

greatly simplifies this procedure since it is always immediately ready for use without the addition of the reducing agent. In addition, it may be shipped and stored conveniently

since refrigeration is not required. Its production on a large scale or commercial basis would not prove difficult if a sufficient need for this material should arise.

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Influence of Heating on the Liberation of Certain Amino Acids from Whole Soybeans.*

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The effect of heating on the nutritive value of soybeans has been studied by a number of investigators. A review of such studies was made recently by Evans and McGinnis.¹ They further showed that the nutritive value of soybean proteins as determined by gain in weight per gram of protein consumed by chicks was increased when the soybean oil meal was autoclaved at 100°C, 110°C or 120°C for 30 minutes but to a smaller degree when it was autoclaved at 130°C for 30 or 60 minutes. The changes in nutritive value were found to parallel the availability of the methionine and cystine of the soybean.

Clandinin *et al.*² reported that heating solvent-extracted soybean flakes in an autoclave at 15 lb pressure for 4 minutes resulted in a meal of high nutritive value for the chick; however, when the heating was prolonged to

4 hours a decrease in the nutritive value was observed. These changes were shown by Riesen *et al.*³ to be related to an increase in the amount of each of the essential amino acids liberated by pancreatic hydrolysis with the short period of heating and a decrease with the prolonged heat treatment.

More recently Klose *et al.*⁴ reported that treatment of raw soybeans with steam at 15 lb pressure gave a product of maximum nutritional value for the rat after 10 to 15 minutes while heating beyond this time resulted in a gradual decrease owing to a resultant deficiency of methionine, lysine and leucine. Steam treatment at about atmospheric pressure for 30 minutes gave a product showing a slightly higher maximum and there was little decrease in nutritive value on prolonged heating.

Soybeans have been used in the Chinese diet for centuries and one of the methods of cooking is to boil in water for 2 to 3 hours until the beans are quite soft. The present investigation was designed to determine the effect of boiling soybeans in water at different periods of time on the liberation of certain essential amino acids by acid and by enzymatic digestion.

Methods. The soybeans used were recently harvested, dried seeds of the Hawkeye

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¹ Evans, R. J., and McGinnis, J., *J. Nutrition*, 1946, **31**, 449.

² Clandinin, D. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Poultry Sci.*, 1947, **21**, 150.

³ Riesen, W. H., Clandinin, D. R., Elvehjem, C. A., and Cravens, W. W., *J. Biol. Chem.*, 1947, **167**, 143.

⁴ Klose, A. A., Hill, B., and Fevold, H. L., *Food Tech.*, 1948, **2**, 201.

TABLE I.
Effect of Time of Boiling on the α -amino Nitrogen Liberation from Soybeans.

Time of boiling hr	Time of digestion, hr	α -amino nitrogen (g %)			
		Acid digest	Pancreatic digest		
			0	6	25
	0	4.51	.42	0.68	1.00
	¼	—	.44	0.99	1.48
	1	—	.54	1.63	2.00
	2	4.56	.49	1.57	2.12
	3	4.75	.52	1.60	2.10
	4	4.70	—	—	—
	12	4.52	.49	1.51	2.01

variety.[†] They were washed free from dirt wiped free from moisture and then air dried at room temperature. Weighed samples of whole beans were soaked in water for 3 hours and then boiled in water (about 5 times the weight of the beans), under reflux for varying periods of time. The soaked and the heated soybeans with liquid were then homogenized in a Waring blender for about one-half hour at short intervals to prevent heating of the emulsion.

These emulsions were used for subsequent acid and alkaline hydrolysis by autoclaving at 15 lb with final volume at 20 ml of 3 N hydrochloric acid per 0.5 g soybean protein for 10 hours and 10 ml of 5 N sodium hydroxide per 0.5 g of soybean protein for 15 hours, respectively. Alkaline hydrolysis was carried out for the assay of tryptophan.

Enzymatic hydrolysis was carried out by shaking the emulsion with desiccated and defatted whole pancreas (VioBin 3 $\times$ USP Trypsin) in disodium phosphate buffer (pH 8.3) with toluene at 37°C for 0, 6 and 25 hours. The quantities used were 100 mg whole pancreas and 50 ml of 0.2 M Na_2HPO_4 to one gram of protein. Other details were similar to those described by Riesen *et al.*³

The liberation of α -amino nitrogen from the acid and pancreatic hydrolysates was determined by the nitrous acid micromethod of Van Slyke.

Amino acid assays were carried out according to the method of Henderson and

Snell⁵ except that in the present experiment *Leuconostoc mesenteroides* P60 was used for leucine and tryptophan while *Leuconostoc citrovorum* 8081 was used for arginine, cystine, histidine and methionine. Lilly's liver concentrate (Reticulogen 20 $\times$ USP) was added to the media for the latter organism at a level of 0.4 ml per 500 ml of medium (Steele *et al.*⁶).

Results and Discussion. Liberation of α -amino nitrogen. As shown in Table I the total α -amino nitrogen found in soybean after acid hydrolysis was unaffected by boiling for different periods of time up to 12 hours. With pancreatic digestion the amount of α -amino nitrogen liberated from raw soybean and that which had been boiled for 15 minutes was small but reached a maximum when the soybean was heated for one hour. Prolonged heating for periods of 2, 3 or 12 hours produced practically no difference in the total α -amino nitrogen liberated. These results are similar to those reported previously³ for solvent-extracted flakes except that in the previous experiment heating at the higher temperature for 4 hours resulted in a decrease in α -amino nitrogen released by pancreatin. The fact that boiling for 15 minutes brought about a smaller release of α -amino nitrogen by pancreatin than boiling for longer periods is added evidence that the trypsin inhibitor was not the sole cause of the smaller release from raw soybean. This confirms the

⁵ Henderson, L. M., and Snell, E. E., *J. Biol. Chem.*, 1948, **172**, 15.

⁶ Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., *J. Biol. Chem.*, in press.

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TABLE II.
Liberation of Amino Acids from Raw and Boiled Whole Soybeans.

Boiling time, hr	Amino acid N content of soybean (% of total N)*			% amino acids liberated by pancreatin (25 hr digestion)		
	0	3	12	0	3	12
α -amino nitrogen	67.3	71.0	67.5	22.2	44.2	44.5
Arginine	14.3	14.8	14.5	28.1	61.1	55.4
Cystine	0.50	0.58	0.57	30.2	76.6	60.6
Histidine	4.13	4.82	4.74	21.1	44.7	43.1
Leucine	4.41	4.22	3.99	30.0	45.7	50.7
Lysine	6.88	6.87	6.88	26.6	53.7	50.5
Methionine	0.60	0.62	0.63	33.5	72.3	63.9
Tryptophan	0.60	0.61	0.57	35.4	79.0	78.3
Total†	31.4	32.5	31.9	28.5	55.4	53.2

* Total nitrogen of soybean determined by Kjeldahl method was 6.69 g %.

† Amino acid N totals are sums of the percentage of the amino acids N liberated by acid or alkaline hydrolysis. Pancreatic hydrolysis totals are obtained by dividing the total amount of individual amino acids liberated enzymatically by the total liberated by acid or alkaline hydrolysis.

finding of Riesen *et al.*³ and also Westfall and Hauge⁷ although the latter workers believe that the trypsin inhibitor was the chief cause of poor utilization of the protein by the mouse.

Amino acid levels. The results of heating on the individual amino acid value of soybeans in the raw state and after boiling for 3 and 12 hours are shown in Table II. It will be noted that after acid hydrolysis the amino acid values for raw soybeans are essentially the same as those boiled for 3 or 12 hours. Riesen *et al.*³ who used prolonged heating at 15 lb pressure found a decrease in the liberation of lysine, arginine and tryptophan. The difference may be due to the fact that the temperature in the experiment of Riesen *et al.* was much higher than that of the present experiment.

Recently Patton *et al.*⁸ reported that boiling of soy globulin in 5% glucose with refluxing for 24 hours caused a considerable loss in 4 out of 10 essential amino acids determined, namely lysine, arginine, tryptophan and histidine, while boiling in water resulted in no change in these amino acids. Our results with whole soybean were similar to theirs with boiling in water and would indicate that the sugars and other carbohydrates in soybeans do not play a part in the loss of amino

acids during boiling, although browning did occur in samples boiled longer than 3 hours. It appears that the loss of certain amino acids during boiling in the presence of glucose reported by Patton *et al.*⁸ does not have a counterpart in the whole soybean moiety.

Block *et al.*⁹ showed that baking of a cake mix of flour, sugar, egg white, lactalbumin, hydrogenated vegetable oil, dried yeast, molasses and salt with 25% of its calories as protein caused a decrease in the protein efficiency. The decrease they believe was possibly due to a partial destruction of lysine which occurred during the baking, or to a formation of a new peptide linkage by a reaction of the free carboxyl groups of the dicarboxylic amino acids with the α -amino groups of lysine, the new peptide linkage being resistant to enzymatic digestion but not to acid hydrolysis. Since during baking the temperature on the surface of the cake may be considerably higher than 100°C, a partial destruction of lysine may occur similarly to that shown by Riesen *et al.*³ On the other hand the molasses may furnish a considerable amount of glucose to cause destruction of lysine as reported by Patton *et al.*⁸

Pancreatic digestion. The effect of boiling the soybean on the liberation of amino acids

⁷ Westfall, R. J., and Hauge, S. M., *J. Nutrition*, 1948, **35**, 379.

⁸ Patton, A. R., Hill, E. G., and Foreman, E. M., *Science*, 1948, **108**, 659.

⁹ Block, R. J., Cannon, P. R., Wissler, R. W., Steffee, C. H., Jr., Straube, R. L., Frazier, L. E., and Woolridge, R. L., *Arch. Biochem.*, 1946, **10**, 295.

was quite evident after 25 hours of pancreatic digestion (Table II). The total α -amino nitrogen released from the sample boiled for 3 hours was double that of the raw sample. Prolonged boiling for 12 hours resulted in no further change. Similar release occurred with each of the 7 amino acids studied. Since previous work indicated that the 7 amino acids tested in this study might be susceptible to damage by heat and in view of the present findings no further study was made of the remaining essential amino acids.

The results of the present study do not support the view that methionine is released at a greater rate than leucine and lysine after heating (Melnick *et al.*¹⁰). It was shown here as well as in a previous report³ that heat increases the release of all amino acids from soybean protein by pancreatin to a similar extent. It would appear that the sulfur amino acid deficiencies in raw soybean previously reported by Hayward *et al.*¹¹ are due to a limited content *per se* of these amino acids in the soybean protein. The low quantities of cystine, methionine and tryptophan in both raw and heated soybean are evident from Table II. Supplementation of the protein with cystine and/or methionine should increase the nutritional value of both raw and heated soybean. Furthermore since all amino acids are less available in raw soybean the critical nature of the sulfur amino acid deficiencies in this protein is even more striking than in the heated soybean.

It is of interest to point out that the percentage increase of total amino acids released by pancreatin over the percentage of

α -amino nitrogen is considerably smaller than that reported previously.³ This may be explained by the fact that the organisms used in the present experiment did not utilize peptides to such an extent as those organisms employed in earlier experiments.

The results of our study show that the method of cooking soybean used in China does increase the nutritive value of soybean by allowing a greater enzymatic release of essential amino acids and that there is no destruction on prolonged boiling up to 12 hours. In view of this and the fact that cooking at higher temperature (autoclaving at 15 lb) results in a deleterious effect on certain amino acids reported previously,³ cooking of soybean in a pressure cooker should not be introduced until future studies have proven that under the ordinary cooking condition with a pressure cooker no harmful result is obtained.

Summary. A study was made on the effect of boiling on the liberation of amino acids by acid and enzymatic hydrolysis using microbiological assay methods.

Boiling up to 12 hours did not affect the release by acid hydrolysis of α -amino nitrogen and of 7 essential amino acids studied.

Boiling for 15 minutes caused a slight increase in the release of α -amino nitrogen and amino acids by pancreatic digestion but boiling for 1 hour and longer up to 12 hours resulted in a marked increase. The increase in the enzymatic liberation of amino acids for periods of over one hour was about twice that liberated from raw soybean. Prolonged boiling up to 12 hours with definite browning did not result in a decrease in enzymatic liberation of amino acids from the "overcooked" soybean.

¹⁰ Melnick, D., Oser, B. L., and Weiss, S., *Science*, 1946, **103**, 326.

¹¹ Hayward, J. W., Steenbock, H., and Bohstedt, G., *J. Nutrition*, 1936, **11**, 219.