Correlation of Amino Acid Indexes with Nutritional Quality of Several Soybean Fractions[1]

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ABSTRACT

Amino acid compositional studies were made on several soybean fractions. An essential amino acid index and a requirement index were calculated, based on the amino acids of the soybean fractions, and the results failed to demonstrate a good correlation between the chemical indexes and protein efficiency ratio (PER) values. Available lysine was determined for the fractions, and it also failed to correlate with PER. The residue, which is the most nutritious fraction as measured by growth and PER, contained more cystine and consequently more total sulfur amino acids. This apparently accounts for the superior utilization of the residue protein.

The biological evaluation of a protein foodstuff in the organism for which it is intended is recognized as the ultimate test. Such evaluation is not always possible, particularly in humans. Much testing of proteins has been done in small animals, in particular the rat. Although studies in rats require much less time and are less expensive than a similar study in humans, there is still a need for a short, routine assay that can be performed in a day or two. To this end, many nutritionists and biochemists have been working for years. In this connection, it is well recognized that protein quality is dependent upon its amino acid make-up, and although nutritionists and biochemists have been aware of this for years, their efforts to develop a chemical index of protein quality have been generally disappointing. At times many different procedures have looked promising, but with further study many exceptions have been found.

Earlier research by Stillings and Hackler (1) and Hackler and Stillings (2) indicates that once the antinutritional factors known to be present in raw soybeans are destroyed, then very good correlations are obtained between protein efficiency ratios (PER) and protein scores based on the amino acids.

Hackler *et al.* (3) in 1963 reported on the utilization of several soybean fractions by weanling rats in PER studies. The results were somewhat surprising, since they indicated that the residue, or water-insoluble fraction, was the most nutritious. Therefore, this investigation was undertaken to ascertain if the balance of the essential amino acids and protein scores calculated from them would account for this observation. Also of interest was evaluation of the available lysine content of these fractions.

MATERIALS AND METHODS

Preparation of Soybean Fractions

In all studies, certified Clark variety soybeans have been used in the

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preparation of the soybean fractions. Figure 1 shows graphically the prepara-

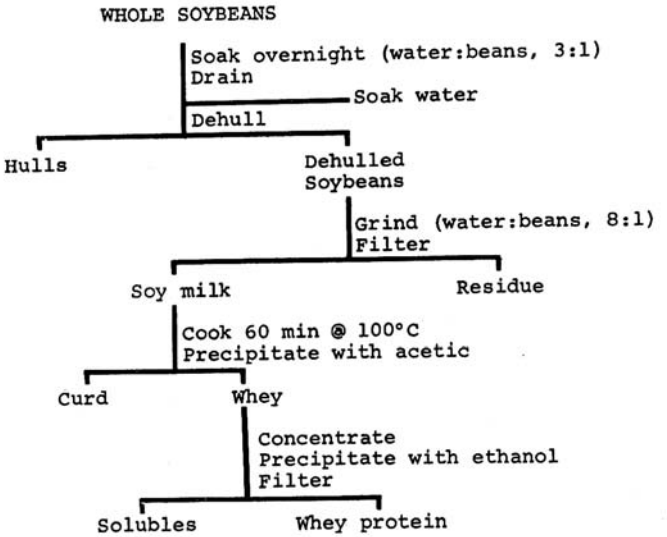

Fig. 1. Preparation of soybean fractions.

tion of the samples. The residue or water-insoluble fraction contains 23.5% of the solids and 13.5% of the protein; the soy milk contains 65.0 and 83.0%, respectively. The curd and whey protein fractions are prepared from the soy milk, and they contain 74.0 and 4.5%, respectively, of the protein from the original starting soybean. The crude protein, lipid, and moisture contents of the freeze-dried soybean fractions are shown in the table below. Lipid composition was determined by ethanol-chloroform extraction, with a slight modification of the AOAC procedure (4) for lipid.

	Crude Protein (N × 6.25) %	*Lipid* %	*Water* %
Soybeans (dehulled)	44.3	25.8	0.8
Residue	23.1	17.3	4.5
Soy milk	50.0	28.5	3.0
Curd	57.5	36.0	2.5
Whey protein	55.0	4.0	7.2
Soak water	18.6	2.0	3.6
Hulls	15.4	6.3	9.6

Additional details on the distribution of the solids and protein have been reported (3).

Analytical Methods

Amino acid contents of the soy milk samples were determined by ion-exchange column chromatography of acid hydrolysates in an automated analyzer. Samples were prepared for the tryptophan analysis by extraction for 20 hr. with a methanol-chloroform mixture (v./v.) to remove all interfering substances (5). Tryptophan was then determined by procedure N of the method of Spies and Chambers (6). Cystine was converted to cysteic acid

as described by Moore (7). The cysteic acid was then quantitatively chromatographed on an automated amino acid analyzer and calculated as cystine with aspartic acid as the reference. Available lysine was determined by the method of Carpenter (8). By this procedure the epsilon amino group of lysine reacts with fluorodinitrobenzene.

The essential amino acid index (EAAI), as modified by Mitchell (9), and the requirement index (RI), as described by Rama Rao *et al.* (10), were calculated for the soybean fractions. These chemical indexes were compared with PER. The PER values were determined in growth studies with weanling rats in which the soybean fraction was added to an otherwise complete diet. Diets contained 10% crude protein (N × 6.25) and were fed *ad lib.* to groups of 10 rats each for 28 days. Since the soybean fractions differed in protein and fat contents, adjustments were made in the amounts of dextrose and corn oil to maintain a constant calorie-to-protein ratio. Additional details of our experimental methods in handling rats for growth studies have been described previously (3).

RESULTS AND DISCUSSION

The results on the amino acid composition of the soybean fractions are reported in Table I as g. of amino acid per 16 g. nitrogen. The lysine

TABLE I

AMINO ACID COMPOSITION OF THE SOYBEAN FRACTIONS

	Residue	Soy Milk	Curd	Whey Protein	Hulls	Soak Water
	g./16 g. N[a]	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*
Lysine	5.09	6.05	5.85	8.84	5.98	2.90
Histidine	2.57	2.53	2.35	3.45	2.41	1.71
Arginine	6.19	7.16	6.97	8.39	5.27	5.29
Aspartic acid	11.5	11.0	11.1	11.2	10.0	11.6
Threonine	4.10	3.87	3.66	5.20	3.52	3.26
Serine	4.90	4.84	4.91	4.40	5.12	3.02
Glutamic acid	15.6	16.9	17.8	22.1	14.2	9.15
Proline	4.82	4.73	4.88	4.05	5.10	3.60
Glycine	4.21	3.92	3.93	4.52	6.11	3.08
Alanine	3.97	3.94	4.02	4.31	3.97	3.73
Valine	5.01	4.78	4.71	3.18	4.61	3.06
Cystine[b]	2.34	1.61	1.71	2.74	1.38	2.51
Methionine	1.02	1.44	1.35	2.17	1.01	0.50
Isoleucine	4.53	4.79	4.89	2.88	3.89	2.46
Leucine	8.02	7.89	8.00	3.96	6.72	4.21
Tyrosine	3.00	3.90	3.72	3.82	3.16	2.44
Phenylalanine	4.93	4.87	4.83	2.00	3.95	3.18
Tryptophan	1.64	1.35	1.08	1.77	1.31	0.64

[a] Nitrogen (N) was determined by the microKjeldahl procedure (3).

[b] Cystine was analyzed by conversion to cysteic acid as described by Moore (7).

content of the residue is lower than that found in soy milk, 5.09 *vs.* 6.05 g./16 g. N, respectively. However, the total sulfur amino acid content is somewhat higher in the residue. Hackler *et al.* (3) reported the residue superior to the other fractions as measured by growth and PER. Since the sulfur amino acids are generally recognized as being first-limiting in soybeans (9), this would suggest that the increased quantity of the total sulfur

amino acids accounts for the increased growth and PER. Other possibilities are that the balance of the essential amino acids may be better in the residue, the release of the essential amino acids during digestion is important, or perhaps some other factor accounts for the difference.

The effect of length of fermentation, deep-fat frying, and steaming on the amino acids present in tempeh (mold-digested soybeans) and their correlation with protein quality have been reported by Stillings and Hackler (1). Also, Hackler and Stillings (2) have reported the effect of length of cooking at 93° and 121°C. on the amino acids present in soy milk and their correlation with protein quality. They have reported a high correlation in products that have received enough heat to inactivate or destroy the "toxic" or antinutritional compounds present in soybeans. Since these soybean fractions have been heat-processed for 60 min. at 100°C., all of the heat-labile, antinutritional substances have been destroyed; thus, one would expect, from previous research on soybean products (1,2), a good correlation between protein scores and PER values of the soybean fractions.

Protein scores of the soybean fractions based on the essential amino acid index and the requirement index are shown in Tables II and III, respectively.

TABLE II

PROTEIN SCORES OF SOYBEAN FRACTIONS BASED ON ESSENTIAL AMINO ACID INDEX

	RESIDUE	SOY MILK	CURD	WHEY PROTEIN	HULLS	SOAK WATER
Lysine	72.7[a]	86.4	83.6	126.3	85.4	41.4
Histidine	107.1	105.4	97.9	143.8	100.4	71.3
Threonine	95.3	90.0	85.1	120.9	81.9	75.8
Isoleucine	58.8	62.2	63.5	37.4	50.5	31.9
Leucine	87.2	85.8	87.0	43.0	73.0	45.8
Valine	69.6	66.3	65.4	44.2	64.0	42.5
Methionine	25.5	36.0	33.8	54.3	25.3	12.5
Cystine	97.5	67.1	71.3	114.2	57.5	104.2
Total SAA	52.5	47.7	47.8	71.4	37.3	45.3
Phenylalanine	78.3	77.3	76.7	31.7	62.7	50.5
Tyrosine	66.7	86.7	82.7	84.9	70.2	54.2
Total P + T	73.4	81.2	79.2	53.9	65.8	52.0
Tryptophan	109.3	90.0	72.0	118.0	87.3	42.7
EAAI	75.2	75.1	72.8	70.8	66.2	70.6

[a] Value for each amino acid is expressed as a percent of the reference.

The EAAI indicates that the residue and soy milk fractions are comparable in nutritional quality, followed by the curd, whey protein, soak water, and hulls. On the other hand, the RI indicates only a very slight drop in nutritional quality between the residue and the curd (85.5 and 84.7, respectively). However, by both procedures the whey protein, soak water, and hulls are definitely inferior to the other fractions.

To compare further the EAAI and RI, the protein scores of the most limiting amino acids by each procedure have been summarized in Tables IV and V, respectively. It is obvious from the results in Table IV (EAAI) that the residue, soy milk, curd, and hulls are most limited in the sulfur amino acids, whereas the whey protein contains more of the sulfur amino acids, and it is first-limiting in phenylalanine, followed by isoleucine, leucine, and

valine. The soak-water fraction is generally deficient in most of the amino acids as measured by the EAAI.

TABLE III

PROTEIN SCORES OF SOYBEAN FRACTIONS BASED ON REQUIREMENT INDEX

	RESIDUE	SOY MILK	CURD	WHEY PROTEIN	HULLS	SOAK WATER
Lysine	56.6[a]	67.2	65.0	98.2	66.4	32.2
Histidine	102.8	101.2	94.0	138.0	96.4	68.4
Threonine	82.0	77.4	73.2	104.0	70.4	65.2
Isoleucine	82.4	87.1	88.9	52.4	70.7	44.7
Leucine	114.6	112.7	114.3	56.6	96.0	60.1
Valine	91.1	86.9	85.6	57.8	83.8	55.6
Methionine	63.8	90.0	84.4	135.6	63.1	31.3
Cystine	68.8	47.4	50.3	80.6	40.6	73.8
Total SAA	67.2	61.0	61.2	98.2	47.8	60.2
Phenylalanine	117.4	116.0	115.0	47.6	94.0	75.7
Tyrosine	100.0	130.0	124.0	127.3	105.3	81.3
Total P + T	110.0	109.3	108.8	69.4	96.5	78.1
Tryptophan	149.1	122.7	98.2	160.9	119.1	58.2
RI	85.5	85.9	84.7	81.6	78.9	82.1

[a] Value for each amino acid is expressed as a percent of the reference.

TABLE IV

PROTEIN SCORES OF MOST LIMITING AMINO ACIDS IN SOYBEAN FRACTIONS AS DETERMINED BY ESSENTIAL AMINO ACID INDEX[a]

AMINO ACID	RESIDUE	SOY MILK	CURD	WHEY PROTEIN	HULLS	SOAK WATER
Methionine	25.5	36.0	33.8	....	25.3	12.5
Total SAA	52.5	47.7	47.8	....	37.3	45.3
Phenylalanine	....	....	....	31.7	....	50.5
Tyrosine	....	....	....	....	....	54.2
Total P + T	....	....	....	53.9	....	52.0
Isoleucine	....	....	....	37.4	....	31.9
Leucine	....	....	....	43.0	....	45.8
Valine	....	....	....	44.2	....	42.5
Lysine	....	....	....	....	....	41.4
Tryptophan	....	....	....	....	....	42.7

[a] Value for each amino acid is expressed as a percent of the reference.

TABLE V

PROTEIN SCORES OF MOST LIMITING AMINO ACIDS IN SOYBEAN FRACTIONS AS DETERMINED BY REQUIREMENT INDEX[a]

AMINO ACID	RESIDUE	SOY MILK	CURD	WHEY PROTEIN	HULLS	SOAK WATER
Methionine	63.8	....	....	....	63.1	31.3
Cystine	....	47.4	50.3	....	40.6	....
Total SAA	....	61.0	61.2	....	47.8	60.2
Phenylalanine	....	....	....	47.6	....	....
Isoleucine	....	....	....	52.4	....	44.7
Leucine	....	....	....	....	....	60.1
Valine	....	....	....	57.8	....	55.6
Lysine	56.6	....	65.0	....	66.4	32.2

[a] Value for each amino acid is expressed as a percent of the refe.ence.

The RI puts more emphasis on lysine than does the EAAI, and this fact is reflected in Table V. The RI indicates that lysine is the first-limiting amino acid in the residue, followed by methionine. Since the RI mean values are approximately 10% higher (for example, the residue has a RI of 85.5, as opposed to 75.2 for the EAAI), this difference has been allowed for in listing the most limiting amino acids in Tables IV and V. In other words, an upper limit of 55 was arbitrarily set for the EAAI and 65 for the RI.

The results on the available lysine content of the soybean fractions are shown in Table VI. The whey protein contains the most available lysine,

TABLE VI

AVAILABLE LYSINE CONTENT AND RECOVERY OF ADDED LYSINE IN VARIOUS SOYBEAN FRACTIONS

		LYSINE ADDED	TOTAL FOUND	RECOVERY	
	mg./g.	*mg./g.*	*mg./g.*	*%*	*g./16 g. N*
Residue	6.34	7.451	11.50	83.4	3.73
Soy milk	20.8	12.61	30.14	90.2	5.33
Curd	21.8	12.11	32.68	96.4	4.58
Whey protein	38.2*	11.452	49.46	99.6	7.01
Hulls	5.65	4.057	7.451	76.8	4.00
Soak water	2.17	1.830	2.348	58.7	1.98

followed by soy milk, curd, hulls, residue, and soak water. Recovery of added epsilon-DNP-L-lysine HCl was determined in the soybean fractions. The results (Table VI) indicate a very wide range of recoveries (from 58.7 for lysine in the soak water to 99.6 for lysine in whey protein). The results tend to indicate that as the purity of the protein increased, there was a concurrent increase in recovery of added lysine.

A summary of the results on PER, EAAI, RI, and available lysine are presented in Table VII. The results on the EAAI, RI, and available lysine are

TABLE VII

COMPARISON OF THE NUTRITIONAL QUALITY OF THE SOYBEAN FRACTIONS AS MEASURED BY PER, EAAI, RI, AND AVAILABLE LYSINE

	RESIDUE	SOY MILK	CURD	WHEY PROTEIN	HULLS	SOAK WATER
PER[a]	2.71	2.11	2.20	1.93	....	....
EAAI	75.2	75.1	72.8	70.8	66.2	70.6
RI	85.5	85.9	84.7	81.6	78.9	82.1
Available lysine[b]	3.73	5.33	4.58	7.01	4.00	1.98

[a] Casein, fed as a standard reference, had a PER of 2.86, dehulled soybeans 2.51.
[b] Available lysine is expressed as g./16 g. N.

somewhat disappointing in that they do not correlate better with PER. Owing to the low quantity of protein in the hulls and soak water, no PER values were obtained for them. The data indicate that further research is needed to more clearly define the nutritional requirements before chemical estimates of biological value can be used routinely as a measure of protein quality. However, a disappointment, such as the one described in this paper, should not discourage future research on the perfection and/or development of a

chemical estimate of protein quality, because there is a real need for a procedure that can be used routinely for assessing protein quality in a short period of time.

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