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AMINO ACIDS IN SOYBEANS

Amino Acid Composition of Soybean Protein Fractions

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Soybean meal is of great economic importance because of its use in human foods and because it is a source of high-quality protein in formulating animal feeds. Amino acid analyses were made on soluble soybean protein and on acid-precipitated and heat-coagulated fractions. Mostly ion exchange chromatographic methods were used. The heat-coagulated fraction contained larger amounts of nearly all of the nutritionally essential amino acids than the total water-soluble protein. The amounts of amino acids in the water-soluble and acid-precipitated fractions were similar to reported values for the meal.

More than 6,000,000 tons of soybean meal are sold annually in mixed feeds. To improve feed efficiency, to stimulate use of isolated soybean protein, and to increase the consumption of meal products in foods, more exact information is required about the amino acid composition of soybean protein fractions. Such information is important because of the nutritionally essential nature of some amino acids. The variation of their requirement by different animal species (*2*) and the increasing evidence that the amount and kind of nonessential amino acids present affect the total amino acid required (*1*), are further reasons why exact composition needs to be determined.

Analyses of soybean protein by recently developed ion exchange chromatographic methods would be of value for comparison with previously reported analyses of the amino acid composition of whole soybean protein made mostly by microbiological methods. Insofar as is known, no amino acid analyses of different protein fractions from the soybean have been made. For the above reasons, the total water-soluble protein, the heat-coagulated fraction of the protein, and the acid-precipitated fraction were analyzed for their amino acid content.

Materials and Procedure

Preparation of Protein Fractions. Three protein isolates were investigated for their amino acid composition: the total water and dilute alkali-soluble protein, hereafter called soluble protein, representing more than 95% of the total protein in the soybean (*10, 12*); the acid-precipitable protein (*11*), representing about 60% of the soluble protein; and the heat-coagulable protein of whey (*13*), which is 7 to 9% of the soluble protein.

Soluble protein was prepared by extracting 20 grams of hexane-defatted meal at room temperature with 400 and 200 ml. of water successively, then twice more with 200 ml. of water adjusted to pH 8.0 with sodium hydroxide. The combined solutions were dialyzed for about 80 hours at 5° C. with several changes of distilled water, and the volume was reduced by pervaporation at room temperature. This solution was dialyzed again, further reduced in volume by pervaporation, and finally dried by lyophilization.

Acid-precipitated protein was prepared by a double extraction with water to meal ratios of 10 to 1 and 5 to 1, and the protein was precipitated from the combined extracts at pH 4.4 with hydrochloric acid. The curd was washed twice and dried by lyophilization.

Heat-coagulable protein, sometimes called albumin, was prepared from whey recovered from the acid-precipitated protein. The whey was adjusted to pH 8.0 to precipitate phosphorous compounds (*13*), heated for 15 minutes at 75° C., dialyzed, and then clarified by centrifugation. When the solution was adjusted to pH 5.0, the heat-coagulable protein was precipitated, recovered in a centrifuge, and dried by lyophilization. Whey proteins prepared by minor variations of this procedure gave the same amino acid composition.

Elementary Composition of Protein Fractions. Table I gives the elementary analysis of the protein samples analyzed for amino acids, as well as the variety of soybeans and crop year from which the samples were obtained. Total nitro-

Table I. Source and Elementary Composition of Various Protein Samples Analyzed

Variety	Crop Year	Preparation	Percentage[a] Nitrogen	Phosphorus	Sulfur	Ash
Lincoln	1952	Soluble protein	15.8	0.42	0.81	1.9
Hawkeye	1955	Soluble protein	16.1	0.52	0.93	2.0
Lincoln	1952	Heat-coagulated	16.1	...	...	...
Hawkeye	1950	Heat-coagulated	15.9	0.0	...	...
Adams	1954	Heat-coagulated	15.9	0.0	0.89	0.1
Adams	1955	Heat-coagulated	15.9	0.0	1.05	0.2
Lincoln	1952	Acid-precipitated	16.5	1.2	0.90	2.6

[a] Calculated on dry basis. Average values for at least two determinations.

gen, sulfur, and phosphorus were determined by A.O.A.C. methods (*1*, *8*).

Method of Hydrolysis. Samples of the proteins, 50 mg. each, were hydrolyzed 25 or 70 hours by heating in a bath held at 125° to 135° C. with 25 ml. of constant-boiling hydrochloric acid which had been redistilled in all-glass apparatus. The hydrochloric acid was removed with a rotary evaporator under vacuum; the residue was then made up to 10 ml. To determine cystine, the protein was treated with performic acid (*9*) before hydrolysis.

Methods of Amino Acid Analyses. The greater part of the amino acids were determined by the chromatographic procedure described by Moore and Stein (*7*). Tryptophan was determined on the unhydrolyzed protein by the method of Spies and Chambers (*14*); methionine by biological assay (*15*); and cystine, after oxidation to cysteic acid, by the method of Schram, Moore, and Bigwood (*9*). Proline was determined in the effluent from a short chromatographic column, described by Moore and Stein (*7*), by the colorimetric method of Chinard (*3*). Almost all recoveries of known amino acids were within 5% of the known amount of amino acid used to test each procedure.

Results and Discussion

Amino Acid Composition of the Protein Fractions. Data are reported in Table II as grams of amino acid per 16 grams of nitrogen. All results are on a moisture- and ash-free basis. Those values for which average deviations are given represent the averages of two to six determinations on 25- and 70-hour hydrolyzates. When values from the 70-hour hydrolysis were consistently higher than from the shorter reaction time, the figures from the longer hydrolysis were taken as the more accurate because an extended hydrolysis was apparently required to completely free the amino acid. The isoleucine values for the 25-hour hydrolysis were 80 and 88% of the value reported for the soluble protein and the heat-coagulated protein, respectively. In cases where consistently lower values were obtained with 70-hour hydrolysis, indicating amino acid destruction, the value considered the most accurate was obtained by extrapolation to zero-hydrolysis time as recommended by Block and Weiss (*2*). Values obtained by the 70-hour hydrolysis were about 70% of the corrected value for serine and 90% for glutamic and aspartic acids. No loss of threonine was detected. Very erratic values for methionine and cystine were obtained by chromatographic procedures on both 25- and 70-hour hydrolyzates. For this reason, methionine was determined by microbiological assay, and the amount of cystine was estimated after oxidation to cysteic acid. Their sulfur content computed from the values obtained accounts for 75 to 90% of the total sulfur, indicating loss of amino acids during hydrolysis or measurement unless other sulfur-containing material was present.

The ammonia formed during hydrolysis and the possible amide nitrogen present in the protein were estimated from the ammonia elution peak. This ammonia nitrogen was 10% of the total nitrogen in the soluble protein sample from the Lincoln variety, and it was 6.7% of the total nitrogen of the heat-coagulated protein sample from the Hawkeye and Adams varieties. Using these values, the total nitrogen recovered as amino acids and ammonia nitrogen was 95% for the soluble protein and 100.3% for the heat-coagulated protein.

Comparison of Protein Fractions. Results vary among the three different protein fractions for which amino acid compositions were determined. The heat-coagulated fraction showed markedly higher nutritionally essential amino acid content when compared with the soluble and acid-precipitated proteins except for histidine and arginine. The percentage of arginine was lower while histidine remained about the same. This heat-coagulated protein fraction represents from 7 to 9% of the soybean protein. From a nutritional standpoint, it is a very high quality protein.

The acid-precipitated fraction from the Lincoln soybean gave slightly lower values than did the soluble protein for eight of the essential amino acids and gave slightly higher values for leucine and tryptophan. Hence, the soluble protein may be of greater nutritional value than the acid-precipitated fraction. The limited testing of protein fractions from different soybean varieties gave no clear-cut evidence of varietal differences in amino acid composition.

Table II. Amino Acid Composition of Soybean Protein Fractions

	Soluble		Acid-Precipitated	Heat-Coagulated	
	Lincoln, g./16 g. N av. dev.	Hawkeye, g./16 g. N	Lincoln, G./16 G. N Av. Dev.	Hawkeye and Adams,[a] g./16 g. N av. dev.	Lincoln, g./16 g. N
Arginine	6.46 ± 0.12	7.3	6.19 ± 0.01	5.49 ± 0.34	5.4
Histidine	2.59 ± 0.12	2.6	2.10 ± 0.10	2.64 ± 0.15	2.6
Lysine	6.67 ± 0.36	6.9	6.07 ± 0.22	7.76 ± 0.23	8.4
Tyrosine	4.37 ± 0.26	3.8	3.91 ± 0.03	5.62 ± 0.42	4.8
Tryptophan	1.35 ± 0.04	1.5	1.45 ± 0.03	2.71 ± 0.07	...
Phenylalanine	5.61 ± 0.16	5.6	5.37 ± 0.13	6.59 ± 0.22	6.2
Cystine	1.36 ± 0.06	1.4	1.22 ± 0.01	1.73 ± 0.29	...
Methionine	...	1.34	1.40	1.57	1.6
Serine	5.97[b]	...	5.72[b]	5.16 ± 0.05	4.9
Threonine	3.93 ± 0.18	3.9	3.60 ± 0.10	4.67 ± 0.09	4.5
Leucine	8.13 ± 0.12	8.2	9.11 ± 0.35	10.51 ± 0.18	...
Isoleucine	5.26[c] + 0.01	...	...	6.62[c] ± 0.17	...
Valine	5.57 ± 0.21	...	5.44 ± 0.34	7.34 ± 0.25	7.1
Glutamic acid	18.52 ± 0.38	20.1	20.80	14.68[b]	...
Aspartic acid	11.28 ± 0.07	11.9	12.03 ± 0.11	13.48[b]	...
Glycine	4.60 ± 0.19	5.0	4.49 ± 0.25	5.54 ± 0.25	4.9
Alanine	4.03 ± 0.02	4.2	3.85	5.28 ± 0.23	5.1
Proline	5.32	...	5.57	5.80	...

[a] Average of determinations from 1 sample of Hawkeye and 2 samples of Adams.
[b] Value obtained by extrapolation to zero hydrolysis time.
[c] Data from 70-hour hydrolyzed protein.

Amino Acid Content of Soluble Protein Compared with Soybean Meal. The values obtained on the soluble protein, which represents more than 95% of the protein in soybean meal, were compared with those on soybean meal as reported by Block and Weiss (*2*). They summarized compositional data obtained by 12 different investigators who used mostly microbiological methods of assay. Values reported herein for aspartic acid, cystine, glycine, leucine, methionine, phenylalanine, tryptophan, tyrosine, and valine (Table II) agree more closely with the higher values they reported. Alanine and isoleucine values agree more closely with their lower values. Values for arginine, glutamic acid, histidine, lysine, serine, threonine, and proline are about the same as the average values reported. Values in this study agree with those reported by Lyman, Kuiken, and Hale (*6*) for the essential amino acid content of soybeans, soybean meal, and crude soybean protein, determined by microbiological assay, with the exception of those for leucine, which were slightly higher. Values for methionine are in the same range as those reported by Krober (*5*) in his work on variables influencing the methionine content of soybeans.

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RADIATION STERILIZATION OF FOODS

Biological Value of Gamma Irradiated Corn Protein and Wheat Gluten

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Effect of gamma-ray irradiation and of heat-cooking on the nutritive value of corn protein and wheat gluten was studied. Irradiation did not produce off-flavors or odors in corn or wheat gluten; the lysine in corn or wheat gluten and the tryptophan in corn were not destroyed. Radiation-sterilized corn and wheat gluten were completely acceptable for the growing rat during a 20-day feeding period. Irradiation, like heat-cooking, did not affect the digestibility of corn protein or the digestibility of wheat gluten. The biological value of corn protein and of wheat gluten were also not affected. However, irradiation of corn at 9.30 million rad lowered its digestibility by 5% but did not lower its biological value.

BEFORE any new food processing method can be put into commercial use, foods so processed must be proven satisfactory with respect to the effect of the processing on wholesomeness (lack of toxicity), acceptability (palatability), and nutritive value of both the micro- and macro-nutrients. Irradiation sterilization has little or no deleterious effect on the nutritive value of some animal and legume proteins for the growing rat (*3, 4*). The protein sources tested were milk, beef, peas, and Lima beans, all of which are deficient in sulfur amino acids. While irradiation did not affect the digestibility of the biological value of the beef and Lima bean proteins, it lowered the biological value of milk and pea proteins by about eight percentage points. Cystine and methionine seemed to be particularly sensitive to ionizing radiation (*2*). A nutritional study of two typical cereal proteins, corn and wheat, in which the limiting amino acids are generally lysine and/or tryptophan, was undertaken. The changes due to gamma irradiation and conventional heat cooking, respectively, in the digestibility and the biological value of corn and wheat gluten are reported.

Experimental

Illinois high protein corn was selected for studying the effect of irradiation on the nutritive value of a protein deficient in tryptophan—and lysine. Even though high protein corn has been reported to be lower in biological value than low protein corn, owing to a significant increase in the zein proportion of the kernel (*9*), the former was used, because when only 50% of the corn is included in the diet it provides 10% protein, and thus its high protein content makes it convenient for formulating a balanced ration. Tryptophan—and lysine—continue to remain the limiting amino acids in the high protein corn.

The corn was finely ground and the corn and wheat gluten—which was obtained from a commercial source—were suspended in water, in each case, at a 35% concentration, canned in No. 2 cans, and frozen. Some of these canned samples were gamma-ray irradiated at the desired dose. Wheat gluten was irradiated at 2.79 million rad, while corn samples were treated at 2.79 million rad and 9.30 million rad, respectively. (One rad is defined as 100 ergs of radiant energy absorbed per gram of material irradiated.) A portion of nonprocessed suspended corn and wheat gluten, respectively, was cooked in an autoclave for 4 minutes at 15 pounds pressure. To obtain nutritional data on these samples owing to cafeteria methods of feed processing, all samples of corn and wheat gluten, including the controls, were then dried in vacuum at room temperature and finely reground. Proximate analysis on the nonprocessed, heat, and irradiation processed corn and wheat gluten samples indicate (Table I) that irradiation did not result in significant loss of nitrogen in corn or wheat gluten.

The corn and wheat gluten samples were analyzed microbiologically for lysine using *L. mesenteroides-P-60* (*1*) after hydrolysis with 2.5*N* hydrochloric acid at 15 pounds pressure for 6 hours. The corn samples were also analyzed microbiologically for tryptophan (*5*) after hydrolysis with 6*N* barium hydroxide; *Streptococcus fecalis* R. was the organism employed; and light transmittance was read at 650 mμ in a Coleman spectrophotometer.

The biological value method of Mitchell (*6, 7*) with certain modifications was used to measure the nutritive value of the corn protein and the wheat gluten. The vacuum-dried samples of corn and wheat gluten—whether nonprocessed, heat-processed, or irradiation-processed—were incorporated into balanced diets to provide 10% protein (N × 6.25), on a moisture-free basis (Table II).

The modification of the Mitchell method consisted of using two instead of three feeding periods. The first experimental period of 20 days was