Chinese Tallow Nut Protein. I. Isolation, Amino Acid, and Vitamin Analysis.

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THE fruit of the Chinese tallow tree, *Sapium sebiferum,* contains high quality drying oil, tallow, and protein in addition to fiber and hulls. The oil (4, 6, 11, 13) and tallow (3, 5, 9, 11) have been rather thoroughly investigated. Also various processing methods have been proposed for the recovery of the tallow and the oil (7, 12), but to date the authors have noted no reference on the recovery and utilization of the protein. The present work describes experiments which are preliminary to the industrial and nutritional investigations for this protein material.

Isolation of Protein

The tallow tree nuts, which are described in detail by Potts and Bolley (11), were separated from trash and twigs by a combination of screening, blowing, and hand picking. The tallow was removed by counter-current extraction with hexane in the pilot plant extractor described by Harris (2). The nuts were cracked with a Bauer mill. Separation of the hulls from the oily meats by simple mechanical means was difficult. However the meats were easily separated from the heavier hulls by flotation in ethylene dichloride. For this reason ethylene dichloride was used as the oil solvent.

The tallow nut flour, which remained after the removal of the oil, contained from 70 to 85% protein (N x 6.25) depending on the degree of hull separation. This material was used for the isolation of protein after nitrogen peptization at various pH levels was determined. The nitrogen peptization studies were conducted as described by Painter and Nesbitt (10), using 3 grams of tallow nut flour in 100-ml. volume and a 2-hour shaking period. As shown in Fig. 1, the protein is quite insoluble at pH 4 to pH 6 and is very soluble on the alkaline side of pH 7. The protein fraction was isolated by dissolving it in water (3 parts of flour to 100 parts of water) at 25°C. and pH 8.5, filtering, and precipitation at pH 5.5. The precipitate was separated by decantation and centrifugation and was dehydrated with acetone. After drying under vacuum, it yielded a white fluffy protein. Other preparations, which were dissolved at more alkaline pH values, were slightly colored and granular.

Amino Acid and B Vitamin Analyses

Two materials were assayed for amino acids. One was a high protein flour or concentrate prepared by screening the extracted meats. This preparation contained 13.0% nitrogen on the moisture free, ash free basis. The second material was the protein prepared as described above and contained 17.7% nitrogen on the moisture free, ash free basis. All amino acids were determined by microbiological methods (Table I). The amino acid content of casein is included in Table I for convenience in comparing it with the tallow nut protein. The striking differences are the

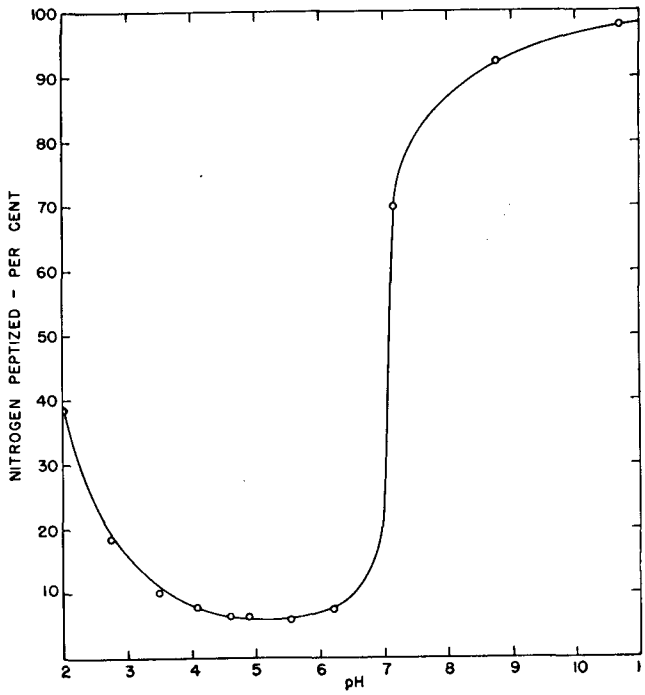

FIG. 1. Peptization of nitrogen in tallow nut flour in aqueous solutions at different pH values.

larger amounts of arginine, aspartic acid, cystine, and glycine in the tallow nut protein compared to casein, and the smaller amounts of lysine and methionine. The increased glycine would be advantageous in poultry feed. It appears that the amino acids are rather well balanced for good nutrition and that feeding tests would be desirable to determine the availability of the amino acids and to ascertain if any toxic substances are present. However, it is anticipated that the protein may be more valuable for certain commercial uses.

Of the microbiological amino acid assay methods used in obtaining the data in Table I the only ones that caused any difficulty were those for alanine, cystine, proline, and serine. The inability to get reproducible assay curves from day to day made it difficult to obtain desirable results although the results reported for these four amino acids represent averages of assay values that checked rather closely. The antagonism between serine and threonine and the possible implications of this antagonism on the assay for serine by some lactic acid bacteria has already been reported by the authors (8). As was the case for most of the amino acid assays, previously reported microbiological methods for the determination of the B vitamins were found to be adqeuate for the two materials assayed.

Table II shows the composition of the crude flour and the isolated tallow nut protein with respect to some of the more common B vitamins. The flour

TABLE I

Amino Acid Content of Chinese Tallow Nut Flour and Protein

Amino acid	Assay organism [a]	Basal medium [b]	Flour [c]	Protein [d]	Casein (1)
			%[e]	%[e]	%[e]
Alanine	F	(15)	1.5	2.2	2.8
Arginine	F	(17, 18)	12.0	16.6	4.2
Aspartic acid	M	(16)	8.3	11.7	6.3
Cystine	M	(19)	0.8	1.3	0.3
Glutamic acid	A	(20, 21)	13.0	17.3	24.2
Glycine	M	(19)	3.6	4.9	0.6
Histidine	F, M	(17, 18)	1.9	2.9	2.5
Isoleucine	A, F	(18, 22)	4.2	5.9	6.5
Leucine	A, F	(18, 22)	5.2	7.4	9.9
Lysine	F, M	(17, 18)	1.9	2.6	7.9
Methionine	F, M	(17, 18, 23)	1.1	1.6	3.5
Phenylalanine	A, D	(18, 22)	3.4	4.8	5.2
Proline	M	(17, 24)	3.2	4.3	8.0
Serine	F, M, D, C	(8, 18, 25, 26)	3.8	5.3	7.5
Threonine	F	(17, 18)	2.6	3.7	4.1
Tryptophan	A, F	(23)	1.0	1.4	1.4
Tyrosine	C, M	(23)	2.4	3.7	6.9
Valine	A, F	(17, 18, 22)	5.4	7.8	6.7

[a] A = *Lactobacillus arabinosus*
C = *Lactobacillus casei*
D = *Lactobacillus delbrueckii*
F = *Streptococcus faecalis* R
M = *Leuconostoc mesenteroides* P-60
[b] Figures in parentheses refer to the bibliography.
[c] Moisture, 10.11%; ash, 6.45%; nitrogen, 10.86%; N (ash and moisture free), 13.0%.
[d] Moisture, 8.75%; ash, 1.08%; nitrogen, 15.96%; N (ash and moisture free), 17.7%.
[e] Moisture free and ash free basis.

compares favorably with whole wheat flour in the content of the B vitamins (Table II). However, in the preparation of the protein there is appreciable loss of biotin, inositol, niacin, pantothenic acid, and riboflavin. Losses in thiamin, pyridoxine, and folic acid are much smaller.

TABLE II

Vitamin Content of Chinese Tallow Nut Flour and Protein

Vitamin	Assay organism [a]	Basal medium [b]	Flour	Protein	Whole wheat flour (14)
			γ/g.	γ/g.	γ/g.
Biotin	C	(27)	0.29	0.04	0.058
Folic acid	C	(27)	0.60	0.37	2.0[c]
Inositol	Ce	(28)	750.0	225.0	1890.0
Niacin	C	(27)	29.8	9.9	46.0
Pantothenic acid	C	(27)	2.4	0.41	13.0
Pyridoxine	S	(28)	17.3	13.9	2.2
Thiamin	F	(29)	100.7	80.8	5.6
Riboflavin	C	(27)	3.6	0.74	1.8

[a] C = *Lactobacillus casei*
F = *Lactobacillus fermentum*
S = *Saccharomyces carlsbergensis*
Ce = *Saccharomyces cerevisiae*
[b] Figures in parentheses refer to the bibliography.
[c] *Potency 40,000—equivalent to approximately 0.5 γ folic acid.*

Conclusion

The protein of the Chinese tallow nut is quite easily isolated by employing rather mild conditions. On the basis of amino acid values presented, both the protein and flour should be suitable as a protein feed. The relatively low lysine and methionine content however would limit the usefulness of the protein as a supplement to cereal grains. The relatively high thiamin content of the protein, in addition to the presence of smaller amounts of the other B vitamins determined, suggests the possible use of this material in the enrichment of flour for the baking industry. The industrial possibilities in addition to the food value of the protein are yet to be determined.

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