale (Molina and Lachance, 1973) from the Heyman meal by the enzymic-chemical method.

The amino acid analyses were performed by the Wisconsin Alumni Research Foundation (WARF), Madison, Wisc. utilizing an amino acid analyzer and the automated ion-exchange method of Spackman et al. (1958).

The protein score for each essential amino acid was calculated (Pike and Brown, 1967) with the exception that only the methionine content rather than the total sulfur amino acids (methionine plus cystine) was taken into account due to the fact that no reliable data could be obtained for the cystine content of both the extracted and the original meal proteins. Whole egg was used as the reference protein.

Biological evaluation. For the biological evaluation, five isonitrogenous (by calculation) diets were prepared according to the AOAC (1965). The diets contained either the protein extract or the original coconut meal or casein or lactalbumin as the only protein source. Two diets were prepared with the protein extract, one containing the standard (AOAC) 1% crude fiber while an extra 3.85% crude fiber was added to the other diet in order to simulate the fiber content (4.85%) of the diet prepared with the untreated coconut meal.

Table 1—Percent composition of the original coconut meal and of the fiber free-coconut protein extract (dry basis)

	Protein (N X 6.25)	Crude fiber	Ash	Carbohydrates[a]	Ether extract
Coconut meal	21.90	11.85	5.05	47.35	13.85
Extracted product	31.80	0	8.62	43.73	15.85

[a] Carbohydrates by difference

Table 2—Percent composition and nitrogen content of the isonitrogenous diets evaluated

	Diets[a]				
Ingredients	CM	PE	PEF	Casein	Lactalbumin
Coconut meal	44.20				
Freeze dried extract		29.81	29.81		
Casein				10.70	
Lactalbumin					12.12
Cottonseed oil	2.35	3.43	3.43	8.00	8.00
Salt mixture[b]	2.93	2.51	2.51	5.00	5.00
Vitamin mixture[b]	1.00	1.00	1.00	1.00	1.00
Nonnutritive fiber[c]	4.85	1.00	4.85	1.00	1.00
Water	1.78	4.06	4.06	5.00	5.00
Sucrose	42.89	58.19	54.34	69.30	67.88
% Nitrogen	1.59	1.42	1.51	1.47	1.37

[a] Named after their protein source. CM = original coconut meal; PE = Protein (Coconut) extract; PEF = Protein (Coconut) extract plus 3.85% extra fiber.
[b] Same composition as that indicated in AOAC (1965) official method for the biological evaluation of protein quality according to which the diets were formulated.
[c] The 4.85% fiber content of the CM diet was that contributed by the coconut meal itself. No extra fiber was added to this diet.

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Table 3–Amino acid composition of the freeze-dried extract and of the original coconut meal (mg/g of material)

Amino acid	Coconut meal[b]	Protein extract[b]
Alanine	7.59 (233)	12.30 (275)**
Ammonia	0.81 (25)	1.79 (37)
Aspartic acid	13.20 (404)	23.40 (488)
Cystine	N.C.[c]	N.C.[c]
Glutamic acid	31.00 (950)	55.00 (1146)
Glycine	5.95 (182)	11.30 (235)
Proline	4.70 (144)	8.32 (173)
Serine	6.84 (210)	14.10 (294)
Tyrosine	3.53 (108)	6.78 (141)
Arginine[a]	23.20 (711)	42.70 (890)
Histidine[a]	2.43 (74)	5.60 (117)
Isoleucine[a]	5.04 (154)	8.96 (187)
Leucine[a]	9.88 (303)	17.70 (369)
Lysine[a]	5.46 (167)	11.40 (238)
Methionine[a]	3.09 (95)	4.41 (92)
Phenylalanine[a]	6.00 (184)	11.10 (231)
Valine[a]	7.76 (238)	13.50 (281)
Threonine[a]	4.75 (146)	8.36 (174)
Tryptophan[a]	1.38 (42)	2.33 (49)

[a] Ten essential amino acids
[b] Figures in brackets represent mg of amino acid per gram of N.
[c] N.C. = not calculated.

A no-protein diet was also prepared having the same nutrients percentages with the exception of additional sucrose being substituted for the protein removed.

The carbohydrate source was sucrose (Domino sugar obtained locally). The salt mixture (U.S.P. XVII), vitamin mixture (AOAC), cottonseed oil, nonnutritive fiber (cellulose type) used in the preparation of the diets were obtained from General Biochemicals (Chagrin Falls, Ohio). The casein and lactalbumin used were also purchased from the same company. The proximate composition of the original coconut meal and the extracted material is given in Table 1. The percentage composition of the diets prepared and their nitrogen content are given in Table 2. All diets were mixed in a liquid-solid blender (Patterson-Kelley Co., East Stroudsburg, Pa.).

The animals used in this study were male, weanling (55–60g) Sprague-Dawley rats, 27 days of age for the net protein utilization (NPU) and net protein retention (NPR) estimations and 40–50g, 21 days of age for the PER and apparent NPU evaluations. They were housed in individual stainless steel wire screen cages in a temperature controlled room. The lights were set for 12 hr of light and 12 hr of dark.

Ten animals per diet were used for the PER and apparent NPU tests and seven animals per diet were used for the NPU and NPR tests. A group of nine animals matched by weight with those used for the NPU and NPR tests were placed on the no-protein diet.

Diets and water were administered ad libitum. Food consumption and animal weights were recorded twice a week.

The PER was estimated following the method described by AOAC (1965). The NPR values were calculated according to Campbell (1963). The apparent NPU (or Productive Protein Value, PPV) values were estimated according to Cremer (1963) after an experimental period of 28 days in five out of the ten animals used for the PER tests. The NPU values were determined according to Miller (1963).

RESULTS & DISCUSSION

THE AMINO ACID composition of both, the freeze dried extract and the coconut meal is given in Table 3. The extract had a higher content (mg of amino acid per gram of extract) of every essential amino acid than the original coconut meal. The higher content of essential amino acids was also observed when the results were expressed as mg of amino acid per gram of nitrogen, except in the case of methionine which gave a slightly lower figure (92 mg/g of N) for the extract than for the coconut meal (95 mg/g of N).

The lysine content of the coconut meal (5.46 mg/g of material or 167 mg/g of N) indicates this to be the probable limiting amino acid, agreeing with the findings of Curtin (1958). However, the extract had a higher and more satisfactory lysine content (11.4 mg/g of extract or 238 mg/g of N) thus making it less probable that lysine is the limiting amino acid. The fact that the lysine content of the extracted proteins is much higher than that of the original coconut meal is of nutritional importance considering that the arginine content of both the extract (42.7 mg/g of extract or 890 mg/g of N) and the coconut meal (23.2 mg/g of material or 711 mg/g of N) is quite high. O'Dell and Regan (1963), Jones et al. (1966), O'Dell and Savage (1966) and Stutz et al. (1971) have clearly shown a lysine-arginine antagonism to occur in different animals. Further, it should be noted that although the arginine content of the extract was higher than that of the coconut meal, the increase was proportionately smaller than that obtained in the case of lysine. From a practical standpoint the high lysine content of the extract when compared to that of the coconut meal is also interesting, because

Table 4–Protein score[a] of the coconut meal (CM) and coconut protein extract (PE)

Material	ILe	Leu	Lys	Phe	Tyr	Met	Thr	Trp	Val	Total
					Amino acid content (mg/g of N)					
PE	187	369	238	231	141	92	174	49	281	1762
CM	154	303	167	184	108	95	146	42	238	1437
Whole egg	415	553	403	365	262	197	317	100	454	3066
					Percentage of total essential amino acids					
PE	10.61	20.94	13.50	13.11	8.00	5.22	9.88	2.78	15.95	
CM	10.72	21.09	11.62	12.80	7.52	6.61	10.16	2.92	16.56	
Whole egg	13.54	18.04	13.14	11.90	8.55	6.42	10.34	3.26	14.80	
					Protein score for amino acid					
PE	78.36	100	100	100	93.56	81.31	95.55	85.27	100	
CM	79.17	100	88.43	100	87.95	100	98.26	89.57	100	

[a] Calculated according to Pike and Brown (1967)

it will allow the blending of the extract with other edible protein materials which may not necessarily have a high lysine content because this amino acid is commonly limiting in many plant proteins.

The protein score for each essential amino acid of both the freeze-dried coconut protein extract and the coconut meal is given in Table 4. The essential amino acid content and the percentage of total essential amino acids of the fiber-free coconut protein extract (freeze dried), coconut meal and whole egg (Pike and Brown, 1967) is included.

It is interesting to note that while the total essential amino acid content of the extracted proteins was higher than that of the coconut meal the percentage of such essential amino acids remains relatively similar. These results, however, indicate that a higher amount of essential amino acids would be supplied by the extract than by the coconut meal when both materials are considered on an isonitrogenous basis.

These results also indicate that isoleucine is the most limiting amino acid for both the extract and the coconut meal used, with a protein score of 78.36 and 79.17, respectively. It is also noteworthy that while tyrosine, threonine and tryptophan appear to be limiting in both cases, methionine is limiting only in the extract and lysine only in the coconut meal. In this respect it should be noted that the amino acid methodology for methionine (as well as tryptophan and cystine) tend to give lower values, thus the values of methionine in the extract and that of tryptophan for both extract and coconut meal could be higher than those reported here. However, the fact that lysine is limiting only in the coconut meal and not in the extract is quite evident. The protein extract appears to have, percentagewise, a higher lysine content than that of the whole egg reference standard.

Table 5 presents protein quality results for the protein extract, the original meal and the extract with 3.85% fiber added to simulate the fiber content of the original meal diet. The protein quality results obtained for the control isonitrogenous diets (by calculation) prepared utilizing casein and lactalbumin are included.

It is evident that by all the methods used to assess protein quality, the protein extracted by the enzymic-chemical method (Molina and Lachance, 1973) was consistently superior to that of the original coconut meal, even when fiber equal to that of the original meal was added to the extracted product. The lower values when fiber was added illustrates the direct effect of the high fiber content of the coconut meal on protein nutritive value. These results are in accordance with the findings of Rama Rao et al. (1965) who reported that the biological value of the coconut proteins decreased considerably as the fiber content of the material increased.

The differences between the values obtained for the proteins of the extract and those of the coconut meal are, in all cases, statistically significant. In the case of the PER values the difference is statistically significant at a 95% confidence level ($\alpha = 0.05$). Thus, the biological assays of protein quality clearly indicate that the protein is available and that the presence of fiber can compromise the final result even when the test diets have similar nitrogen contents.

Although the zero fiber content of the extract appears to be a definitive factor in enhancing biological value one cannot disregard the possibility that the change in the essential amino acid profile during the protein extraction could account, partly at least, for the improvement in nutritive value especially when considering that the extracted protein has a higher content of essential amino acids (mg/g of N) than the coconut meal proteins (see Table 4), and that the lysine-to-arginine ratio is relatively higher in the former (0.27) than in the latter (0.23).

The higher nitrogen intake observed in the animals on the coconut meal diet when compared to those on the extract diet could possibly be explained by a combination of two factors: (1) the higher content of essential amino acids (mg/g of N) of the latter diet; and (2) the cathartic effect of the relatively higher fiber content of the former diet when compared to the latter diet. Although this second factor has been shown clearly to compromise the final results, little is known as to what extent the improved amino acid profile could affect the intake. The consistently improved protein biological values for the extract and the extract plus 3.85% fiber probably represent a benefit attributable to a more balanced amino acid profile.

Another possibility is that the enzymatically extracted protein, being partially broken down by the proteolytic enzyme (ficin), is more digestible than the protein in the original coconut meal.

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Table 5—Protein nutritive of the coconut protein extract compared to the original coconut meal, casein lactalbumin

	HSD[a]	No. of animals	CM[b]	PE[c]	PEF[d]	Casein	Lactalbumin
NPU	1.92	7	47 ± 0.65	56 ± 1.07	52 ± 0.85	69 ± 1.00	95 ± 1.41
NPR	0.60	7	3.81 ± 0.28	4.49 ± 0.36	3.94 ± 0.33	3.72 ± 0.32	6.71 ± 0.21
NPU app	2.97	5	47 ± 0.72	55 ± 1.51	49 ± 1.68	58 ± 0.75	—
PER	0.45	10	2.69 ± 0.10	3.09 ± 0.10	2.91 ± 0.17	2.86 ± 0.08	—
PER corrected			2.36	2.72	2.55	2.50	—

[a] "Honestly significant difference," calculated according to Tukey's w-procedure ($\alpha = 0.01$).
[b] CM = Original coconut meal.
[c] PE = Coconut protein extract.
[d] PEF = Extract plus 3.85% extra fiber.

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