AMINO ACID COMPOSITION OF HEAT-PROCESSED SOYMILK AND ITS CORRELATION WITH NUTRITIVE VALUE[1]

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ABSTRACT

Investigations were made to evaluate the effect of heat-processing time and temperature on changes in amino acids of soymilk protein and, also, to evaluate the possible correlation of essential amino acid index and requirement index with nutritional value as measured *in vivo*. Two processing temperatures (93° and 121°C.) were studied. Also investigated was the effect of spray-drying inlet temperature on the amino acids of soymilk. The results indicate that soymilk heat-processed for as long as 4 hr. at 93°C. caused no significant changes in amino acid composition. On the other hand, decreases in some of the amino acids (especially cystine) were observed when the processing temperature of 121°C. was used. Furthermore, it was found that spray-drying inlet temperature is critical, and that the amino acids destroyed during spray-drying are different from those observed when soymilk was heated at 121°C. It is quite apparent from the results that heat-processing conditions must be carefully controlled to produce soymilk of highest nutritional quality.

Research on soybean products has been stimulated in recent years as a means of combating protein malnutrition in many of the underdeveloped countries. Of the legumes, soybeans have received the most emphasis because of their high content of protein and their good quality. Since protein deficiency is more severe during the early stages of growth, considerable emphasis has been placed on the nutritional quality of soymilk protein. Soymilk is of particular interest because it is used in infant feeding in the United States, especially when an infant is allergic to cow's milk.

Recently, Hackler *et al.* (1) and Stillings and Hackler (2) have reported that heat-processing temperature and time may alter the nutritional quality of protein and its amino acid composition. Protein efficiency ratio of heat-processed soymilk has been shown to be dependent upon both time and temperature (1). Stillings and Hackler (2) showed that the amino acid composition of deep-fat-fried tempeh (soybeans, fermented with *Rhizopus oligosporus* Saito) changed with longer cooking time after 3 min.

The present study was initiated to evaluate the effect of various heat-processing techniques on the amino acid contents of soymilk (a water-extract of soybeans). Since the availability of amino acids in a protein is responsible for protein quality, it seemed desirable to attempt to relate the amino acid

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composition of the heat-processed soymilk products to an *in vivo* estimate of protein quality. Therefore, this investigation was undertaken to learn to what extent variations in the nutritional quality of the protein in a water-extract of soybeans might be related to its amino acid composition.

Materials and Methods

Preparation of Soymilk Samples. In all studies, certified Clark variety soybeans were used in the preparation of the soymilk samples. Soybeans were soaked overnight in three times their weight of water; the beans were then ground with water through a 0.023-in. screen of a disintegrator (Model RA-4K53, Rietz Mfg. Co., Santa Rosa, Calif.). The resulting slurry was passed through a plate filter to remove the water-insoluble residue, and pressed. The soymilk was immediately cooked in a heat-exchanger for the desired time, at either 93°C. (first study) or 121°C. (second study); it was then concentrated to approximately 16% solids in a vacuum evaporator, and dried.

In a third study to evaluate the effect of spray-drying inlet temperature on nutritional quality and amino acid composition of the protein in soymilk, the samples were cooked for 10 min. at 121°C. in a heat-exchanger prior to dehydration. Additional details relating to the preparation of the various soymilk samples and measurement of nutritive value have been published previously by Hand *et al.* (3) and Hackler *et al.* (1), respectively.

Analytical Methods. Amino acid contents of the soymilk samples were determined with a Model 120 Beckman-Spinco amino acid analyzer. Samples of soymilk containing 35–40 mg. protein were placed in test tubes for hydrolysis in a 10-ml. volume of 6*N* HCl for 22 hr. Prior to hydrolysis, the tubes were evacuated and sealed in a semisolid or frozen state. After hydrolysis, the samples were filtered through prewashed Whatman filter paper, and 1 ml. of the hydrolysate was dried in a vacuum desiccator under sodium hydroxide pellets. Five milliliters of pH 2.2 sodium citrate buffer was added, and the samples were then stored at 0°C. until analyzed.

Tryptophan was determined by procedure N of the method of Spies and Chambers (4). Prior to tryptophan analysis, the samples were extracted for 20 hr. with a methanol-chloroform mixture (v./v.) to remove all interfering substances (5). Cystine was analyzed by conversion to cysteic acid as described by Moore (6). The cysteic acid was then quantitatively chromatographed on the amino acid analyzer and calculated, with aspartic acid as the reference.

The essential amino acid index (EAAI), as modified by Mitchell (7), and the requirement index (RI), as described by Rama Rao *et al.* (8), were calculated for the heat-processed soymilk samples.

Protein efficiency ratio (PER) was determined from growth studies in which the protein sample (soymilk) was fed as the sole source of protein. Diets contained 10% crude protein ($N \times 6.25$) and were fed *ad libitum* to groups of 10 rats each for 28 days. Additional details of our experimental methods in handling rats for growth studies have been described previously (9).

Results and Discussion

The results on the amino acid composition of the soymilk samples processed from 15 to 240 min. at 93°C. (Table I) show that cooking the samples for

TABLE I
EFFECT OF PROCESSING SOYMILK AT 93°C. ON AMINO ACID COMPOSITION

	Minutes				
	15	30	60	120	240
	g./16 g. N	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*
Lysine	6.20	6.25	6.20	6.20	6.01
Histidine	2.54	2.59	2.57	2.51	2.53
Arginine	7.77	7.81	7.90	7.66	7.64
Aspartic acid	11.8	11.8	12.0	11.9	11.6
Threonine	3.84	3.87	3.95	3.85	3.79
Serine	5.22	5.30	5.27	5.23	5.32
Glutamic acid	18.7	19.0	18.8	18.6	18.2
Proline	5.00	5.01	4.96	5.11	5.08
Glycine	4.23	4.25	4.28	4.24	4.16
Alanine	4.40	4.41	4.44	4.43	4.32
Valine	4.85	4.85	4.95	4.80	4.86
Cystine[a]	1.70	1.72	1.74	1.76	1.70
Methionine	1.39	1.39	1.38	1.38	1.45
Isoleucine	5.10	5.10	5.12	5.14	5.07
Leucine	8.25	8.29	8.28	8.18	8.14
Tyrosine	3.86	3.95	3.94	3.90	3.81
Phenylalanine	5.17	5.13	5.11	5.11	5.08
Tryptophan	1.33	1.44	1.47	1.40	1.32
EAAI	77.8	78.7	78.9	78.2	77.3
RI	86.6	86.7	86.9	86.7	86.7

[a]Cystine was analyzed by conversion to cysteic acid as described by Moore (6).

as long as 4 hr. had no significant effect on amino acid composition, EAAI, and RI. Figure 1 shows graphically the correlation of PER with EAAI and RI. The results indicate that once optimum nutritional quality as measured by PER is obtained, a high correlation results. This suggests that *in vitro* estimates of protein quality for foodstuffs containing antinutritional factors are of no value until these substances have been rendered inactive.

Results for the second study on the effect on amino acid composition, EAAI, and RI of heat-processing soymilk at 121°C. are shown in Table II. Cystine and tryptophan decrease as the cooking time is increased from 0 to 120 min. Cystine is the most susceptible to damage (decrease is immediate), whereas a decline in tryptophan is noted only with soymilk cooked for 60 min. or longer at 121°C. These two amino acids also account for the slight drop in the EAAI and RI (Table II). The correlation of PER, EAAI, and RI is presented graphically in Fig. 2. As mentioned previously, once

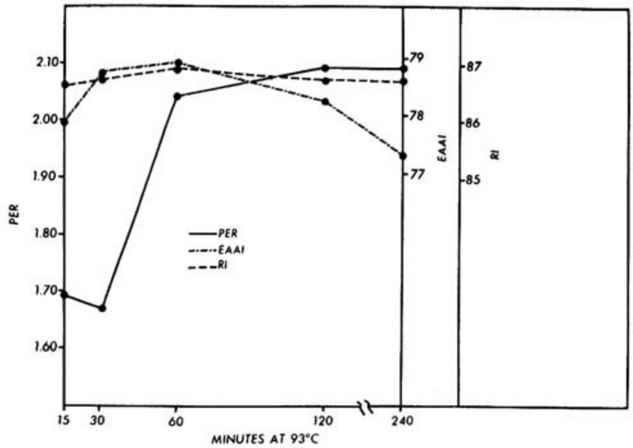

Fig. 1. Effect of heat-processing time at 93°C. on protein efficiency ratio (PER), essential amino acid index (EAAI), and requirement index (RI).

TABLE II
EFFECT OF PROCESSING SOYMILK AT 121°C. ON AMINO ACID COMPOSITION

	Minutes						
	0	5	10	20	40	60	120
	g./16 g. N	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*
Lysine	5.45	5.60	5.54	5.46	5.60	5.56	5.46
Histidine	2.44	2.45	2.44	2.40	2.47	2.37	2.40
Arginine	7.20	7.10	7.14	7.12	7.17	7.23	7.18
Aspartic acid	11.0	10.8	10.7	10.8	11.2	11.1	11.0
Threonine	3.56	3.48	3.50	3.61	3.55	3.57	3.62
Serine	4.84	4.82	4.80	4.83	4.88	4.72	4.72
Glutamic acid	16.7	16.8	17.0	17.0	17.1	16.7	16.4
Proline	4.87	4.93	5.03	5.00	4.82	4.85	4.82
Glycine	4.00	4.02	3.99	4.04	4.05	3.91	3.99
Alanine	4.21	4.15	4.19	4.25	4.29	4.16	4.26
Valine	4.67	4.60	4.74	4.74	4.74	4.67	4.60
Cystine[a]	1.70	1.57	1.62	1.62	1.44	1.28	1.17
Methionine	1.46	1.44	1.48	1.45	1.44	1.47	1.45
Isoleucine	4.87	4.86	4.88	4.85	4.89	4.84	4.89
Leucine	8.10	8.08	8.08	8.05	8.01	7.80	8.02
Tyrosine	3.85	3.89	3.88	3.84	3.86	3.86	3.88
Phenylalanine	5.22	5.10	5.24	5.22	5.16	5.06	5.23
Tryptophan	1.46	1.48	1.43	1.42	1.45	1.26	1.28
EAAI	76.9	76.1	76.5	76.3	75.3	73.5	73.5
RI	85.2	84.5	85.1	84.8	84.2	83.7	83.0

[a]Cystine was analyzed by conversion to cysteic acid as described by Moore (6).

optimum PER is obtained, EAAI and RI run essentially parallel with protein efficiency.

These results might be interpreted to mean that the drop in PER was directly related to a decrease in cystine, followed by a decrease in tryptophan,

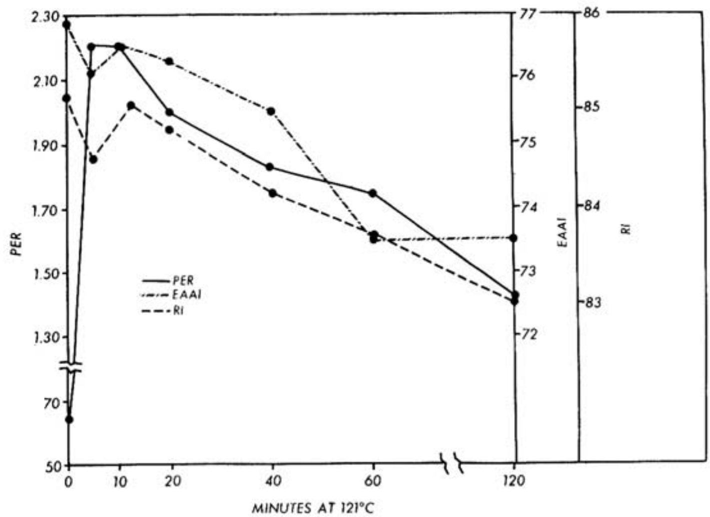

Fig. 2. Effect of heat-processing time at 121°C. on protein efficiency ratio (PER), essential amino acid index (EAAI), and requirement index (RI).

since it is generally recognized that the total sulfur amino acid content of soybeans limits the utilization of its protein.

The results in Tables I and II and Figs. 1 and 2 suggest that there is a very critical time-temperature relationship in the PER curve of soymilk processed at 121°C. It is of interest to note that the highest PER value for soymilk processed at 121°C. is somewhat greater than the best PER value found for soymilk processed at 93°C. Since the results in Tables I and II represent different preparations, it was first thought that this might account for the slight increase in PER at 121°C. However, in other research (1) from this laboratory, a single preparation of some soymilk was heat-processed at 93° and 121°C. and there was a slight increase in PER in that study also, although the increase was only 6% as opposed to 12% in the results reported here.

In an earlier report (10) it was observed that the available lysine content of soymilk samples that had been processed for 0, 5, 10, 20, 40, 60, and 120 min. at 121°C. started decreasing with the sample cooked for 40 min., whereas no drop in total lysine was found in this study. Although a high correlation was reported for available lysine and PER, it is questionable whether the available lysine decrease in the earlier report was responsible for the decrease in nutritional quality. The decrease in cystine would appear to be a better explanation for the drop in PER, since the sulfur amino acids are considered first-limiting in soybeans (11).

A third study was made to determine the effect of spray-drying temperature (inlet) on amino acid composition, PER, EAAI, and RI. Neither cystine nor methionine was altered by spray-drying; however, the results (Fig. 3) show a decrease in PER, EAAI, and RI when a drying temperature (inlet)

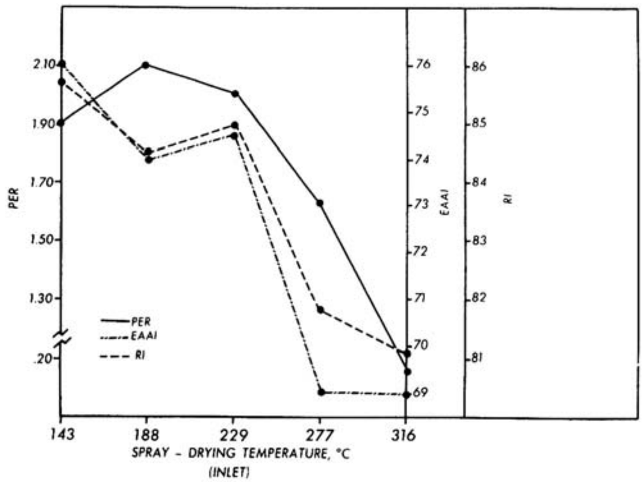

Fig. 3. Effect of spray-drying (inlet) temperature on protein efficiency ratio (PER), essential amino acid index (EAAI), and requirement index (RI).

of 277°C. or higher was used. Thus, it would appear that the sulfur amino acids are not first-limiting in the spray-dried soymilk. It is quite apparent that using an inlet temperature of 277°C. or higher causes a decrease in several amino acids: lysine, 16%; histidine, 16%; arginine, 4%; threonine, 8%; serine, 11%; proline, 8%; phenylalanine, 11%; tyrosine, 15%; and tryptophan, 4%. The preceding amino acids showed decreases of 41, 14, 20, 8, 12, 11, 6, 15, and 12% respectively at 316°C.

Of particular interest is the fact that cystine is not affected by spray-drying inlet temperature. This suggests that some other amino acid (or acids) is now first-limiting or that some other factor (or factors) is influencing the utilization of the protein; or perhaps an interaction of amino acid deficiency and some other factor or factors.

The correlation of PER, EAAI, and RI for spray-dried soymilk samples is presented in Fig. 3. Since the soymilk had been cooked for sufficient time (10 min. at 121°C.) to destroy the antinutritional constituents present in it, EAAI and RI more closely parallel PER here than they did in Figs. 1 and 2.

It should be pointed out that the soymilk samples used for the amino acid data in Tables I, II, and III represent different preparations, and this may account for the differences in amino acid composition of the underheated or properly heat-processed samples.

These investigations support the generally recognized phenomenon that *in vitro* estimates of protein quality work are valid only in well-defined systems. Our results indicate that for legumes that contain antinutritional factors that must be destroyed by heat, EAAI and RI do not provide a good estimate of protein quality. However, they both appear satisfactory once the

TABLE III
EFFECT OF SPRAY-DRYING INLET TEMPERATURE ON AMINO ACID COMPOSITION

	143°C.	188°C.	229°C.	277°C.	316°C.
	g./16 g. N	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*
Lysine	5.60	5.47	5.49	4.69	3.28
Histidine	2.38	2.35	2.37	1.99	2.04
Arginine	7.18	7.11	7.16	6.86	5.74
Aspartic acid	11.3	10.5	11.0	10.6	10.5
Threonine	3.70	3.63	3.63	3.40	3.42
Serine	4.96	4.68	4.85	4.40	4.37
Glutamic acid	17.8	16.9	17.4	16.3	16.5
Proline	4.99	4.76	4.91	4.58	4.46
Glycine	4.10	3.95	3.97	3.75	3.92
Alanine	4.24	4.11	4.13	3.83	4.05
Valine	4.78	4.53	4.55	4.37	4.47
Cystine[a]	1.68	1.74	1.70	1.72	1.65
Methionine	1.50	1.41	1.42	1.41	1.48
Isoleucine	4.88	4.71	4.80	4.53	4.59
Leucine	7.92	7.56	7.67	7.34	7.52
Tyrosine	3.92	3.74	3.65	3.32	3.32
Phenylalanine	5.00	4.77	4.78	4.45	4.69
Tryptophan	1.30	1.28	1.37	1.25	1.15
EAAI	76.1	74.0	74.5	69.1	69.0
RI	85.7	84.5	84.6	81.8	81.1

[a]Cystine was analyzed by conversion to cysteic acid as described by Moore (6).

antinutritional substances are destroyed, although it should be pointed out that the range in the calculated values is very narrow.

From the results reported here, plus earlier research from this laboratory on available lysine content of heat-processed soymilk (10), it appears that losses in amino acids are influenced by various conditions during heat-processing. The results reported here indicate essentially no change in any of the amino acids under milk heat-processing conditions (93°C.), but heat-processing soymilk at 121°C. causes a decline in cystine and tryptophan (Table II) and available lysine (10) but no change in total lysine. However, subjecting soymilk to spray-drying inlet temperatures between 143° and 316°C. caused a decline in total lysine and available lysine (10) but no change in cystine. These results appear to indicate that cystine is susceptible to destruction under high-moisture conditions, but that it is stable to dry heat.

Since dehydration is immediate in a spray-dryer, the preceding assumes that the observed changes took place after dehydration was completed. These different responses to heat for lysine and cystine are important observations, since lysine and total sulfur amino acids (methionine and cystine) are the amino acids most likely to be deficient in diets throughout the world.

It has also been reported (2) that deep-fat frying of tempeh (soybeans fermented with *Rhizopus oligosporus* Saito) in corn oil preheated to 196°C. for 1, 3, 5, and 7 min. causes a decline in lysine at 5 and 7 min. and in cystine at 7 min.

It is quite apparent from the results reported that heat-processing may alter the amino acid composition of soymilk. Additional research is needed to evaluate the effect of various heat-processing conditions on changes in amino acids and their effect on nutritive value.

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