Studies on a Vegetable Protein Mixture Based on Peanut and Soya Flours

M. NARAYANA RAO, R. RAJAGOPALAN, M. SWAMINATHAN, and H. A. B. PARPIA, Central Food Technological Research Institute, Mysore, India

Abstract

A vegetable protein mixture made up of 50% edible peanut flour and 50% full-fat soya flour and fortified with 1% each of l-lysine and dl-methionine and adequate amts of essential vitamins and minerals has been developed. Two formulations, 1) unseasoned and 2) seasoned have been developed for use in sweet and savoury preparations; both the formulations keep well for more than 9 months at 37C when stored in hermetically sealed cans. The protein mixture contains 49.2% protein which possesses a PER of 2.80 and NPU of 72.3; these values compare favourably with those for skim milk powder. Growth studies on albino rats and feeding trials on school children have shown that the protein mixture has a significant supplementary value to poor cereal-based diets. The mixture fortified with methionine alone is equally effective as skim milk powder for the treatment of kwashiorkor.

Introduction

PROTEIN MALNUTRITION, affects a great majority of pre-school children in the low income groups in most of the technically underdeveloped areas of the world (1). Milk and other protective foods of animal origin are in short supply (2) in these regions and efforts have been made by a number of workers (3–6) in developing highly nutritive supplements based on plant protein sources like edible oil seed residues, legume flours, etc., for supplementing the diets of children and other vulnerable groups. Plant proteins in general are lacking in one or more of the essential amino acids and possess a nutritive value lower than that of milk proteins (7). However, it is possible to prepare protein supplements of high nutritive value by suitably blending two or more vegetable proteins, which mutually supplement each other or by fortification with the essential amino acids in which they are deficient (8,9). Synthetic lysine and methionine are at present available in commercial quantities and can be advantageously used for fortification of protein foods. The protein supplements so far developed contain proteins of moderate nutritive value (10,11). There is need for standardizing protein blends of high nutritive value approaching that of milk proteins and which can be used in place of milk in the treatment and prevention of protein malnutrition in children.

The results of studies on the preparation, chemical composition and nutritive value of a vegetable protein mixture based on peanut and soya flours fortified with lysine and methionine and with adequate amts of vitamins and minerals are presented in this paper.

Experimental

Processing of Raw Materials

Peanut Flour. This was prepared from good quality peanut kernel free from spoilt seeds according to Subrahmanyan et al. (12). The process consisted of the following steps: 1) light roasting of peanut kernels freed from foreign matter at about 90–100C for 5 min; 2) removal of testa using a blanching machine; 3) separation of shrivelled, spoilt and fungus-attacked seeds by hand picking; 4) pressing the oil in a screw press; and 5) powdering the resulting cake in a hammer mill. The product had a light cream colour with a pleasant nutty odour and acceptable taste.

Full Fat-Soya Flour. Decorticated soya bean *dhal* (split dehusked legumes) was soaked in water for 1–2 min and autoclaved at 15 psi for 30 min. It was then debittered by treatment with sodium bicarbonate. The processed *dhal* was dried at 50–55C in a cabinet drier and powdered in a hammer mill to pass through 50 mesh. The full-fat soya flour so prepared was slightly yellowish brown in colour and had a pleasant odour and taste. The flour, as tested by the method of Anson (13) was completely free from tryptic inhibitor.

Seasoning Premix. A mixture of coriander seeds (60 parts), black pepper (30 parts), turmeric (9 parts) and asafoetida (1 part) was lightly roasted with a small quantity of hydrogenated fat and powdered to 60 mesh.

Process Details

The protein food was prepared in two forms: 1) unseasoned and 2) seasoned. The unseasoned protein food was prepared by blending peanut flour (50 parts) and full-fat soya flour (50 parts) in a suitable mixer. The calcium salts, vitamins A and D, thiamine and

TABLE I

Chemical Composition [a] of Protein Mixture

Constituent	Protein mixture		Skim milk powder
	Seasoned	Unseasoned	
Moisture (g)	7.4	7.6	3.1
Protein (N × 6.25) (g)	47.8	49.2	35.0
Fat (g)	15.8	15.5	1.0
Carbohydrate (g)	18.4	18.5	53.1
Calcium (g)	0.93	0.98	1.30
Phosphorus (g)	0.72	0.75	1.05
Iron (mg)	7.4	6.2	0.6
Thiamine (mg)	2.2	2.3	0.35
Riboflavin (mg)	3.1	3.3	1.39
Niacin (mg)	11.6	12.2	1.1
Vitamin A[b] (I.U.)	3000	3000	—
Vitamin D[b] (I.U.)	300	300	

[a] Values per 100 g.
[b] Added values.

TABLE II

Essential Amino Acid Composition of Protein Mixture

Amino acid (g /16g N)	Protein mixture	Skim milk powder	FAO reference protein pattern
Arginine	8.9	3.7	—
Histidine	2.4	2.7	—
Lysine	6.9	7.9	4.2
Methionine	3.1	2.5	2.2
Cystine	1.6	0.9	—
Total S-amino acids	4.7	3.4	4.2
Phenylalanine	5.0	4.9	2.8
Tryptophan	1.2	1.4	1.4
Threonine	3.3	4.7	2.8
Valine	5.1	7.0	4.2
Leucine	6.9	10.0	4.8
Iso-leucine	4.7	6.5	4.2

riboflavin were added to the above as suitable premix. The lysine and sulphur amino acid contents of the mixture were 4.9 and 2.7 g/16 g N, respectively. The protein food was also fortified with 1% each of l-lysine and dl-methionine so as to raise the lysine content to 6.9 g/16 g N and sulphur amino acid content to 4.7 g/16 g N. The unseasoned product was flavoured with vanillin at 0.05% level. The seasoned form was prepared by adding the seasoning premix at 3% level and common salt at 2% level to the mixture.

Chemical Composition. The chemical composition of the protein food (seasoned and unseasoned forms) determined according to standard methods of AOAC (14) is given in Table I; the composition of skim milk powder is given for comparison.

Shelf-Life Studies. The protein food packed in hermetically sealed cans was stored at 37C for a period of 9 months. Control samples were kept at 0C. The samples were analysed periodically once in 3 months, for peroxide value, fat acidity, thiamine and vitamin A. The organoleptic quality of the protein food was tested by a panel of eight judges selected from among the Institute staff.

Amino Acid Composition. The essential amino acid composition of the protein food and of skim milk powder determined according to Krishnamurthy et al. (15) are given in Table II.

Evaluation of the Nutritive Value. Growth studies on albino rats and feeding trials on school children have been carried out to evaluate the nutritive value of the protein food.

The results of studies on the protein efficiency ratio and net protein utilization of the food at 10% level of protein intake as determined according to Osborne et al. (16) and Miller and Bender (17) are given in Table III. Since the incidence of protein malnutrition is very high in children whose diets consist predominantly of maize and tapioca (18), the supplementary value of the protein food to a poor maize-tapioca diet was studied in albino rats. The protein food was incorporated at a level to provide 10% extra protein. In addition, histological examination of the livers was also carried out. The results are given in Table IV.

Supplementary Value to the Diets of School Children. A controlled feeding trial over a period of 5½ months was carried out in a local boarding home to assess the effect of a daily supplement of 40 g of this protein food on the growth and nutritional status of school children, belonging to the low-income groups of the population. Forty girls, aged 5–12 years, were selected for the experiment. They were paired on the basis of initial height and weight and the members of each pair allotted at random to the control and experimental groups. The two groups received the boarding home diet, which consisted mainly of cereals and only small amts of legumes, milk and meat. Each child in the experimental group received a daily supplement of 40 g of protein food as against 40 g of rice. The

TABLE III[a]

Protein Efficiency Ratio and Net Protein Utilization of the Protein Mixture

Source of protein in diet	PER[b]		NPU[c]	
Protein mixture	2.80	±0.068 (21 df)	72.3	±2.23 (21 df)
Skim milk powder	2.96		79.9	

[a] Data includes two more groups, results of which are not included in the table.
[b] Mean values for 8 males in each group; duration of experiment, 4 weeks
[c] Mean values for 8 males in each group; duration of experiment, 10 days.

TABLE IV[a]

Gain in Body Weight of Rats on a Maize-Tapioca Diet Supplemented with the Protein Mixture

Diet	Weekly gain in weight (g)[b]		Feed efficiency ratio[c]	
Maize-tapioca diet	−0.15		−0.003	
Maize-tapioca diet + protein mixture	19.9	+0.94 (30 df)	0.26	±0.01 (15 df)
Maize-tapioca diet + skim milk powder	18.6		0.26	

[a] Data include one more group, results of which are not included.
[b] Mean values for 6 males and 6 females in each group; statistical analysis was carried out omitting the maize-tapioca diet.
[c] Mean values for 6 males in each group; statistical analysis was carried out, omitting the maize-tapioca diet.

nutrients provided in the diets are given in Table V. At the end of the feeding period, height, weight, hemoglobin, red blood cell count and nutritional status of the children were determined and their nutritional status assessed. The results are presented in Table V. The results of a metabolism study to determine the retention of nitrogen, calcium and phosphorus carried out on eight children in each group, when the feeding trial had been in progress for a period of 3 months is given in Tables VI and VII.

Efficacy of the Protein Food in the Treatment of Kwashiorkor. The protein food fortified with methionine alone has been successfully used in the treatment of kwashiorkor in children, 2–5 years old. The patients were clinically examined, immediately on admission. Except for traces of albumin, their urine was normal. No intestinal parasites (except roundworm ova in some subjects) were found in the stools. Anaemia was present to varying degrees in all patients. Blood samples were analysed for the following constituents soon after admission; cholesterol and thymolturbidity according to King and Wootton (19), hemoglobin and red blood cell count according to Reddy et al. (20).

The protein food was reconstituted with six to eight times its weight of water and sweetened with cane sugar. The supplements were administered orally in four to five doses daily. The quantity of protein feeds was gradually increased until each child received 30 g protein from the food by the end of the first week of treatment. Additional calories were provided in the form of cooked rice, bread and vegetable soup. The protein derived from these sources was about 8–10 g daily. The protein intake by the subjects was approx 4–5 g per kilogram body weight. The calorie intake was about 120 per kilogram body weight. Specimens of blood were taken on admission and on the 10th and 30th day of treatment for estimation of serum proteins according to King and Wootton (19).

TABLE V

Increases[a] in Height, Weight, Haemoglobin Content and Red Blood Cell Count

	Control[c] (Rice diet)	Experimental[d] (Rice diet + Protein mixture)	Differences in the increase ± standard error
Height (cm)	1.04	2.21	1.17[b]±0.20
Weight (kg)	0.66	1.92	1.26[b]±0.07
Haemoglobin (g/100 ml)	0.45	1.21	0.76[b]±0.10
R.B.C. count (10^6/cu mm)	0.19	0.38	0.19[b]±0.05

[a] Mean values for 20 girls aged 5–12 years in each group: duration of experiment 5½ months.
[b] Highly significant.

The nutrient intake in the two groups was as follows:

	c	d
Protein (g)	39.9	57.1
Calcium (g)	0.27	0.65
Riboflavin (mg)	0.56	1.90
Vitamin A (I.U.)	882	2222
Calories (cal)	1520	1521

TABLE VI

Nitrogen Metabolism [a]

Group	Nitrogen intake		Nitrogen excreted			Nitrogen balance		
	Total (g)	mg/kg	Urinary (g)	Faecal (g)	Total (g)	(g)		mg/kg
Control (rice diet)	6.35	298	3.23	1.63	4.86	1.49	±0.20 (7 df)	70
Experimental (rice diet + protein mixture)	9.13	427	5.28	1.31	6.59	2.54		119

[a] Mean values per day; average of 8 girls, aged 9–10 years, per group.

Results and Discussion

Chemical Composition and Shelf-Life Studies (Table I)

The protein food contains about 50% protein and substantial amts of vitamins and minerals. A supplement of 40–50 g of the food will supply one third to one half the daily requirements of proteins, vitamins and minerals for children. Both the seasoned and unseasoned forms of the food kept well for over a period of 9 months at 37C when packed in hermetically sealed cans. The loss of vitamin A and thiamine during the period were 25% and 15%, respectively. The peroxide value (7.8) and fat acidity (6.1) of the seasoned protein food at the end of 9 months' storage were slightly lower than the values (9.7 and 6.3, respectively) for unseasoned protein food; this may be due to the antioxidant activity of the spices (22) present in the seasoned food.

Amino Acid Composition (Table II)

The protein food contained lesser amts of all the essential amino acids, except arginine, phenylalanine and total sulphur amino acids than milk proteins but judged by the FAO reference protein pattern (23) the food was deficient only in tryptophan.

Protein Efficiency Ratio and Net Protein Utilisation (Table III)

The protein efficiency ratio (2.80) and net protein utilization of the food (72.3) at 10% level of protein intake are comparable to those (2.96 and 79.9) obtained for skim milk powder. While there was no significant difference in the PER of the protein food and skim milk powder, the values for NPU were significant at 5% level ($P < 0.05$).

Supplementary Value to Maize-Tapioca Diet (Table IV)

The rats fed on the maize-tapioca diet did not grow but lost weight during the experimental period. Incorporation of the protein food in the diet to provide 10% extra protein made up the deficiencies in the diet and caused a marked improvement in the growth rate of rats. The growth of rats receiving the supplements of protein food (19.9 g/wk) was slightly higher than that (18.6 g/wk) of rats receiving skim milk powder. The livers of rats receiving the diet based on maize and tapioca showed cytoplasmic vacuolation and periportal fatty infiltration, whereas those of rats receiving the protein food or skim milk powder supplements had a normal histological appearance. The results obtained show that the protein food is as effective as skim milk powder in making up the deficiencies in a maize-tapioca diet and in preventing liver damage. The periportal fatty infiltration observed in livers of rats receiving the maize-tapioca diet is mainly due to the deficiency of protein in the diet (24).

TABLE VII

Calcium and Phosphorus Metabolism [a]

Group	Intake (mg)	Excretion (mg)			Balance (mg)	
		Urinary	Faecal	Total		
Calcium						
Control (rice diet)	267	25	210	235	32	±25.7 (7 df)
Experimental (rice diet + protein mixture)	634	65	386	451	183	
Phosphorus						
(Control (rice diet)	826	357	314	671	155	±52.2 (7 df)
Experimental (rice diet + protein mixture)	1180	263	529	792	388	

[a] Mean values per day; average of 8 girls, aged 9–10 years, per group.

Feeding Trials on School Children (Table V)

A highly significant ($P < 0.001$) increase in the height, weight, haemoglobin content and red blood cell count of blood as well as improvement in nutritional status of the subjects receiving a 40 g daily supplement of the protein food was observed as compared with the control group. Sixteen children in the experimental group improved in their nutritional status as compared with only five children in the control group. Further, four children in the control group deteriorated in their nutritional status while none in the experimental group showed any deterioration. The children in the experimental group have also retained significantly larger amts of N, Ca and P than those receiving the control diet (Tables VI and VII).

Treatment of Kwashiorkor in Children

After a few days of treatment the edema began to decrease and disappeared completely as treatment was continued. The average time taken for the disappearance of the edema was 11.4 days, as compared to 12.3 days obtained in the case of skim milk powder in an earlier study (21). Diarrhoea began to subside within a week of the institution of protein therapy in a large number of cases. The average time taken for the disappearance of edema was 8.5 days, as compared to 9.8 days obtained for skim milk powder in an earlier study. The slightly longer time taken for the disappearance of diarrhoea in the case of skim milk powder may be because lactose is not well tolerated by kwashiorkor children (25). The rate of regeneration of serum proteins and albumin (2.38 and 1.92 g by the 30th day of treatment) on the protein food are slightly lower than those (2.88 and 2.20 g, respectively) reported for skim milk powder. This may be due to the fact that the protein food contains lesser amts of lysine and threonine than skim milk powder. The results indicate that the protein food is quite effective in initiating a cure of kwashiorkor.

Conclusions

The results of investigations reported in this paper have demonstrated that it is possible by fortification with lysine and methionine to prepare a vegetable protein mixture of high nutritive value, comparable to that of skim milk powder, which can be used in the effective treatment and prevention of protein malnutrition in children. Though lysine and methionine are at present available in commercial quantities, their cost is still high and restricts their use in the preparation of processed protein supplements for the supplementary feeding programmes envisaged in the technically developing countries. Much headway has yet to be made in the technology of these products and in organising distribution. Large scale production and consumption of processed protein foods, suitably fortified with vitamins and minerals, will help considerably in making up the many dietary deficiencies and in improving the health of children and other vulnerable sections of the population in several developing countries. It is gratifying to note that several international organizations like FAO, UNICEF and WHO are helping various governments in the production and use of protein-rich foods for the prevention and treatment of protein malnutrition in children.

ACKNOWLEDGMENT

Miss Myna Panemangalore, and Drs. S. Venkat Rao, T. R. Doraiswamy and R. K. Bhagavan assisted in these studies. This study was supported by grants-in-aid from PL 480 funds, USPHS.

REFERENCES

1. Jelliffe, D. B., "Infant Nutrition in Tropics and Subtropics", WHO monograph No. 29, Geneva, 1955.
2. Food and Agriculture Organization, "Production Year Book," Vol. 16, FAO, Rome, 1962.
3. Subrahmanyan, V., M. Narayana Rao and M. Swaminathan, Proc. Nat. Inst. Sci. (India), 26A (suppl 1), 99–112 (1960).
4. Scrimshaw, N. S., and R. Bressani, Fed. Proc. *20* (suppl 7), 80–88 (1961).
5. Autret, M., and A. G. van Veen, Amer. J. Clin. Nutr. *3*, 234–243 (1955).
6. Behar, M., R. Bressani and N. S. Scrimshaw, in "World Reviews of Nutrition and Dietetics," Vol. 1, Editor, G. H. Bourne, Pitman Medical Publishing Co., London, 1959, pp. 77–101.
7. Kuppuswamy, S., M. Srinivasan and V. Subrahmanyan, "Proteins in Foods," Special Report No. 33, Indian Council of Medical Research, New Delhi, 1958.
8. Dean, R.F.A., "Plant Proteins in Child Feeding," Special Report Series No. 279, Medical Research Council, London, 1953.
9. Howe, E., in "Meeting Protein Needs of Infants and Children," Publication No. 843, National Research Council, National Academy of Sciences, Washington, D.C., 1961, pp. 495–507.
10. Subrahmanyan, V., G. Rama Rao, S. Kuppuswamy, M. Narayana Rao and M. Swaminathan, Food Science *6*, 76–80 (1957).
11. Scrimshaw, N. S., J. Amer. Dietet. Assoc. *35*, 441–448 (1959).
12. Subrahmanyan, V., D. S. Bhatia, G. S. Bains, M. Swaminathan and Y. K. Raghunatha Rao, Bull. Cent. Fd. Technol. Res. Inst. *3*, 180–183 (1954).
13. Anson, M. L., J. Gen. Physiol. 22, 79–89 (1938).
14. AOAC, "Official and Tentative Methods of Analysis," AOAC, 8th edition, Washington, 1955.
15. Krishnamurthy, K., P. K. Tasker, T. N. Ramakrishnan, R. Rajagopalan and M. Swaminathan, Ann. Biochem. Exp. Med. (India) *20*, 73–76 (1960).
16. Osborne, T. B., L. B. Mendel and E. L. Ferry, J. Biol. Chem. *37*, 223–228 (1919).
17. Miller, D. S., and A. E. Bender, Brit. J. Nutr. 9, 382–388 (1955).
18. Trowell, H. C., J. N. P. Davies and R. F. A. Dean, "Kwashiorkor," Edward Arnold Publishers Ltd., London, 1954.
19. King, E. J., and I. D. P. Wootton, "Micro-analysis in Medical Biochemistry," J & A Churchill Ltd., London, 1956.
20. Reddy, S. K., T. R. Doraiswamy, A. N. Sankaran, M. Swaminathan and V. Subrahmanyan, Brit. J. Nutr. *8*, 17–21 (1954).
21. Bhagavan, R. K., T. R. Doraiswamy, N. Subramanian, M. Narayana Rao, M. Swaminathan, D. S. Bhatia, A. Sreenivasan and V. Subrahmanyan, Amer. J. Clin. Nutr. *11*, 127–133 (1962).
22. Sahasrabudhe, M. R., and D. S. Bhatia, Sci. Cult. (Calcutta) *18*, 384–385 (1953).
23. Food and Agriculture Organization, "Protein Requirements," Nutrition Studies No. 16, FAO, Rome, 1957.
24. Sriramachari, S., Indian J. Med. Res. *50*, 920–951 (1962).
25. Dean, R. F. A., Bull. World Health Org. *14*, 798–801 (1956).

[Received August 12, 1964—Accepted March 25, 1965]

Continuous Recovery of Acid Oil

LOIS S. CRAUER, The De Laval Separator Company, Poughkeepsie, New York

Abstract

A continuous process for acidulation of all types of caustic soapstocks is described. The soap is diluted, heated, acidulated and centrifuged to yield three products: acid oil, acid water and a sludge phase. Typical performance data on various grades of soapstocks and the recovered products are given.

Introduction

CONTINUOUS PROCESSING has been practiced in the Vegetable Oil Industry for three decades, but is a recent development in the acidulation of soapstock for recovery of acid oil.

Soapstock acidulation by the batch method is widely practiced, but is a marginal operation (1). It is time-consuming, a labor problem, often a hodgepodge of miscellaneous equipment, high in reagent demand, creates disposal problems and too frequently may produce a degraded acidified oil.

Today, however, with technological developments, such as, corrosion-resistant metals, automatic control systems and new types of processing equipment, acidulation of soapstock in a continuous system has become a reality. A fair price for quality acid oil and demand for a nutritive meal additive have given the process economic advantages. Under the impetus of legal regulations for waste disposition, continuous acidulation is becoming a necessity.

For the past four years, The De Laval Separator Company has been developing and extensively testing on a variety of soapstocks from many sources, a process for continuous recovery of acid oil in its pilot plant. A schematic flow sheet of this development appears in Figure 1.

General Process

The caustic soapstock, received directly from the refinery, is blended in a surge tank with a diluent in order to provide a homogeneous mixture of proper viscosity range as feed stream to the system. The di-

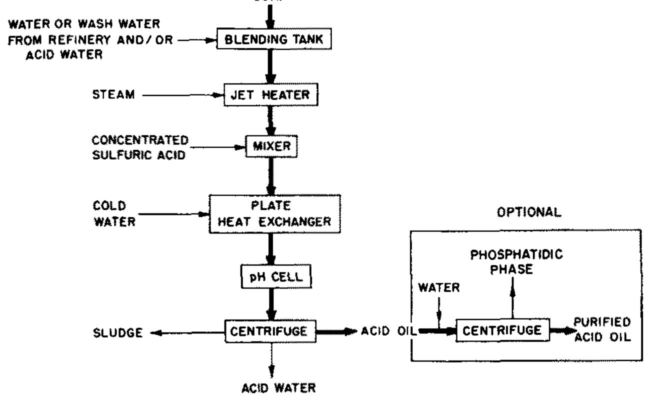

FIG. 1. Schematic flow diagram for continuous recovery of acid oil.