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STUDIES ON PROCESSED PROTEIN FOODS BASED ON BLENDS OF GROUNDNUT, BENGALGRAM, SOYABEAN AND SESAME FLOURS AND FORTIFIED WITH MINERALS AND VITAMINS

II. AMINO ACID COMPOSITION AND NUTRITIVE VALUE OF THE PROTEINS

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In the preceding paper, the results of studies on the preparation, chemical composition and shelf-life of protein foods based on blends of groundnut, Bengal gram, soya and sesame flours and fortified with limiting amino acids, calcium salts and essential vitamins have been reported[1]. The present paper deals with studies on the amino acid composition and nutritive value of the protein present in the protein foods.

Experimental

Materials : The protein foods based on (i) a blend (40 : 40 : 20) of groundnut, Bengal gram and sesame flours and (ii) a blend (40 : 30 : 30) of groundnut, soya and sesame flours, fortified with l-lysine and dl-methionine, calcium salts and vitamins A and D, thiamine and riboflavin were prepared as described in an earlier paper.[1]

Amino acid composition : The essential amino acid composition of protein foods and of skim milk powder determined according to the methods of Krishnamurthy *et al.*[2] is given in Table I.

Animal experiments :

Protein efficiency ratio : The protein efficiency ratio (PER) of the foods at 10% level of protein intake was determined by the rat growth method of Osborne,

Table I. *Essential amino acid composition of Protein Foods based on blends of sesame, Bengal gram, soya bean and groundnut flours*

Amino acid (g)/16gN	Protein Food I[a]		Protein Food II[b]		Skim milk powder	Human milk*
	Unfortified	Fortified[1]	Unfortified	Fortified[2]		
Arginine ...	9.6	9.6	9.6	9.6	3.7	—
Histidine ...	2.0	2.0	2.3	2.3	2.7	—
Lysine ...	3 8	6.3	4.3	6 3	7.9	6.3
Methionine ...	1.6	2.6	1.7	2.3	2.5	2.2
Cystine ...	1.7	1.7	2.0	2.0	0.9	2.1
Total sulphur amino acids ...	3.3	4.3	3.7	4.3	3.4	4.3
Phenylalanine ...	5.9	5.9	5.9	5.9	4.9	4.6
Tryptophan ...	1.1	1.1	1.3	1.3	1.4	1.6
Threonine ...	3.0	3.0	3.1	3.1	4.7	4.6
Valine ...	4.7	4.7	5.0	5.0	7.0	6.6
Leucine ...	7.1	7.1	7.4	7.4	10.0	8.9
Isoleucine ...	5.0	5 0	5.2	5.2	6.5	6.4

* FAO Nutrition Studies No. 16. Food and Agriculture Organization, ROME 1957.

a. Blend of 1 : 2 : 2 of sesame, Bengalgram and groundnut flours.

b. Blend of 3 : 4 : 4 of sesame, soya and groundnut flours.

1, 2. The protein foods I and II were fortified with l-lysine (2.5g/16gN and 2.0g/16gN respectively) and dl-methionine (1.0g and 0.6g/16gN respectively) to raise the levels of these amino acids to that of human milk proteins.

Mendel & Ferry.[3] Male albino rats (Wistar strain) 28 days old, were allotted to different groups in a randomised block design. Each group contained 8 rats. The composition of the experimental diets is given in Table II. The methods used for the preparation of the diets and the feeding of the animals were the same as those of Tasker *et al*[4] The rats were weighed weekly and records of food intake of the animals were maintained. The values for PER calculated from the data obtained are given in Table III.

Table II. *Percentage composition of experimental diets*

	Nitrogen free diet	Protein food diets I	Protein food diets II	Skim milk powder diet III
Protein Foods*	—	25.9	21.6	—
Skim milk powder	—	—	—	26.8
Salt mixture†	2.0	2.0	2.0	2.0
Vitaminised starch††	1.0	1.0	1.0	1.0
Groundnut oil	9.0	9.0	9.0	9.0
Corn starch	87.0	61.1	65.4	60.2
Vitaminised oil ††	1.0	1.0	1.0	1.0

* Protein food I- based on 1 : 2 : 2 blend of sesame flour, Bengal gram and groundnut flour, (with or without fortification with lysine and methionine).
Protein Food II-based on 3 : 3 : 4 blend of sesame flour, full fat processed soya flour and groundnut flour, (with or without fortification with lysine and methionine)
† Hubbel, Mendel, and Wakeman salt mixture supplemented with $ZnCo_3$ 0.115g per 100g of salt mixture.
†† Vitaminised starch and oil of Chapman *et al*[5]

Net protein utilization : The net protein utilization (NPU) of the protein foods was determined according to Miller and Bender[5] at 10% level of protein intake. Male albino rats (28 days old) weighing 40-45g were alloted in a randomised block design to different groups Each group contained 8 animals One group received a nitrogen-free diet while the other groups received the diets containing 10% protein from the protein foods and skim milk powder for a period of 10 days. At the end of 10 days, the animals were sacrificed. After the removal of the contents of the gastro-intestinal tract by squeezing, the rats were minced in a meat mincer and dried to constant weight in a drier at 90-95°C. The carcass nitrogen was estimated and the NPU and NPU (st) values were calculated according to the formula used by Panemangalore *et al.*[6] The net protein ratio (NPR) values were also calculated according to Panemangalore *et al.*[6] The results are presented in Table IV.

Results

Amino acid composition (Table I): The limiting amino acids in protein foods I and II were lysine, sulphur amino acids and threonine. When compared with human milk proteins, the protein foods fortified with l-lysine and dl-methionine were limiting in threonine.

Protein efficiency ratio (Table III): The protein efficiency ratios (PERs) of protein foods I and II were 2.29 and 2.40 (at 10% protein level), 2.15 and 2.33 (at 15% protein level) and 1.86 and 1.80 (at 20% protein level) as compared with values of 3.10, 2.23 and 1.50 respectively obtained for milk proteins. Fortification of the protein foods with l-lysine and dl-methionine increased the PERs of the protein foods to 2.41 and 2.57 and these were less than the PER (3.10) of milk proteins.

Net protein utilization (Table IV) : The NPU values for the protein foods I and II were 52 7 and 57.9 as compared with 70.1 obtained for milk proteins Fortification with l-lysine and dl-methionine brought about a slight increase in the NPU values.

Net protein ratio (Table IV) : Table IV shows that the net protein ratios (NPRs) of the protein foods were 3.61 and 3.81 and were less than that (4.47) obtained for skim milk powder.

Discussion

The results reported in this paper have shown that protein foods based on 40 : 40 : 20 blend of groundnut, Bengal gram and sesame flours and 40 : 30 : 30 blend of groundnut, soya and sesame flours possessed fairly high PER (2.29 and 2.41). These values are higher than that reported for Indian multipurpose food[7] (1.8) and

Table III. *Protein efficiency ratio of the Protein Foods based an blends of sesame, Bengalgram, soyabean and groundnut flours*
(Mean values for 8 males in each group ; Duration of experiment-4 weeks)

Source of protein in diet	Protein level %	Initial body weight (g)	Protein intake (g)	Gain in body weight (g)	Protein efficiency ratio†	
Protein Food I :	10	46.8	35.05	80.5	2.29 ± .05 (97.df)	
	15	46.5*	50 61*	108.7*	2.15 ± .06 (97 df)	
	20	46.8	70.85	131.9	1.86	
Protein Food I + lysine + methionine :	10	46.8	35.29	85.2	2.41	
	15	46.9	53.58	127.1	2.37	
	20	46.6	70.52	129.4	1.84	
Protein Food II :	10	46.6	35.10	84.5	2.40	
	15	46.6	51.78	120.4	2.33	
	20	46.8	71.67	128.8	1.80	± .05 (97.df)
Protein Food II + lysine + methionine :	10	46.6	38.26	98.2	2.57	
	15	46 6	53.81	121.6	2.26	
	20	46.6	71.18	131.9	1.85	
Skim milk powder :	10	46.2	36.62	113.8	3.10	
	15	46.6	52.89	118.0	2.23	
	20	46.6	61.82	92.9	1.50	
Critical Differences at :						
One tailed 5% level					0.12[1]	0.13[2]
1% ,,					0.17	0.19
0.1% ,,					0.22	0.26
Two tailed 5% ,,					0.14	0.16
1% ,,					0.18	0.21
0.1% ,,					0.24	0.27

[1]. To be used for comparison between means other than protein food I at 15% level.
[2]. To be used for comparison between protein food I at 15% level with any other group.
* As one sample value was missing the means are based on only 7 observations.
† One sample value was missing in the second group. It has been estimated and standard error calculated according to formula given in *Design and analysis of experiments* by O. Kempthorne p. 175, Wiley Publications 1952.

Table : IV *Net protein utilization of Protein Foods based on blends of sesame, Bengal gram, soyabean and groundnut flours*
(Mean values for 8 male rats in each group ; level of protein 10% ; duration of experiment 10 days)

Group No.	Source of protein in the diet	Gain in weight (g)	Nitrogen intake (g)	Body nitrogen (g)	$NPU_{(op)}$		$NPU_{(Std)}$		PER		NPR	
1	N-free diet	-5.3	0.032	1.017	—		—		—		—	
2	Protein Food I	24.2	1.317	1.689	52.70		55.53		2.94		3.61	
3	Protein Food I+ lysine + methionine	24.2	1.286	1.670	53.25		56.70		3.01		3.70	
4	Protein Food II	24.6	1.281	1.731	57.87		62.14		3.04		3.81	
5	,, ,, +lysine+methionine	28.4	1.358	1.827	61.95		67.27		3.26		4.01	
6	Skim milk powder	33.8	1.408	1.953	70.11		79.38		3.85		4.47	
	Standard error				±2.62 (28df)		±3.25 (28df)		±0.15(28df)		±0.17(28df)	
					a	b	a	b	a	b	a	b
	Critical difference at 5%			...	6.31	7.6	7.82	9.42	0.36	0.43	0.41	0.49
	,, ,, 1%			...	9.15	10.25	11.35	12.71	0.52	0.58	0.59	0.66
	,, ,, 0.1%			...	12.64	13.63	15.68	16.90	0.71	0.77	0.81	0.88

a : One tailed test b : Two tailed test

for protein food based on 1 : 1 blend of groundnut and soya flours[6] (1.99) and nearly equal to that (2.39) of Indian multipurpose food formula C[8] (based on 60 : 20 : 20 blend of groundnut flour, Bengal gram and skim milk powder). The results indicate that it is possible to replace skim milk powder in the Indian multipurpose food formula C by a blend of sesame and Bengal gram or sesame and soya bean flours without lowering the protein efficiency ratio. This finding is of great practical and economic importance to India and other developing countries in which skim milk powder is costly and is not available in adequate quantities. Fortification of the protein foods with l-lysine and dl-methionine brought about a significant increase in the PER. Fortification with these amino acids, however, will be possible when they are available at low cost.

Summary

1) The protein efficiency ratios (PERs) of two protein blends (Blend I based on 40 : 40 : 20 of groundnut, Bengal gram and sesame flours and Blend II based on 40 : 30 : 30 blend of groundnut, Soya bean and sesame flours) at 10, 15 and 20% levels of protein intake were 2.29 and 2.40, 2.15 and 2.33 and 1.86 and 1.80 respectivly as compared with corresponding values of 3.10, 2.23 and 1.50 obtained for milk proteins. Fortification with l-lysine and dl-methionine increased the PERs at 10% level to 2.41 and 2·57.

2) The net protein utilization (NPU) values (at 10% protein level) for the two protein blends were 52.7 and 57.9 as compared with a value of 70.1 obtained for milk proteins. The net protein ratios (NPRs)of the two protein foods were 3.61 and 3.81 as compared with a value of 4.47 for skim milk powder.

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REFERENCES

1. Guttikar, M. N., Panemangalore, M., Narayana Rao, M. and Swaminathan, M. (1965), *J. Nutr. Dietet.*, **2**. 21.
2. Krishnamurthy, K., Tasker, P. K., Ramakrishnan, T. N., Rajagopalan, R. and Swaminathan, M. (1960), *Ann, Biochem. Expt. Med.*, **20**, 73.
3. Osborne, T. B., Mendel, L. B. and Ferry, E. L. (1919), *J. Biol. Chem.*, **37**, 223.
4. Tasker, P. K., Joseph, A. A., Ananthaswamy, H. N., Indiramma, K., Narayana Rao, M., Swaminathan, M., Sreenivasan, A. and Subrahmanyan, V. (1962), *Food Science*, **11**, 173.
5. Miller, D. S. and Bender, A. E.(1955), *Brit. J. Nutr.*, **9**, 382.
6. Panemangalore M., Balaji Rao, M., Narayana-Rao, M., Rajagopalan, R., Chandrasekhara, B.S., Swaminathan, M., Sreenivasan, A. and Subrahmanyan, V. (1964), *J. Nutr. Dietet.*, **1**, 4.
7. Shurpalekar, S. R., Moorjani, M. N., Lahiri, N. L., Indiramma, K., Swaminathan, M., Sreenivasan, A. and Subrahmanyan, V. (1962), *Food Science*, **11**, 42.
8. Joseph, K. Narayana Rao, M., Swaminathan, M., Indiramma, K. and Subrahmanyan, V. (1960), *Ann. Biochem. Exp. Med.*, **20**, 243.