Tryptophan in Soybean Meal and Soybean Whey Proteins[1]

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ABSTRACT

Pronase hydrolysates of soybean meal, whole-whey protein, and heat-coagulated and supernatant proteins prepared from whey heated at 75°C. for 30 min. were analyzed for tryptophan by two different colorimetric methods. Dilutions of the hydrolysates were reacted with either *p*-dimethylaminobenzaldehyde or acetic acid containing ferric ions. In the second method, a reference standard was developed, based on isolated protein, that effectively blanked out high absorbance arising from reaction of concentrated sulfuric acid with soybean constituents. Values in g. tryptophan per 16 g. N were: 1.583 and 1.597 for soybean meal, 1.707 and 1.440 for whole whey, 2.433 and 2.380 for heat-coagulated protein, and 1.173 and 1.063 for supernatant protein. Standard deviation was 0.046 g. tryptophan per 16 g. N and least significant difference (95% level) between methods was 0.080. Tryptophan values for the whole-whey and supernatant proteins are significantly different by both methods. Calculations based on values of amino acid analysis of these soybean samples and the mean of the tryptophan values obtained by the two colorimetric methods show that soybean meal, whole-whey, heat-coagulated proteins, and supernatant protein contain 120, 113, 153, and 90% of the tryptophan content of the hen's egg essential amino acid pattern (mg. per g. total EAA).

From current figures estimated for production of soy protein concentrates and isolates, Rackis et al. (1) determined that about 14 million lb. protein as soybean whey is disposed of as waste. Rackis et al. (2,3) concluded that whole-whey protein may be a source of edible protein because of the good balance of essential amino acids. To obtain more information on the potential value of whey proteins as a source of edible protein, a determination was made of the tryptophan content of whole-whey protein and heat-coagulated and supernatant proteins prepared from whey (1).

Since problems with high color blanks and poor recovery of added tryptophan were encountered by Rackis et al. (1,2) in determining the tryptophan content of soybean meal and whey samples with the procedure of Spies and Chambers (4), Pronase hydrolysis, before colorimetric analysis, was used. This paper describes an adaptation of the Spies procedure (5) for Pronase hydrolysis. The tryptophan content of the hydrolysates is then determined by two different colorimetric methods. The methods involve reaction of the diluted hydrolysates with *p*-dimethylaminobenzaldehyde (DMAB) according to Spies and Chambers (6) or with acetic acid containing ferric ions according to Opieńska-Blauth et al. (7). The second method was changed to simplify the procedure and to minimize color interference from nonprotein constituents.

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[1]Presented in part at the 55th Annual Meeting, Minneapolis, October 1970. Contribution from the Northern Regional Research Laboratory, Peoria, Ill. 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture.

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MATERIALS AND METHODS

Materials

Dehulled, defatted flakes were prepared from certified seed-grade soybeans (Amsoy 1967 crop) purchased from Farmer City Grain Company, Farmer City, Ill. The flakes were ground in an agate mortar before hydrolysis with Pronase. Whey fractions—whole-whey protein, heat-coagulated protein, and supernatant protein—were prepared from the flakes according to the procedures described by Rackis et al. (1).

Mann-assayed L-tryptophan (Mann Research Laboratories, New York, N.Y.) served as standard. Crystalline bovine serum albumin was purchased from Sigma Chemical Company, St. Louis, Mo., and Pronase from Calbiochem, Los Angeles, Calif.

Reagent grade chemicals were: sulfuric acid (sp. gr. 1.84) and glacial acetic acid, lot B253 (Allied Chemical, Morristown, N.J.), and ferric chloride, $FeCl_3 \cdot 6H_2O$ (Fisher Scientific Company, Fair Lawn, N.J.). DMAB from Eastman Organic Chemicals, Rochester, N.Y., was repurified according to the procedure of Adams and Coleman (8), except for elimination of the step involving addition of water before neutralization of an acid solution of DMAB with sodium hydroxide. Sodium nitrite, U.S.P. (J. T. Baker Chemical Co., Phillipsburg, N.J.) was used without further purification.

Nitrogen and Moisture Analyses

Nitrogen was determined by the Kjeldahl micromethod with catalysts potassium sulfate and mercuric oxide. Moisture was measured by heating the sample for 2 hr. at 110°C. in an Abderhalden vacuum drier.

Pronase Hydrolysis

About 250-mg. samples of defatted meal, whole-whey protein, and heat-coagulated protein and 400-mg. samples of supernatant protein and bovine serum albumin were each placed into 50-ml. nylon centrifuge tubes; 7.0 ml. aqueous Pronase (2.0 mg. per ml.), 2 drops of toluene, and one glass bead were added. Each tightly stoppered tube was placed in the neck of a 250-ml. wide-mouthed Erlenmeyer flask. The flasks were clamped onto a Psychrotherm shaker and incubated at 35°C. for 72 to 96 hr. in an incubator shaker. After hydrolysis, the samples were transferred to smaller tubes and centrifuged at 20°C. for 30 min. at approximately 12,350 × g. Dilutions of the clear supernatants were analyzed for tryptophan and nitrogen. A Pronase control, containing 2 drops of toluene, was run along with the samples to determine contribution of Pronase to tryptophan and nitrogen analyses. Bovine serum albumin, hydrolyzed along with each set of experiments, served as a hydrolysis control and as a standard for tryptophan recovery studies.

Tryptophan Determination

Tryptophan content of the hydrolysates was determined by the colorimetric methods of Spies and Chambers (6) and Opieńska-Blauth et al. (7). Defatted soybean meal was included in our study to test the usefulness of the methods outlined on material of lesser protein content and to compare the tryptophan value

of our meal with literature values for meal processed by other methods.

Dilutions from three separate Pronase hydrolysates of each sample were analyzed in triplicate by procedure B of Spies and Chambers (6), except that 0.08% sodium nitrite was used for reaction II. This percentage of sodium nitrite gave maximum color yield with the soybean samples. Absorbance of the color complex was read at 590 nm. on a Beckman model B spectrophotometer. Blank solutions were similar to test solutions, except that DMAB was omitted.

The Fe^{+3}-acetic acid procedure of Opieńska-Blauth et al. (7) was simplified and changed to minimize color interference. To simplify the procedure, the Fe^{+3}-acetic acid and concentrated sulfuric acid were mixed at least 24 hr. before analysis and 4 ml. of this composite reagent was added to the diluted hydrolysate. Addition of the composite reagent, rather than the individual reagents, did not change the slope of the standard curve for tryptophan significantly (95% confidence limit). An advantage, besides that of dispensing one reagent rather than two, is that less heat is evolved when the composite reagent is added to the diluted hydrolysate. The temperature of the solution rises to approximately 59°C. for a few seconds while dispensing the composite reagent but falls rapidly below 50°C. upon mixing. Temperatures above 50°C. decompose glyoxylic acid, formed by oxidation of acetic acid with ferric ions, to formaldehyde and CO_2 according to Fearon (9). The resulting formaldehyde either retards formation of the tryptophan complex with glyoxylic acid or reacts with tryptophan to yield a color complex that absorbs at a different wave length maximum (9). To minimize color interference, blank solutions consisting of 1 ml. diluted hydrolysate reacted with 4 ml. 50% sulfuric acid containing 56 mg. Fe^{+3} per liter replaced reagent blanks. Absorbance of the color complex was read at 545 nm. on a Beckman DU spectrophotometer and then converted to weight of tryptophan from a standard curve of tryptophan dilutions similarly treated.

We and others in our Laboratory found that certain lots of glacial acetic acid, even after distillation, gave no color reaction with tryptophan standards when used according to either the original or changed test procedure. This problem merits further investigation.

RESULTS

Solubilization of Nitrogen by Pronase Hydrolysis

Nitrogen content and amount of nitrogen solubilized by Pronase hydrolysis of soybean samples and bovine serum albumin are presented in Table I. Hydrolysis times of 72 to 96 hr. were needed to obtain maximum nitrogen solubility from the starting materials. Because nitrogen solubility was not increased significantly when soybean samples were hydrolyzed with Pronase in 0.1M phosphate buffer at pH 7.5, Pronase was dissolved in water for our hydrolyses.

Tryptophan Recovery

To determine whether tryptophan is degraded during Pronase hydrolysis for 72 to 96 hr., we added tryptophan as bovine serum albumin to each of the soybean samples before hydrolysis. The total amount of tryptophan found (BSA + soybean sample) represented 97 to 104% of the calculated value (Table II).

The suitability of both colorimetric methods for determining tryptophan in soybean meal and protein hydrolysates was demonstrated by adding known amounts of L-tryptophan to each of the hydrolysates (Table III). Recoveries based on amount found vs. amount calculated ranged from 98 to 105%.

Tryptophan Content of Soybean Samples and Bovine Serum Albumin

Tryptophan content of soybean meal, whey proteins, and bovine serum albumin is shown in Table IV. These values were not corrected for the amount of tryptophan in the solids remaining after hydrolysis. Tryptophan in these solids was determined by procedure N of Spies and Chambers (4), with reaction I time of 24 hr. Tryptophan content of the solids from the whey proteins amounted to 1.2 to 3.1% of the total sample tryptophan. The solids remaining from

TABLE I. NITROGEN CONTENT AND AMOUNT OF NITROGEN SOLUBILIZED BY PRONASE HYDROLYSIS OF SOYBEAN SAMPLES AND BOVINE SERUM ALBUMIN

Sample	Nitrogen Content[a] %	Hydrolysis Time hr.	Hydrolysate Nitrogen[b] %
Soybean meal	9.3	96	82.4–92.6
Whole whey	13.7	72	90.8–95.8
Heat-coagulated protein	14.8	96	90.3–95.9
Supernatant protein	14.4	72	83.6–89.7
Bovine serum albumin	15.7	72; 96	94.4–97.1

[a]Dry basis.

[b]Percentage of total sample nitrogen corrected for Pronase nitrogen; range of three replications.

TABLE II. RECOVERY OF TRYPTOPHAN ADDED TO SOYBEAN SAMPLES BEFORE HYDROLYSIS[a]

Sample	Method	Tryptophan (γ/sample) Endogenous	Added	Calculated	Found	% Recovery, Found/ Calculated
Soybean meal	1[b]	1,676	1,212	2,888	2,796	96.8
	2[c]	1,644	1,118	2,762	2,716	98.3
Whole whey	1	2,848	1,224	4,072	4,112	101.0
	2	2,314	1,102	3,416	3,544	103.7
Heat-coagulated protein	1	4,118	1,170	5,288	5,368	101.5
	2	4,114	1,100	5,214	5,116	98.1
Supernatant protein	1	1,786	1,224	3,010	3,036	100.9
	2	1,474	1,092	2,566	2,596	101.2

[a]Tryptophan was added as crystalline bovine serum albumin. The recorded values are based on 200 mg. each for soybean samples and added tryptophan.

[b]Dimethylaminobenzaldehyde (DMAB).

[c]Fe^{+3}-acetic acid.

TABLE III. RECOVERY OF TRYPTOPHAN FROM HYDROLYZED SOYBEAN SAMPLES

Sample	Method	Tryptophan (γ/ml. of Diluted Hydrolysate) Endogenous	Added	Calculated	Found	% Recovery, Found/ Calculated
Soybean meal	1[a]	57	10	67	68	101
		57	20	77	77	100
	2[b,c]	12	13	25	25	100
		12	21	33	33	100
Whole whey	1	39	10	49	48	98
		39	20	59	62	105
	2	16	13	29	29	100
		16	21	37	37	100
Heat-coagulated protein	1	80	10	90	89	99
		80	20	100	101	101
	2	10	13	23	23	100
		10	21	31	32	103
Supernatant protein	1	41	10	51	52	102
	2	8	21	29	29	100

[a]DMAB.

[b]Fe^{+3}-acetic acid.

[c]All hydrolysates analyzed with Fe^{+3}-acetic acid required greater dilution than those analyzed with DMAB.

TABLE IV. COMPARISON OF TRYPTOPHAN CONTENTS OF PRONASE-HYDROLYZED SAMPLES DETERMINED BY TWO COLORIMETRIC METHODS

Test Material with Hydrolysis Time	Method g. Tryptophan/16 g. N[a] DMAB	Fe^{+3}-Acetic Acid
96 hr.		
Bovine serum albumin	0.663[b]	0.637[b]
Soybean meal	1.583	1.597
Heat-coagulated protein	2.433	2.380
72 hr.		
Bovine serum albumin	0.643	0.580
Whole whey	1.707*	1.440*
Supernatant protein	1.173*	1.063*

[a]Values based on hydrolysate nitrogen (see Table I) and corrected for tryptophan content of Pronase.

[b]Values given are the means of triplicate analyses on three hydrolysates. Standard deviation = 0.046. Least significant difference between methods (95% level) = 0.080.

Pronase-hydrolyzed soybean meal yielded an atypical pink-brown color after reaction with DMAB. Consequently, the amount of tryptophan in these solids was not estimated.

There were no significant differences between methods at 96 hr. However at 72 hr. the Fe^{+3}-acetic acid method gave significantly lower values than the DMAB method for both whole-whey and supernatant proteins. This result provides evidence of a method-hydrolysis time (product) interaction for these soybean

proteins. Variation between the four bovine serum albumin values is not significant. The standard deviation based on variation between samples run on different days is 0.046 g. per 16 g. N or roughly 5 to 7% relative error. Except for the good agreement with soybean meal, the values resulting from the Fe^{+3}-acetic acid method were lower than those from the DMAB method.

DISCUSSION

Comparison of our tryptophan values of 1.58 to 1.60 g. per 16 g. N for Pronase-hydrolyzed soybean meal with literature values for hydrolyzed meals shows our values are higher. Slump and Schreuder (10) report tryptophan values for soybean meal of 1.21 to 1.50 g. per 16 g. N after barium hydroxide hydrolysis, separation of the hydrolysate on a Sephadex G-25 column, and colorimetric analysis of the column effluent with ninhydrin. Miller's (11) tryptophan value was 1.09 g. per 16 g. N after barium hydroxide hydrolysis of the meal and analysis of the hydrolysate with DMAB. Sternkopf (12) evaluated several hydrolytic methods in combination with colorimetric methods and microbiological assay to determine tryptophan content of grain legumes including soybeans. His results ranged from 0.93 g. per 16 g. N on a papain-hydrolyzed sample to 1.36 g. per 16 g. N on samples hydrolyzed by either barium or sodium hydroxide followed by microbiological and colorimetric determinations. Critical comparison of the literature values cannot be made to assess the accuracy of our results since different batches of soybean meals and different conditions were used. The tryptophan value for crystalline bovine serum albumin hydrolyzed by Pronase and analyzed with DMAB was 0.545 g. per 16 g. N (13). Spies (13) also obtained a value of 0.594 g. per 16 g. N after alkaline hydrolysis followed by the same DMAB procedure, which value compares more favorably with our values for bovine serum albumin.

Upon comparison of the results on Pronase-hydrolyzed samples (Table IV) with those on unhydrolyzed samples by Rackis et al. (1), the tryptophan values of all the hydrolyzed samples are higher, except for the value of hydrolyzed whole whey determined with the Fe^{+3}-acetic acid method. Since we had an excellent recovery of tryptophan added to the hydrolysates (see Table III), interfering compound(s), reported by Rackis et al. (2), that caused poor recovery of tryptophan must remain behind with the precipitates.

To determine whether tryptophan is a limiting amino acid in the soybean samples analyzed, the Food and Agriculture Organization of the United Nations (FAO) system (14) was adopted, where each essential amino acid is reported as a proportion of the total of essential amino acids (mg. per g. total EAA). These values were then converted to percentages of hen's egg essential amino acid pattern. Calculations based on the mean of the tryptophan values by the two colorimetric methods and values for the essential amino acids on the same samples obtained by us (1) show that soybean meal, whole-whey, and heat-coagulated proteins possess 120, 113, and 153% of the tryptophan content of the hen's egg essential amino acid pattern (mg. per g. total EAA). Supernatant protein on the same basis has 90% of the tryptophan content. Therefore tryptophan is a limiting amino acid in this protein. Except for the 113% for whole-whey protein, these percentages are significantly higher than those presented previously (1).

Acknowledgments

We thank Mrs. Karen Jones for nitrogen and moisture determinations and William F. Kwolek for statistical analyses of our data.

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[Received August 24, 1970. Accepted November 13, 1970]