Biochemical and Nutritional Assessment of Different Varieties of Soybeans

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Over 100 different commercial varieties and experimental strains of soybeans were initially screened for antitryptic and hemagglutinating activities. On the basis of these data, 26 samples representing low, medium, and high levels of these two activities were selected for further study which included measurements of antichymotryptic activity, cystine and methionine content, weight of pancreas of rats fed diets containing these soybean samples, and protein efficiency ratios (PER). Of all the parameters so examined only the weights of the pancreas showed a significant inverse correlation with PER. The possibility is suggested that there exists in raw soybeans a factor(s) which has no demonstrable antitryptic activity *in vitro* but which is nevertheless capable of causing pancreatic hypertrophy and an inhibition of growth. No positive correlation between total sulfur–amino acid content and PER of several heat-treated soybean samples was noted.

The importance of the soybean as a protein supplement for animal feed and its potential value for human food is well recognized. However, one of the principle drawbacks in the utilization of soybean protein, aside from its deficiency of the sulfur-containing amino acids, is the presence of a number of natural constituents which may adversely affect its nutritive value. These include protease inhibitors, hemagglutinins, saponins, and antivitamins (Liener, 1969). Although heat treatment will effectively eliminate most of these undesirable substances, careful control of processing conditions is essential to prevent both functional as well as nutritional damage to the protein resulting from excessive heat treatment. On the other hand, the breeding of varieties or strains of soybeans which are genetically deficient in one or more of these antinutritional factors would offer a much more satisfactory long-term solution to this important problem since it would eliminate the uncertainties entailed in the processing of soybeans. In the present study a large number of different commercial and experimental varieties of soybeans were examined for trypsin and chymotrypsin inhibitor activity, hemagglutinating activity, and the S-containing amino acids, cystine and methionine. An attempt was then made to establish what relationship, if any, might exist between these biochemical parameters and the nutritive properties of the protein as revealed by rat feeding studies.

MATERIALS AND METHODS

Soybean Samples. The samples of soybeans used in this study were of three kinds: (1) commercial varieties, (2) advanced breeding lines from the soybean breeding project of the Minnesota Agricultural Experiment Station, and (3) plant introductions maintained by the U.S. Regional Soybean Laboratory, Urbana, Illinois. The seeds of all of these samples were grown on experimental plots of the University. The whole mature seeds were ground in a Wiley mill to pass through a 100-mesh screen and extracted with ten volumes of petroleum ether (bp 60–70° C) at room temperature. After centrifuging off the ether layer, the residue was air-dried at room temperature.

Protein Content. The crude protein content (N × 6.25) of the soybean samples was determined by the Kjeldahl–Wilfarth–Gunning method (AOAC, 1950). The protein content of extracts of the soybean meal was determined by the method of Lowry *et al.* (1951).

Protease Inhibitor Activity. Trypsin inhibitor activity was evaluated in terms of the extent to which a portion of an aqueous extract of the soybean meal inhibited the action of trypsin on benzoyl-DL-arginine-*p*-nitroanilide (Kakade *et al.*, 1969). Chymotrypsin inhibitor activity was measured on the basis of the ability of the aqueous extract to inhibit the digestion of casein by chymotrypsin (Kakade *et al.*, 1970b). Chymotrypsin inhibitor activity may be attributed primarily to the presence of the Bowman–Birk inhibitor which, in contrast to the soybean trypsin inhibitor of Kunitz, is also a potent inhibitor of chymotrypsin (Birk, 1961).

Hemagglutinating Activity. Hemagglutinating activity was determined by the photometric technique of Liener (1955), which measures the ability of soybean extracts to agglutinate rabbit erythrocytes.

Methionine and Cystine. Methionine and cystine were determined as methionine sulfone and cysteic acid, respectively, by the following procedure. To approximately 100 mg of sample in a 50-ml conical flask was added 5 ml of freshly prepared performic acid (Schram *et al.*, 1954). After allowing the flasks to set at 4° C for 16 hr, the performic acid was removed by evaporation on a steam bath for 2 hr. To the dried residue was added 5 ml of 20% HCl, and the suspension was autoclaved at 120° C for 6 hr (Evans and Bandemer, 1967). The HCl was removed by evaporation on a steam bath, the residue dissolved in 5 ml of water, and again reduced to dryness. The latter step was repeated at least once more in order to eliminate completely the HCl. The residue was dissolved in 25 ml of pH 2 buffer, filtered, and 0.5 ml of the filtrate was subjected to amino acid analysis as described by Spackman *et al.* (1958).

Rat Feeding Experiments. The protein quality of the various soybean samples was evaluated in terms of protein efficiency ratio (PER, grams gained per gram of protein consumed). Male weanling rats, 21 days old and weighing 50–55 g, were divided into groups of six animals, distributed

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Table I. Distribution of Antitryptic and Hemagglutinating Activities of 108 Varieties and Strains of Soybeans

	Trypsin inhibitor activity			Hemagglutinating activity		
		Number of samples			Number of samples	
Arbitrary classification	Range of values[a]	Commercial and breeding lines	Plant introductions	Range of values[b]	Commercial varieties	Experimental strains
Low	66–121	7	15	60–182	23	47
Medium	122–177	34	25	183–304	28	4
High	178–233	16	11	305–426	6	0

[a] Expressed as trypsin units inhibited (TUI) per mg protein as defined by Kakade *et al.* (1969a). [b] Expressed as hemagglutinating units (HU) per mg protein as defined by Liener (1955).

Table II. Biochemical Parameters and Nutritional Evaluation of Various Samples of Soybeans

Sample[a]	Trypsin inhibitor activity, TUI/mg protein[d]	Chymotrypsin inhibitor activity, CUI/mg protein[e]	Hemagglutinating activity HU/mg protein[d]	Cystine content[b]	Methionine content,[b] g/16 g of N	Cystine + methionine	Weight of pancreas, g/100 g body weight	PER[c]
A-100	121	48	183	1.9	1.9	3.8	0.63	1.16
Provar	106	48	186	2.1	1.3	3.4	0.59	1.60
Rampage	136	47	313	1.8	1.5	3.3	0.66	1.18
Disoy	100	43	118	1.6	1.5	3.1	0.59	1.65
Flambeau	121	48	180	1.6	1.5	3.1	0.61	1.37
Hark	100	39	156	2.4	1.5	3.9	0.65	1.21
Merit	168	56	165	2.1	1.0	4.1	0.58	1.44
M54-46-4	169	66	323	3.5	1.4	3.9	0.65	1.21
M55-14	183	66	247	2.4	1.3	3.7	0.64	1.26
M55-59	157	52	225	2.5	1.8	4.3	0.61	1.26
M58-14	139	63	309	2.2	1.3	3.5	0.55	1.36
M59-120	154	54	176	2.2	1.3	3.5	0.61	1.23
M60-169	171	55	193	2.0	1.1	3.1	0.57	1.34
M60-217	123	45	167	2.0	1.3	3.3	0.61	1.35
M60-266	117	41	181	2.5	1.5	4.0	0.62	1.47
PI 153206	139	55	43	2.9	1.3	4.2	0.52	1.63
PI 153319	168	59	110	2.2	1.4	3.6	0.67	0.90
PI 180517	150	48	46	2.9	1.3	4.2	0.73	0.74
PI 189880	168	72	37	2.6	1.5	4.1	0.57	1.65
PI 189898	154	63	97	2.7	1.5	4.2	0.66	0.90
PI 189900	152	63	108	2.3	1.4	3.7	0.72	0.76
PI 257428	184	83	122	2.1	1.9	4.0	0.61	1.40
PI 257438	152	70	106	2.4	1.0	3.4	0.59	1.68
PI 257439	139	85	226	2.3	1.3	3.6	0.62	1.34
PI 258383	175	85	172	2.6	1.3	3.9	0.47	1.74
PI 258385	178	82	140	2.6	1.4	4.0	0.53	1.72

[a] Commercial strains are denoted by proper names with the exception of sample A-100, advanced lines from the soybean breeding project of the Minnesota Agricultural Experiment Station by the prefix "M," and plant introductions maintained by U.S. Regional Soybean Laboratory, Urbana, Illinois, by the prefix "PI." [b] Cystine was determined as cysteic acid and methionine as methionine sulfone. See text for details. [c] Actual PER values have been corrected on the basis that the PER of the casein control group is 2.5. [d] See footnotes to Table I. [e] CUI, chymotrypsin units inhibited as defined by Kakade *et al.* (1970a).

as equally as possible with respect to weight. The composition of the diet and other details were the same as described in a previous publication (Kakade *et al.*, 1970a) except that a level of 10% protein was provided by samples of ground soybeans which had not been defatted by extraction with petroleum ether. A control group of rats receiving an identical diet in which casein provided a protein level of 10% was run concurrently. At the end of the experimental period of 28 days the rats were sacrificed, and the pancreas was excised and weighed.

RESULTS

During the course of this investigation 57 commercial varieties and advanced breeding lines and 51 plant introductions of soybeans were examined for trypsin inhibitor and hemagglutinating activities. On the basis of these determinations, these soybean samples were divided into six arbitrary categories representing samples with low, medium, and high antitryptic or hemagglutinating activities as shown in Table I. These data show a high degree of variability in antitryptic and hemagglutinating activities among an essentially random sampling of the soybean specimens which were available to us. The upper and lower limits of the data presented in Table I reveal about a fourfold variation in antitryptic activity and a sevenfold variation in hemagglutinating activity.

Based on this initial screening process, 26 different varieties or strains of soybeans representing each of the six categories shown in Table I were selected for further detailed study. These data are tabulated in Table II. In order to establish whether any correlation existed between any one of these parameters and the nutritive properties of the protein as revealed by PER, the scatter diagrams shown in Figure 1 were prepared, relating each of the following parameters to PER: trypsin inhibitor activity, chymotrypsin inhibitor activity, hemagglutinating activity, total sulfur-containing amino acids, and weights of the pancreas. It is immediately apparent that there is essentially no correlation between any of these parameters and PER, with the notable exception of the weight

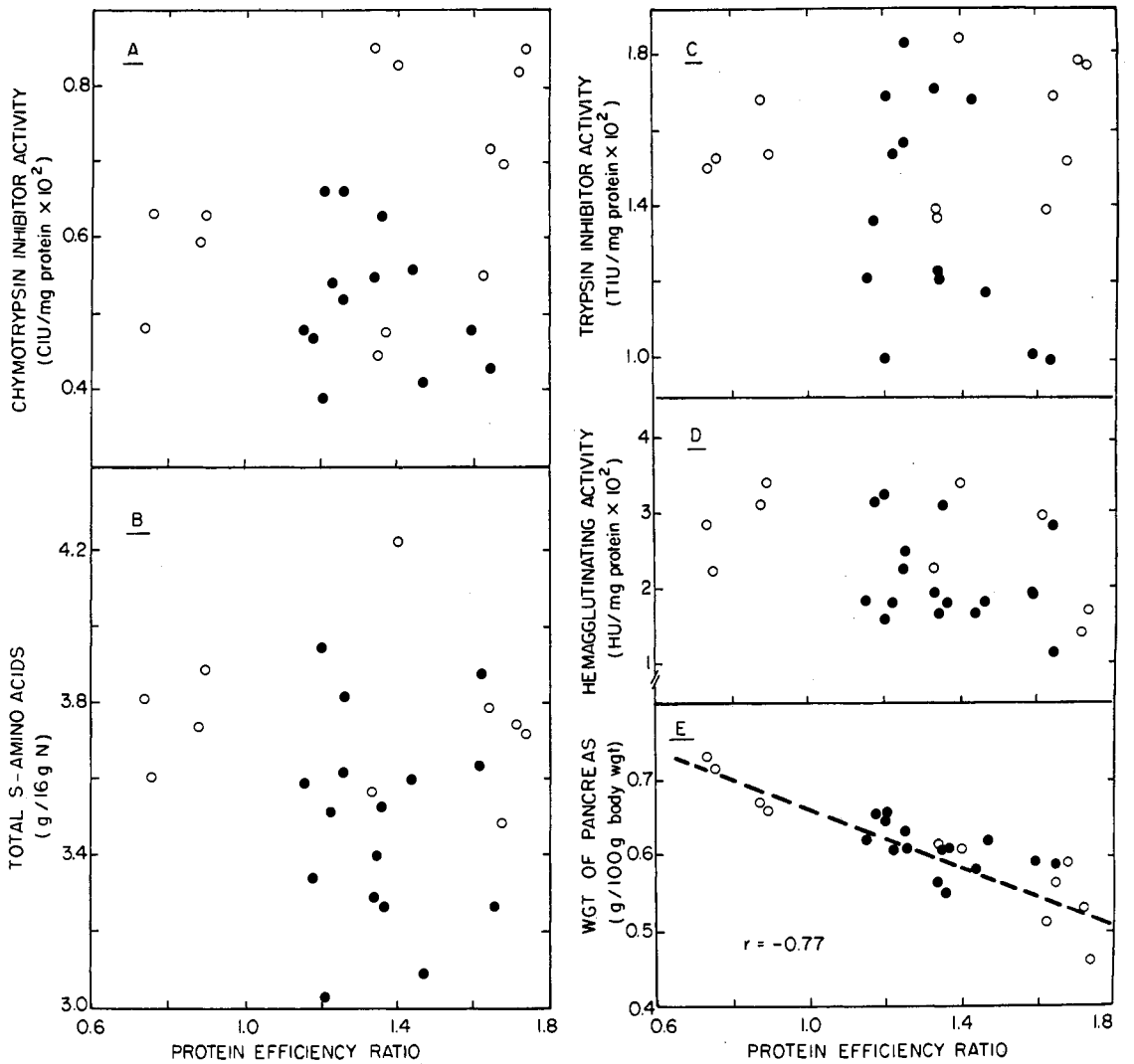

Figure 1. Scatter diagrams relating various biochemical parameters to protein efficiency ratios (PER) of various soybean samples. Solid circles refer to data obtained with commercial varieties, and open circles refer to data obtained with experimental strains (Table II). A, chymotrypsin inhibitor activity; B, total sulfur-containing amino acids; C, trypsin inhibitor activity; D, hemagglutinating activity; E, pancreatic weights

of the pancreas. In the latter instance statistical evaluation of these data revealed a high negative degree of correlation ($r = -0.77$; $P < 0.05$) between weights of the pancreas and PER.

DISCUSSION

The various soybean samples used in this study showed marked differences in nutritive value, the PER values ranging from 0.7 to 1.75, compared to a casein value of 2.5. Such a wide variation should have provided ample opportunity to establish a correlation between a given parameter and PER, should one have existed. Despite the evidence which implicates the trypsin inhibitor and phytohemagglutinin as factors contributing to the poor nutritive value of raw soybean meal (Liener, 1969), no such relationship was apparent in these studies.

In view of the lack of correlation between trypsin inhibitor activity and PER, the finding that there was a significant degree of correlation between the weights of the pancreas and PER was somewhat surprising, since it is generally assumed that the trypsin inhibitors are mainly responsible for hypertrophy of the pancreas and the poor growth of animals fed raw soybeans (Garlich and Nesheim, 1966; Rackis, 1965; Sambeth *et al.*, 1967). A possible explanation might be that the measurement of antitryptic activity *in vitro* does not reflect the true inhibitor content of the soybean. For example, the trypsin inhibitors might be bound in such a fashion that they cannot be extracted by aqueous solvents generally employed for their assay. This might explain why the water-insoluble residue of soybeans, although low in antitryptic activity, inhibited growth and caused pancreatic hypertrophy (Garlich and Nesheim, 1966; Rackis *et al.*, 1963; Saxena *et al.*, 1963). Of course, one cannot exclude the possibility that there may be present in raw soybeans a factor which is totally unrelated to the trypsin inhibitor but which is nevertheless capable of causing pancreatic hypertrophy and an inhibition of growth.

Since the S-containing amino acids, methionine and cystine, are the most limiting amino acids of soybean protein, it was hoped that this study might also uncover a variety or strain of soybeans which would have a higher content of these amino acids than has heretofore been reported. As shown in Table II, however, this expectation was not realized. Methionine values ranged from 1.0 to 1.9 g/16 g of N which does not differ appreciably from the range of values reported in the literature for the methionine content of soybeans as influenced by variety, 1.1–1.6 g/16 g of N (Alderks, 1949;

Table III. The Effect of Heating on the Nutritive Value of Samples of Soybeans Varying Total Sulfur Amino Acid Content

Sample	Protein %	Total S-amino acids,[a] g/16 g of N	PER	
			Unheated[a]	Heated[b]
Disoy	39.6	3.1	1.47	2.66
Provar	41.2	3.4	1.60	2.20
PI 153319	36.6	3.6	0.88	2.46
Hark	39.1	3.9	1.21	2.32
PI 153206	37.5	4.2	1.21	1.95

[a] Taken from data in Table II. [b] Autoclaved at 15 lb/in.^{2} (120° C) for 30 min.

Evans and Bandemer, 1967; Kuiken and Lyman, 1949), location and season, 1.3–1.7 g/16 g of N (Krober, 1956), or protein content, 1.0–1.7 g/16 g of N (Krober and Cartter, 1966).

The influence of variety on the cystine content of soybeans was first studied by Csonka and Jones (1934), who reported a range of 0.7 to 1.1 g/16 g of N by direct chemical analysis. Conversion of cystine to cysteic acid followed by ion-exchange chromatography by the method of Schram *et al.* (1954) or Spackman *et al.* (1958) has given values of 1.6 and 2.6 g/16 g of N, respectively, for a hydrolysate of soybean meal (Rackis *et al.*, 1961). The range of values reported here, 1.6–2.9 g/16 g of N, may therefore be regarded as being within the "normal" range.

The failure to observe a correlation between the S-amino acid content of various soybean samples and their PER could very well have been due to a masking by the various growth inhibitory factors present in the raw bean. It was of interest, therefore, to ascertain whether there might be a positive association between S-amino acid content and the PER of heated soybean samples. It was first established that autoclaving soybeans at 15 lb/in.^{2} (130° C) for 30 min completely destroyed the hemagglutinating activity and eliminated at least 90 to 95% of the trypsin and chymotrypsin inhibitor activities. Five soybean samples representing a range of 3.1 to 4.2 g of methionine plus cystine per 16 g of N were selected for such a study. As shown in Table III, a marked improvement in PER was effected in all cases by heat treatment, but again there appeared to be little if any correlation with the level of the total S-amino acids which they contained. Although not shown in Table III, the average weights of the pancreas of rats fed diets containing the heated soybean samples were not significantly different from those receiving casein. Similar results have been reported by Rackis (1965). Although limited in scope, this study would suggest that factors other than the content of S-amino acids appear to be involved in determining the nutritive value of soybean protein in which heat-labile growth inhibitors have been destroyed.

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