Horsebean as Protein Supplement in Breadmaking. I. Isolation of Horsebean Protein and Its Amino Acid Composition[1]

K. M. PATEL and J. A. JOHNSON[2], Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan

ABSTRACT

A protein isolate was prepared from horsebean (*Vicia faba*) by extraction with water or dilute $Ca(OH)_2$ solution using rapid agitation. Adding 0.25% L-ascorbic acid based on flour weight prevented the alkali extracts and the final isolate from turning green. Protein in the clarified liquor was precipitated after adjustment to pH 4.25 with 6N HCl. Following precipitation and centrifugation, the protein curd was washed with water, centrifuged, and finally freeze- or oven-dried. The final protein isolate was light tan. The protein content of the isolate prepared at laboratory and pilot scale ranged from 71 to 83% (N × 6.25). The total yield based on flour or flake weight was 16.5 to 21%. The yield of the isolates depended on solid:solvent ratio, granulation, extraction time, and temperature. Horsebean flour and horsebean protein isolate were analyzed for amino acid composition. Both sources exhibited a relatively high concentration of essential amino acids. The lysine content in both was 3.5 times that of wheat flour. Isolation of protein resulted in slight losses and shifts in the amino acid concentration. The use of horsebean protein in breadmaking will be discussed in the second paper of this series.

Widespread concern for the serious global food protein shortage has led to intensive efforts toward exploring novel and indigenous protein sources at present not adequately utilized (1—3). Among the diverse solutions proposed to meet it, legumes will probably have a far greater impact toward filling the protein gap than will fish, leaf, or single-cell protein, considering the economics, technology of production, processing, local availability, and acceptability of protein in diet (4). Cereal fortification with lysine-rich protein offers a logical and immediate means to upgrade the quality and quantity of dietary protein (5,6). Legume or oilseed flours requiring a minimum of processing would obviously be economical protein supplements. However, their presence in larger quantities may adversely affect the functional properties of food systems and flavor (7,8). Superior functional properties, low flavor profile, relative freedom from toxic factors and indigestible carbohydrates make protein isolates increasingly attractive as protein supplements (9,10).

Horsebean (*Vicia faba*), a legume high in both protein and lysine, is a major indigenous crop in Morocco. Agricultural production from Morocco for 1964-66, indicated 120,000 metric tons of horsebean production, 20% of which was used as food and 20% as animal feed (11). By rechanneling horsebeans currently used as animal feed, 5,000 to 6,000 metric tons of additional horsebean protein could be made available for human consumption in Morocco. Under a contract between the Agency for International Development and the Department of Grain Science and Industry at Kansas State University, we investigated horsebean as a protein supplement in cereal foods for Morocco.

Preparing protein isolate from soy, peanut, and safflower has been well documented (10). However, little information on horsebean protein is available. Recently, Flink and Christiansen (12) obtained essentially complete horsebean

[1]Contribution No. 850, Department of Grain Science and Industry, Kansas State University, Manhattan. Condensation of thesis presented by senior author in partial fulfillment of requirements for PhD. degree, Kansas State University.

[2]Respectively: research associate and professor.

protein extraction between pH 8 and 10 at room temperature with 1:5 meal:solvent ratio and meal particle size of <0.2 mm. Meal:solvent ratio, length of extraction, temperature, pH, particle size, and nature of solvent reportedly significantly influence extraction efficiency of oilseed and legume proteins (13–16). The undesirable green color of vegetable protein isolated under alkaline conditions has been attributed to oxidation of chlorogenic acid (17). Methods to improve the color of vegetable protein isolates have been based on using reducing agents to prevent polyphenols from oxidizing to corresponding quinones (which condense and covalently bind to reactive protein groups) (18) or using organic solvents to extract color-imparting compounds (19,20).

Toxic factors such as trypsin inhibitor, cyanogenic glucosides, saponins, alkaloids, and hemaglutinin have been identified with legumes. Most are water-soluble and heat-labile (21). A disease, favism, has been associated with horsebean or pollen from horsebean flowers. Its toxic factor has been neither identified nor reproduced in experimental animals. Clinical manifestations of the disease are hemolitic anemia, hemobiourea, and jaundice, resulting in fragility of the red blood cells (21).

MATERIALS AND METHODS

Horsebeans (Scimeco Supermarket, Kansas City, Mo.) were milled to flour (130 μ) and to two coarse granulations (208 and 360 μ). The horsebean flour was defatted by refluxing with petroleum ether in a Soxhlet apparatus for 14 to 16 hr. It was dried at room temperature (20° C.) and stored in plastic bags in fiber drums at 4.5° C. until used. The extraction variables studied included: mode and length of agitation, strength of $Ca(OH)_2$ solvent, solid:solvent ratio, temperature, defatted, versus as-is flour, and particle size.

Horsebean Protein Isolate Prepared on a Laboratory Scale

Protein was extracted using a Waring Blendor and Lightning variable speed (Model F) stirrer. About 50 g. samples of horsebean flour suspended in $Ca(OH)_2$ solution (0.1, 0.02, 0.03M) with 1:5 flour:solvent ratio were blended in a Waring Blendor at speed "one" for 7 min., reaching a final temperature of 51° C. Agitation was continuous except for short stops at 2-min. intervals to read temperature. To prevent excessive foaming, 4 to 5 drops of octanol or antifoam FD-82 (Hodag Chemical Corp., Skokie, Ill.) 15 p.p.m. dispersed in 25 ml. of distilled water was added. To control the final temperature, the Blendor cup, solvent, and flour were initially cooled to 4.5° C.

Horsebean flour samples (50 g.) dispersed in 0.02M $Ca(OH)_2$ solution with 1:10 flour:solvent ratio, in a 1,000-ml. beaker, were extracted with a Lightning stirrer to create a brisk vortex. The variables studied were: temperatures of 9°, 23°, and 26° C.; a solid:solvent ratio of 1:5, 1:10, 1:20; length of extraction ranging from 5 to 240 min.; and particle sizes of 130, 208, and 360 μ. Following the extractions, suspensions were centrifuged at 2,000 × g for 30 min. in an International centrifuge (Model V, No. 28958) without temperature control. In a later study, suspensions were centrifuged at 10,000 × g for 15 min. at 23° C. with a Beckman Model J-21 refrigerated centrifuge (Beckman Instrument Co., Palo Alto, Calif.). Residues were subjected to a second extraction, under conditions similar to the first, except at a 1:5 ratio. To prevent the green coloration of protein isolate, we added 0.25% reducing agents, L-ascorbic acid or sodium

thiosulfate, to the extracting medium; or washed wet curds with 50% isopropyl alcohol, following the procedure of Gheyasuddin et al. (20).

To determine pH of horsebean protein maximum precipitation, 50-ml. aliquots of protein extract were adjusted to a pH value between 6.5 and 3.0 (with 0.5 pH interval) using 1N HCl, while the solution was stirred continuously with a magnetic stirrer. Protein curds were allowed to form and settle for 2 hr. before whey was separated by centrifuging at 10,000 × g for 15 min. Wet curds were washed twice by resuspending them in a volume of tap water equal to the whey volume. Curds were separated from wash water by centrifugation. Filtrate was saved to determine protein lost in washing. Protein curds were freeze-dried at 60° C. for 5 to 6 hr. and at 16° C. for 15 to 20 hr. in a mechanically refrigerated cabinet (Model 10 146 MR-BA, Virtis Co., Inc., Gardiner, N.Y.). Efficiency of protein extraction was expressed as percentage of total flour protein. Total protein extracted minus protein quantity in whey gave protein precipitation value. Adjusting 50-ml. aliquots of protein extract to a pH between 6.5 and 3.0 caused whey proteins to be diluted to a different degree. The difference in dilution was corrected so that constant volume was employed for protein determination. All protein percentages were based on determination of nitrogen by the Kjeldahl method. Nitrogen of horsebean was multiplied by the factor 6.25 while wheat protein percentage was calculated by multiplying by a factor of 5.75.

Horsebean Protein Isolate Prepared on a Pilot Plant Scale

Horsebean flour and flakes (50 lb.) with 0.25% L-ascorbic acid were extracted with 27° C. water at 1:10 ratio for 15 min. and 1 hr., respectively, with mild agitation. The slurry was screened on a 150-mesh screen using a Rotex Screener (Orville Simpson Co., Cincinnati, Ohio). The residue from the screen was reslurried with water at 1:10 ratio, based upon the original flour or flake weight and rescreened. The two liquors were then combined and clarified in a continuous centrifuge. Proteins in the clarified liquor were precipitated at pH 4.25 with 6N HCl. The protein curds were allowed to settle, the supernatant whey then was decanted, and the curd washed with a volume of water of 38° C. equal to the volume of whey. The diluted curd slurry was then concentrated in a continuous centrifuge. The concentrated curd was filtered, pressed, granulated, and dried in a forced draft oven at 66° C. Finally, the protein isolate was ground in a micromill to pass through 40-mesh sieve. Proximate analyses were determined according to AOAC methods (22).

Amino Acid Analysis of Horsebean Flour and Horsebean Protein Isolate

Amino acid analysis was by the method of Spackman et al. and Moore et al. (23,24); we used an automatic amino acid analyzer (Model 120B, Beckman Instrument Co., Palo Alto, Calif.). Tryptophan was determined by the procedure of Sessa et al. (25).

RESULTS AND DISCUSSION

Effect of Extraction Conditions on Extraction Efficiency of Horsebean Protein

Data on conditions influencing extraction efficiency of horsebean protein are presented in Table I. In two successive extractions, high-speed agitation in a

TABLE I. PHYSICAL FACTORS INFLUENCING EXTRACTION EFFICIENCY OF HORSEBEAN PROTEIN

Procedure	Flour treatment	Flour: solvent ratio	$Ca(OH)_2$ conc. M	Extract min.	Extract °C.	% Total Protein 1st ext.	% Total Protein 2nd ext.	pH 1st ext.	pH 2nd ext.	Dry Solids[1] Yield %	Dry Solids[1] Protein[2] %	Extract Color
High-speed blender	Defat.	1:5	0.01	7	51	77.6	13.6	6.8	10.8	16.5	84.4	Light tan; turns green with time and temp.
	Defat.	1:5	0.02	7	51	87.7	7.1	7.1	11.0	19.8	85.0	
	Defat.	1:5	0.03	7	51	78.6	13.6	8.8	11.0	16.7	86.2	
High-speed blender (4.5°C.)	Defat.	1:5	0.02	8	50	92.5	4.9	8.2	10.8	19.7	89.2	
	As-is	1:5	0.02	8	50	91.9	6.5	8.2	11.0	19.5	88.8	
Lightning stirrer	As-is	1:10	0.02	60	26	96.8	...	10.5	...	20.6	84.6	Dark green
	As-is	1:10	0.02	120	26	96.9	...	10.5	...	20.6	84.6	Dark green
	As-is	1:10	0.02	240	26	96.8	...	10.5	...	20.6	85.7	Dark green
	As-is	1:10	0.02	60	9	90.3	...	10.4	...	19.2	85.6	Med. green
	As-is	1:10	0.02	120	9	91.6	...	10.5	...	19.5	85.3	Med. green
	As-is	1:10	0.02	240	9	94.2	...	10.5	...	20.0	85.4	Med. green
	As-is	1:10	0.02	5	23	84.0	...	10.5	...	17.9	85.3	Light tan
	As-is	1:10	0.02	10	23	94.2	...	10.5	...	20.0	85.5	Light tan
	As-is	1:10	0.02	15	23	95.8	...	10.5	...	20.4	85.6	Light tan
	As-is	1:10	0.02	30	23	95.8	...	10.5	...	20.4	85.9	Light tan
	As-is	1:10	0.02	60	23	95.8	...	10.5	...	20.4	86.0	Dark green
	As-is	1:10	0.02	120	23	98.0	...	10.5	...	21.0	85.8	Dark green
Flour: solvent ratio	As-is	1:5	0.02	15	23	88.3	...	8.6	...			
	As-is	1:10	0.02	15	23	94.2	...	10.2	...			
	As-is	1:20	0.02	15	23	92.6	...	11.5	...			
Particle size												
130 μ	As-is	1:10	0.02	15	23	94.2	...	10.2	...			
208 μ	As-is	1:10	0.02	15	23	93.5	...	10.2	...			
360 μ	As-is	1:10	0.02	15	23	86.4	...	10.2	...			
208 μ	As-is	1:10	0.0	15	23	77.9	...	6.8	...			

[1]Moisture-free basis.
[2]N × 6.25.

Waring Blendor for 7 min. to 51° C. with three concentrations of $Ca(OH)_2$ caused 91.2 to 95.0% extraction. The bulk of the proteins (78 to 88%) was dispersed in the first extract. Maximum protein solubility occurred with 0.02M (95%), as compared with 0.01M (91%) or 0.03M (92%) $Ca(OH)_2$ solution. Although this method proved to be efficient for maximum extraction in a very short time, there were major deterrents: rapid rise in temperature, excessive foaming, starch gelatinization, and alkaline extract turning green. The only advantage of chilling the Blendor cup, solvent, and flour seemed to be a resultant protein extraction with only 1 min. of agitation and the temperature not exceeding 51° C. Although octanol efficiently prevented excessive foaming during agitation, its residue in dried protein isolate caused objectionable odor in baked bread; hence, antifoam FD-82 was used. Extraction with water (pH 6.5) lowered the extraction efficiency. Defatting of flour prior to extraction was found to be unnecessary, presumably because of low fat content (2.3%) of horsebean flour.

In general, longer extraction time gave a slightly higher yield. At 23° C., extending extraction time from 5 to 15 min. enhanced protein dispersibility from 84.4 to 95.8% of the total protein. No further solubilization occurred beyond 15 min. up to 1 hr. Nearly 84% of the total protein was extracted within 5 min. of agitation, indicating that the bulk of the protein dispersed rapidly.

At 9° C., extended agitation (from 1 to 4 hr.) increased the protein solubility by 1.3 and 2.6% after 2 and 4 hr., respectively. At room temperature, similar increases were not noted. These data indicate that low temperature depressing effect on protein dispersibility can be corrected by extending extraction time or vigor of agitation. Extractions were substantially greater at high temperatures. Protein content of the extracts prepared at room temperature exceeded that at 9° C. by 6.5, 5.3, and 2.6% after 1, 2, and 4 hr. of extraction, respectively, indicating that the effect of temperature on extractability was more critical than that of time. Protein extraction from soybeans and mung beans was increased substantially by increasing the temperature to 45° C. (13–15), by efficient shearing action of vigorous stirring (13,15), or, in the case of kidney beans, by using a colloid mill (16). Thus, a rapid rise in temperature (from 23° to 51° C. in 7 min.) and a simultaneous shearing action (in a Waring Blendor or agitating briskly in a Lightning stirrer) explain the rapid dispersion of horsebean protein.

Protein was solubilized best at a solid:solvent ratio of 1:10 (94%) and less well at a ratio of 1:5 (88%). Based on yield and convenience in handling liquids, these data justify preference for a 1:10 ratio over others. When flour was subjected to only one extraction, extraction was greater at a 1:20 than at a 1:5 ratio. These findings partly corroborate those of Djang et al. (14) and Smith et al. (15), who found that raising ratios from 1:7 to 1:20 substantially increased protein extraction.

Protein extraction efficiency was a function of particle size. Protein was more nearly solubilized with flour (130 μ) than with coarse granulations (208, 360 μ); dispersible protein values were 94.2, 93.5, and 86.4%, respectively. More efficient extraction with reduced particle size must have resulted from an increase in damage or rupture of cellular membrane surrounding protein bodies and an increase in surface exposed to the extracting medium. Similar observations have been noted by others (13–15).

Figure 1 illustrates the profile of horsebean protein precipitation as a function of pH. Maximum protein precipitation and best color of wet curds were obtained

TABLE II. PROXIMATE ANALYSIS OF WHEAT FLOUR, HORSEBEAN FLOUR, AND HORSEBEAN PROTEIN ISOLATE (14% m.b.)

Ingredient	Moisture %	Protein %	Ash %	Crude Fat %	Crude Fiber %	NSI[1]
Wheat flour						
Strong	11.5	13.3[2]	0.41	0.97	0.29	
Medium	11.7	11.2[2]	0.48	0.92	0.31	
Weak	12.3	8.9[2]	0.49	0.65	0.41	
Horsebean flour	10.1	29.7[3]	3.30	2.26	1.44	85
Horsebean flakes	12.2	29.9[3]	3.20	2.20	1.54	88
Horsebean Protein Isolate						
Lab. scale						
KSU	4.2	81.2[3]	1.34	0.20	0.49	
Central Soya	5.0	80.2[3]	3.71	0.20	0.52	
Pilot plant scale						
Horsebean flour	10.4	73.5[3]	2.40	...	1.16	
Horsebean flakes	7.3	76.5[3]	3.00	...	0.23	

[1]Nitrogen solubility index.
[2]N × 5.75.
[3]N × 6.25.

between pH 4.0 and 4.5. Increasing acidity below pH 4.0 accompanied enhanced protein dispersion and reappearance of green color. Thus, solubility of horsebean proteins is very similar to that of other legume and oilseed proteins.

Color and Yield of Horsebean Protein Isolate

Horsebean protein extracts turned green at alkaline pH. The color intensity was a function of time and temperature, solid:solvent ratio, and solvent alkalinity. Although the extract remained light tan during 15 min. of extraction at 23°C. and subsequent 15 min. centrifugation (23°C.), dried solids were sufficiently colored to impart an objectionable green cast to the bread crumb. Washing protein curds with 50% isopropyl alcohol resulted in a chalky white, powdery material with no cohesive property. However, during steaming or baking, green color redeveloped. Of the two reducing agents added to the alkaline extracting media, sodium thiosulfate did not adequately prevent the color development. L-ascorbic acid kept the extract light tan up to 2 hr. at room temperature and yielded a desirable cream-colored horsebean protein isolate which remained cream-colored after 16 months' storage at 4.5°C.

Dry protein solid yield, which was proportional to extraction efficiency, ranged from 16.5 to 19.8% (flour-weight basis) using a high-speed blender and from 17.9 to 21.0% using the Lightning-type stirrer. Variation in the former values may be attributed to the solvent strength; in the latter, to the length of extraction. Dry protein content ranged from 84.4 to 89%. No relation was observed between protein content of the dry protein preparation and method or length of agitation. In laboratory experiments, major protein losses occurred as

TABLE III. AMINO ACID COMPOSITION OF HORSEBEAN FLOUR (HBF) AND HORSEBEAN PROTEIN ISOLATE (HBPI)

			Net change	Percent of FAO Pattern (25)	
	HBF	HBPI	HBF to HBPI %	HBF	HBPI
Amino acid[1]					
Lysine	6.91	6.46	-6.5	164	154
Histidine	2.68	2.63	-2.1		
Arginine	11.09	9.76	-12.0		
Aspartic acid	11.80	11.77	-0.2		
Threonine	3.79	3.50	-7.8	136	126
Serine	5.18	5.21	+0.6		
Glutamic acid	18.08	18.95	+4.8		
Proline	4.11	4.34	+5.7		
Glycine	4.37	4.07	-6.8		
Alanine	4.54	4.24	-6.7		
Cystine[2]	0.53	0.43	-18.4		
Valine	4.79	4.93	+2.9	114	118
Methionine[2]	0.65	0.61	-6.3	30	28
Isoleucine	4.26	4.38	+2.8	101	104
Leucine	7.91	8.58	+8.4	165	179
Tyrosine	3.40	3.60	+5.8	122	129
Phenylalanine	4.42	4.65	+5.1	158	166
Total S-amino acids	1.18	1.04	-12.0	28	25
Tryptophan[3]	0.88	1.03	+18.0	63	74

[1]Grams amino acid per 100 g. protein corrected to 100% protein recovery.
[2]Cystine and methionine by oxidation (24).
[3]Tryptophan by colorimetric method (25).

whey protein and in wash water, amounting to 18 and 20% of the total flour protein, respectively. Consequently, 82% of the original flour protein was recovered as protein isolate.

Table II summarizes the analytical data obtained on horsebean flour and protein isolate prepared in laboratory and pilot plant. With pilot plant equipment, dry protein yields were only 10 and 13% from flour and flakes, respectively. Major protein losses occurred at two stages: in the screening of the protein extraction slurry and in separating starch from the protein liquor by continuous centrifugation. Greater losses occurred with flour than with flakes, because of the difficulty in separating finely divided residue from the protein extraction slurry during screening and the difficulty in separating starch from protein liquor by continuous centrifugation. Protein content of the isolates made from flour and flakes in pilot plant was 73.5 and 76.5%, respectively; in contrast, protein content of isolates prepared from flour in the laboratory was 80 to 81%.

Amino Acid Analysis

Since protein quality depends on its content of essential amino acids as well as the ratio of these amino acids to each other (26), study of amino acid pattern of horsebean protein was of interest. The amino acid composition of two protein supplements (Table III) shows that, except for leucine, lysine is the most

abundant of the essential amino acids; it is 3.8 times greater than that found in wheat flour. The total sulfur amino acids (methionine plus cystine) were the first limiting, though tryptophan also was below the level in the FAO protein pattern. All other essential amino acids considerably exceeded that of the FAO reference protein: horsebean flour furnished 58 to 65% more lysine, leucine, and phenylalanine, 36% more threonine, 22 to 14% more tyrosine and valine; and horsebean protein isolate supplied 79 and 66% more leucine and phenylalanine, 54% more lysine, and 18 to 29% more valine, tyrosine, and threonine.

Horsebean protein isolate gave lower essential amino acid values than did horsebean flour for lysine, methionine, and threonine by 6 to 8%, and for cystine by 18%. Horsebean protein isolate contained 2.9% more valine, 5 to 8% more tyrosine, phenylalanine, and leucine; and 18% more tryptophan than did horsebean flour. The variation in amino acid content between two forms of horsebean protein supplements could be ascribed to fractionation of the flour proteins during isolate preparation and to losses occurring as whey proteins. Wide variability in essential amino acid losses resulting from protein isolation has been reported. Rackis et al. (27) found that in isolating soy protein, only 2.5% lysine and 20% or more tryptophan and sulfur amino acids were lost. They further observed a higher portion of methionine remaining with whey protein. According to Maga (28), processing from soybean to protein isolate caused a net loss of 13% lysine and 21% sulfur amino acids. On the other hand, Longnecker et al. (29) found little difference in essential amino acid content between soy flour and three soy protein isolates.

The lysine content of horsebean flour was 15% higher than that reported for soy flour and flakes (9,24,28,29) but 8% lower than that for chickpea flour (30). Likewise, horsebean protein isolate contained 15 to 20% more lysine than values reported for soy protein isolate (9,27–29). But sulfur amino acids of horsebean flour and horsebean protein isolate were below that of soy flour, soy protein isolate (9,27–29), and chickpea flour (30).

Acknowledgments

The authors are grateful to E. W. Meyer, Central Soya, Chicago, Ill. for pilot plant preparation of protein isolate, and to the Agency for International Development for financial assistance.

Literature Cited

1. BENDER, A. E., KIHLBERG, R., LOFQUIST, B., and MUNCK, L., eds. Evaluation of novel protein products. Proc. Int. Biol. Prog. (Wenner-Gren Center Symp., Stockholm, 1968, pp. 1–114; 359–380. Pergamon Press: New York.
2. SCRIMSHAW, N. S. World wide importance of protein malnutrition and progress toward its prevention. Amer. J. Pub. Health 55: 1781 (1963).
3. MILNER, M. Protein food problems in developing countries. Food Technol. 16(6): 51 (1962).
4. ANONYMOUS. Fortified foods: the next revolution. Chem. Eng. News 48 (Aug. 10, 1970). FEDS Reprint 2.
5. BRESSANI, R., and ELIAS, L. G. Processed vegetable protein mixtures for human consumption in developing countries. Advan. Food Res. 16: 1 (1968).
6. PARMAN, G. K. Fortification of cereals and cereal products with proteins and amino acids. J. Agr. Food Chem. 16: 168 (1968).
7. MATTHEWS, R. H., SHARPE, E. J., and CLARK, W. M. The use of some oilseed flours in bread. Cereal Chem. 47: 181 (1970).
8. ROONEY, L. W., GUSTAFSON, C. B., CLARK, S. P., and CATER, C. M. Comparison of the baking properties of several oilseed flours. J. Food Sci. 37: 14 (1972).

9. MEYER, E. W. Soy protein concentrates and isolates. In: Soybean protein foods. U.S. Dept. Agr.-Agr. Res. Serv. 71-35 20-27 (1967).
10. BERK, Z. Soy protein concentrates and isolates. In: Food Sci. Technol. Proc. Int. Congr., 3rd, 1970, p. 242.
11. ANONYMOUS. Food production and consumption in Morocco in 1964-66. In: Food balance sheets. FAO: Rome (1971).
12. FLINK, J., and CHRISTIANSEN, I. The production of a protein isolate from *Vicia faba*. J. Food Sci. Technol. 6(3): 102 (1973).
13. NAGEL, R. H., BECKER, H. C., and MILNER, R. T. Some physical factors affecting the dispersion of soybean proteins in water. Cereal Chem. 15: 463 (1938).
14. DJANG, S. S. T., LILLEVIK, H. A., and BALL, C. D. Factors affecting solubilization of the nitrogenous constituents of the mung bean, *Phaseolus aureas*. Cereal Chem. 30: 230 (1953).
15. SMITH, A. K., BELTER, P. A., and JOHNSEN, V. L. Peptization of soybean meal. Effect of method of dispersion and age of beans. J. Amer. Oil Chem. Soc. 29: 309 (1952).
16. HANG, Y. D., WILKENS, W. F., HILL, A. S., STEINKRAUS, K. H., and HACKLER, L. R. Studies on the extraction of proteins from red kidney beans. I. Improvement in filterability of aqueous bean extract. Cereal Chem. 47: 259 (1970).
17. CLANDININ, D. R. Sunflower seed oilmeal. In: Processed plant protein food stuffs, ed. by A. M. Altschul; p. 557. Academic Press: New York (1958).
18. LOOMIS, W. D., and BATTAILE, J. Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry 5: 423 (1966).
19. SMITH, A. K., and JOHNSEN, V. L. Sunflower seed protein. Cereal Chem. 25: 399 (1948).
20. GHEYASUDDIN, S., CATER, C. M., and MATTIL, K. F. Preparation of a colorless protein isolate. Food Technol. 24: 242 (1970).
21. PATWARDHAM, V. N. Pulses and beans in human nutrition. Amer. J. Clin. Nutr. 11: 12 (1962).
22. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (9th ed.). The Association: Washington, D.C. (1960).
23. SPACKMAN, D. H., STEIN, W. H., and MOORE, S. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190 (1958).
24. MOORE, J., SPACKMAN, D. H., and STEIN, W. H. Chromatography of amino acids on sulfonated polystyrene resins. Anal. Chem. 30: 1185 (1958).
25. SESSA, D. J., ABBEY, K. J., and RACKIS, J. J. Tryptophan in soybean meal and soybean whey proteins. Cereal Chem. 48: 321 (1971).
26. NATIONAL RESEARCH COUNCIL, COMMITTEE ON PROTEIN MALNUTRITION. Evaluation of protein quality. Publ. 1100. Nat. Acad. Sci.-Nat. Res. Coun.: Washington, D.C. (1963).
27. RACKIS, J. J., ANDERSON, R. L., SESAME, H. A., and SMITH, A. K. Amino acids in hulls and oilmeal fractions. J. Agr. Food Chem. 9: 409 (1961).
28. MAGA, J. A. Observations on effects of processing and storage on soy flavor. Ph.D. thesis, Kansas State University, Manhattan (1970).
29. LONGNECKER, J. B., WILBUR, H., MARTIN, C., and SARETT, H. P. Improvement in protein efficiency of soybean concentrates and isolates by heat treatment. J. Agr. Food Chem. 12: 411 (1964).
30. SHEHATA, N. The effect of supplementation with chickpea flour on the quality of wheat. Ph.D. thesis, Kansas State University, Manhattan (1969).

[Received February 11, 1974. Accepted May 28, 1974]