Studies on Amino Acid Contents of Processed Soybean

Part XI. Evaluation of Nutritional Losses of Overheated Defatted Soybean Flour

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Effect of heating on the nutritive value of defatted soybean flour has been investigated by animal experiments. Loss due to heat degradation was evaluated in two ways. In the first method, the amino acids lost during overheating were supplemented by cystine and mixture of lysine, arginine, tryptophan, and serine at dietary levels of 1.6% nitrogen, and cystine and mixture of those amino acids plus histidine at dietary levels of 3.2% nitrogen. The other procedure adopted was the absorbent test used with amino acid mixtures based on the pattern of amino acids released by pancreatic hydrolysis of unheated, properly heated, and overheated defatted soybean flour at 6 and 120 hr hydrolysis.

At 1.6% dietary nitrogen level, the nutritive value of overheated soybean flour increased by supplementation with cystine and amino acid mixture, but at the 3.2% nitrogen level only cystine was effective. Supplementation of lost amino acids to overheated flour did not restore the nutritive value to that of the properly heated flour. Based on the amino acids released by pancreatic hydrolysis of unheated, properly heated, and overheated soybean flour after 6 and 120 hr reaction, amino acid mixtures were prepared and tested for their nutritive value. While the nutritive value of amino acid mixture prepared based on the pattern of amino acid liberated by 6 hr digestion of unheated, properly heated, and overheated flour did not show similar trend to that of 3 kinds of flour itself, the nutritive value of the amino acid mixture prepared after the data obtained by 120 hr digestion agreed well with the trend of unheated or heated soybean flour.

The nutritive value was also measured by the nitrogen balance of test animals.

Osborne and Mendel¹⁾ have reported that nutritive value of soybeans improved by mild heating. Improvement in the nutritive quality is partly due to the destruction of trypsin inhibitor(s)^{2~4)} and also to the denaturation

of protein by increasing the digestibility and making sulphur containing amino acids available.^{3,5)} Overheating of soybean flour, on the other hand, not only destroys amino acids^{6,7)} but also decreases the biological

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value.^{8~12)} The loss in nutritive value of soybean flour can be restored by supplementation of diets with lysine and methionine,^{9~11)} or lysine, methionine and cystine.¹²⁾ Iriarte and Barnes¹³⁾ observed that addition of cystine to overheated soybean products improved the nutritive value, but not to its optimum level. It is well known that nutritive value of protein depends not only the pattern of its amino acid composition but also its physiological availability.

Of the several characteristics which determine the nutritional quality of processed protein foods, their digestibility is of paramount importance. For evaluating the nutritional value, the most common procedure is *in vitro* tests. It has been reported that correlation exists between the nutritive value and the index of enzymatic hydrolysis.^{14~19)} Nutritive evaluation of heat processed protein foods by enzymatic digestion procedure is necessary because the amino acids present in the protein may only be partially available or completely unavailable.

The present studies were undertaken to determine the effect of heating on the nutritive value of defatted soybean flour. The experiment was designed with the twin objective of finding out the nutritive value of

overheated soybean flour after supplementing the amino acids lost during heating, and of amino acid mixtures based on the amino acids released by enzymatic digestion.

EXPERIMENTAL

Preparation of diets

A. Heat treatment of soybean flour and amino acid supplementation

a. *Heat treatment of soybean flour.* Defatted soybean flakes were obtained from Ajinomoto Co. Inc. It was prepared by extracting the oil from the raw soybean flakes with *n*-hexane by Lurgi-type extractor. The flakes were ground to pass through a 40-mesh standard screen.

Heat treatment was carried out by autoclaving the defatted soybean flour at 0.35 kg/cm² (108°C) for 1 hr (properly heated)⁶⁾ and at 1.4 kg/cm² (126°C) for 4 hr

TABLE I. DESTRUCTION OF AMINO ACIDS IN DEFATTED SOYBEAN FLOUR SUBJECTED TO DIFFERENT HEAT

Heat treatment	TREATMENTS		(g/16 gN)	
	Un-heated	Properly heated	Overheated	
	Temp. (°C) Time (hr)	(108°C) 1	(126°C) 4	
Amino acids		(a)	(a')	(b) (b')
Glycines	4.3	4.2	4.3	4.4 4.3
Alanine	4.5	4.5	4.5	4.4 4.3
Valine	5.4	5.4	5.3	5.4 5.3
Isoleucine	4.8	4.8	4.8	4.9 4.8
Leucine	8.3	8.2	8.3	8.6 8.4
Aspartic acid	11.9	11.9	11.9	11.9 11.9
Glutamic acid	20.8	20.8	20.6	21.1 21.0
Lysine	7.0	7.0	7.0	5.2 5.6
Arginine	6.5	6.5	6.5	5.2 5.9
Histidine	2.5	2.5	2.5	2.5 2.3
Phenylalanine	4.7	4.7	4.7	4.7 4.7
Tyrosine	3.2	3.2	3.2	3.2 3.2
Proline	5.4	5.5	5.5	5.4 5.4
Tryptophan	1.40	1.40	1.40	1.27 1.12
Methionine	0.95	0.97	0.95	0.94 0.95
Cystine	1.20	1.20	1.20	0.71 0.65
Serine	6.4	6.4	6.4	6.0 6.3
Threonine	4.6	4.6	4.6	4.7 4.6
Total	103.85	103.77	103.65	100.52 100.72

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(overheated)⁶⁾ in the presence of an equal weight of water.⁷⁾ Each sample was lyophilized and ground to pass through a 40-mesh standard screen.

Amino acid composition of the samples was determined by microbiological assay⁶⁾ after acid or alkaline hydrolysis. Amino acid values of unheated, properly heated, and overheated soybean flours are expressed as g per 16 g of total nitrogen of each sample and the data are presented in Table I.

b. Amino acid supplementation. Overheating destroyed cystine, lysine, arginine, tryptophan, and serine (sample b), and also histidine in sample b' of treated soybean flour. Supplementation test with overheated defatted soybean flour was carried out by the addition of amino acids at levels comparable to those destroyed during overheating. The composition of diets is presented in Table II. Heated soybean flour and supplemented diet with amino acid were tested at dietary nitrogen levels of 1.6 and 3.2%.²⁰⁾

B. The diets containing amino acid mixture in accordance with the released amino acid patterns of pancreatic hydrolysates

a. Digestion with pancreatin. The procedure de-

scribed by Melnick¹⁴⁾ and Hoshino *et al.*²¹⁾ was followed in the preparation of samples. The raw and heated defatted soybean flour containing 6 g protein was suspended in 50~60 ml of distilled water and 30 ml of phosphate buffer solution (750 ml of M/5 KH_2PO_4 plus 144 ml of 1 M NaOH, diluted to 1000 ml) of pH 8.4 was added and the volume was made up to 130 ml with distilled water. Then 20 ml of the same buffer containing 200 mg of pancreatin (Hishiyama Seiyaku Co. Inc.) was added, and the reaction mixture was preserved under 10 ml of toluene. The mixture was incubated at 37°C, and the samples were drawn at intervals of 0, 6, 18, 24, 48, and 120 hr to find out the effect of time on enzyme treatment. Soon after drawing the samples, the digests were heated in a boiling water bath for 10 min, cooled, and filtered. The filtrates were adjusted to pH 6.8, diluted to the analytical range of amino acid concentration and frozen until analyzed.

The hydrolysates were assayed by microbiological method described previously.²²⁾ The free amino acid value means the free amino acid available to the test strains of lactic acid bacteria. The data are presented in Table III.

TABLE II. COMPOSITION OF DIETS FOR DIFFERENT GROUPS PREPARED FROM
HEAT TREATED SOYBEAN FLOUR

(%)

Components	Groups	A	B	C	D	E	F	G	H	I
Properly heated soybean flour		20.4 ¹⁾				44.7 ⁵⁾				
Overheated soybean flour			20.3 ²⁾	20.3 ²⁾	20.3 ²⁾		44.3 ⁶⁾	44.3 ⁶⁾	44.3 ⁶⁾	
Supplemental amino acids				0.428 ³⁾	0.473 ⁴⁾			0.74 ⁷⁾	0.85 ⁸⁾	
Salts ⁹⁾		4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Water-soluble vitamins ⁹⁾		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fat-soluble vitamins in soybean oil		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Starch (Potato starch)		69.6	69.7	69.3	69.2	45.3	45.7	45.0	44.8	90.0
Total		100	100	100	100	100	100	100	100	100
Diet N		1.60	1.60	1.68	1.69	3.20	3.20	3.37	3.38	0

1) Sample a in Table I (N: 7.85%).

2) Sample b in Table I (N: 7.88%).

3) 2) plus 221 mg L-Lys·HCl, 166 mg L-Arg·HCl, 10 mg L-Try and 31 mg L-Ser.

4) 3) plus 45 mg L-Cys.

5) Sample a' in Table I (N: 7.10%).

6) Sample b' in Table I (N: 7.22%).

7) 6) plus 350 mg L-Lys·HCl, 150 mg L-Arg·HCl, 83 mg L-His·HCl, 106 mg DL-Try and 49 mg L-Ser.

8) 7) plus 110 mg L-Cys.

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TABLE III. THE EFFECT OF HEATING ON THE LIBERATION OF AMINO

Heat treatment Digestion period (hr)	Unheated							
	0	6	18	24	48	120	0	6
Amino acids								
Glycine	0.05	0.06	0.11	0.14	0.14	0.25	0.05	0.08
Alanine	0.02	0.43	0.78	0.72	0.74	0.70	0.06	0.32
Valine	0.07	0.19	0.31	0.37	0.50	0.85	0.08	0.17
Isoleucine	0.05	0.14	0.29	0.37	0.41	0.73	0.05	0.17
Leucine	0.06	0.18	0.30	0.39	0.66	0.92	0.10	0.32
Aspartic acid	0.12	0.28	0.36	0.44	0.45	0.53	0.14	0.24
Glutamic acid	0.20	0.25	0.34	0.38	0.75	0.82	0.18	0.33
Lysine	0.09	0.12	0.30	0.46	0.56	0.76	0.15	0.32
Arginine	0.14	0.24	0.36	0.34	0.40	0.49	0.21	0.46
Histidine	0.08	0.15	0.26	0.34	0.37	0.67	0.10	0.18
Phenylalanine	0.10	0.21	0.29	0.40	0.82	1.14	0.12	0.39
Tyrosine	0.07	0.56	0.71	0.74	0.81	1.18	0.08	0.49
Proline	0.06	0.08	0.13	0.18	0.31	0.37	0.06	0.10
Tryptophan	0.03	0.31	0.44	0.48	0.39	0.44	0.06	0.22
Methionine	0.02	0.04	0.08	0.12	0.15	0.19	0.02	0.05
Cystine	0.0010	0.0037	0.0038	0.0048	0.0072	0.0145	0.0011	0.0023
Serine	0.07	0.14	0.21	0.29	0.32	0.53	0.12	0.26
Threonine	0.08	0.55	1.32	1.55	1.63	2.07	0.15	0.87
Total	1.3110	3.9337	6.5938	7.7148	9.4172	12.6545	1.7311	4.9723

TABLE IV. AMINO ACID COMPOSITION OF DIFFERENT DIETS COMPOUNDED BY USING AMINO ACID MIXTURES*

Groups	Amino acids					
	D'	E'	F'	G'	H'	I'
Amino acids						
Glycine	1.53	1.60	2.25	1.98	3.22	3.01
L-Alanine	10.93	6.44	2.91	5.53	3.71	4.03
L-Valine	4.83	3.42	3.58	6.72	5.52	5.75
L-Isoleucine	3.56	3.42	2.25	5.77	4.89	4.29
L-Leucine	4.58	6.44	8.98	7.27	8.93	10.37
L-Aspartic acid	7.11	4.83	6.07	4.19	4.74	3.96
L-Glutamic acid	6.36	6.64	5.41	6.48	4.82	6.82
L-Lysine**	3.05	6.44	7.40	6.01	6.97	6.34
L-Arginine**	6.10	9.25	15.72	3.87	11.49	11.91
L-Histidine**	3.81	3.62	3.41	5.29	4.11	3.19
L-Phenylalanine	5.34	7.84	9.56	9.01	8.23	8.39
L-Tyrosine	14.24	9.85	6.32	9.32	7.75	7.33
L-Proline	2.03	2.01	1.91	2.92	3.52	3.30
L-Tryptophan	7.88	4.42	3.58	3.48	3.56	3.04
L-Methionine	1.02	1.01	1.08	1.50	1.56	1.58
L-Cystine	0.09	0.05	0.03	0.11	0.18	0.10
L-Serine	3.56	5.23	5.82	4.19	7.08	8.10
L-Threonine	13.98	17.50	13.72	16.36	9.72	8.50
Total	100	100	100	100	100	100

* Composition was based on the pattern of amino acid released by enzymatic hydrolysis of unheated, properly heated, and overheated soybean flour at 6 and 120 hr digestion.

D'=6 hr digestion of unheated flour; E'=6 hr digestion of properly heated flour; F'=6 hr digestion of overheated flour; G'=120 hr digestion of unheated flour; H'=120 hr digestion of properly heated flour; I'=120 hr digestion of overheated flour.

** Listed here as the free base but added to the diets as the hydrochlorides.

ACIDS FROM SOYBEAN FLOUR BY THE DIGESTION OF PANCREATIN

(g/16gN)

Properly heated				Overheated					
18	24	48	120	0	6	18	24	48	120
0.20	0.33	0.55	0.87	0.10	0.27	0.49	0.51	0.64	0.82
0.58	0.96	0.98	1.00	0.11	0.35	0.58	0.75	0.76	1.10
0.50	0.61	0.99	1.49	0.13	0.43	0.88	0.98	1.30	1.57
0.37	0.62	0.99	1.32	0.07	0.27	0.62	0.67	0.92	1.17
0.83	1.06	1.58	2.41	0.20	1.08	2.26	2.35	2.69	2.83
0.74	1.09	1.20	1.28	0.25	0.73	0.84	0.99	1.10	1.08
0.62	0.86	1.00	1.30	0.24	0.65	1.05	1.32	1.59	1.86
0.81	1.05	1.66	1.88	0.16	0.89	0.97	1.20	1.36	1.73
1.22	1.80	2.69	3.10	0.67	1.89	2.31	2.79	3.35	3.25
0.44	0.60	0.78	1.11	0.15	0.41	0.66	0.69	0.81	0.87
0.82	1.17	1.48	2.22	0.33	1.15	1.36	1.72	1.92	2.29
0.63	1.15	1.40	2.09	0.16	0.76	1.27	1.41	1.91	2.00
0.24	0.49	0.61	0.95	0.10	0.23	0.47	0.58	0.75	0.90
0.40	0.60	0.74	0.96	0.04	0.43	0.71	0.67	0.81	0.83
0.13	0.20	0.26	0.42	0.03	0.13	0.27	0.30	0.37	0.43
0.0053	0.0083	0.0161	0.0476	0.0006	0.0045	0.0095	0.0096	0.0151	0.0265
0.60	1.11	1.35	1.91	0.23	0.70	1.27	1.88	1.69	2.21
1.63	1.86	2.22	2.62	0.37	1.65	1.70	1.86	2.12	2.32
10.7653	15.5683	20.4961	26.9776	3.3406	12.0245	17.7195	20.6796	24.1051	27.2865

TABLE V. COMPOSITION OF DIETS USED IN THE ANIMAL EXPERIMENTS

(%)

Components	Groups									
	A'	B'	C'	D'	E'	F'	G'	H'	I'	J'
Unheated soybean flour (N: 6.65%)	36.10									
Properly heated soybean flour (N: 7.49%)	32.05									
Overheated soybean flour (N: 7.62%)			31.50							
Amino acid mixture*										
D' (N: 12.67%)				18.96						
E' (N: 13.18%)					18.21					
F' (N: 14.21%)						16.89				
G' (N: 12.13%)							19.80			
H' (N: 13.93%)								17.23		
I' (N: 13.61%)									17.64	
Casein (N: 13.37%)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	0
Salts**	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Water-soluble vitamins**	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fat-soluble vitamins in soybean oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Starch (Potato starch)	47.90	51.95	52.50	65.04	65.79	67.11	64.20	66.77	66.36	90.00
Total	100	100	100	100	100	100	100	100	100	100
Diet N	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20	0

* See the Table IV.

** A. E. Harper, *J. Nutr.*, **68**, 405 (1959).

b. Preparation of amino acid mixture. Casein plus soybean flour of different heat treatment or casein plus amino acid mixture was included in diets at the level of 3.2% nitrogen. In all cases, casein was used at a level of 0.80% nitrogen. The amino acid mixtures were prepared, based on the pattern of amino acids released during the enzymatic hydrolysis of unheated, properly heated, and overheated soybean flour after 6 and 120 hr of digestion (Table IV). All amino acids used were L-isomers.

Animal experiments

Male weanling rats of the Wister strain weighing about 50~60 g were used in this study. The rats were maintained on a stock diet for one week, divided into groups of 6 animals each and housed in individual wire-bottom cages. Only 4 animals were used in the group A to D of the experiments (Table II). Food and water were given *ad libitum* for three weeks and the animals were weighed weekly. I and J' groups of rats were fed a protein-free diet, and the figures for nitrogen retention and biological value have been corrected for metabolic and endogenous losses.

Absorbability measurement

Rats belonging to groups E-I (Table II) and A'-J' (Table V) were fed diets of 3.2% nitrogen levels for 3 weeks. Food consumption was recorded daily, and feces and urine were collected for 3 days during the last week. Nitrogen quality and nitrogen balance were calculated by determining nitrogen in feed, feces, and urine by the macro-Kjeldahl method.

RESULTS AND DISCUSSION

Effect of amino acid supplementation to overheated defatted soybean flour

Amino acids lost during heating were supplemented and nitrogen quality and nitrogen balance of overheated flour were determined. Nutritive value of properly heated flour was also found out and the relevant data are presented in Table VI.

a. Nitrogen quality When the dietary nitrogen level was 1.6%, nitrogen efficiency ratio (NER) of properly heated flour (group A) and overheated flour (group B) was 9.54 and 0.83, respectively. Results of group C indicates

that NER of group B could be increased by the addition of mixture of amino acids lost during heating, *i.e.* lysine, arginine, tryptophan, and serine, even without cystine. Even though the NER of Group D diet (group C diet plus cystine) increased, it did not reach the level of group A. In the same experiment when the level of nitrogen was increased to 3.2% in the diets, NERs of properly heated flour (group E) and overheated flour (group F) were 8.74 and 4.85, respectively. Reduced NER of the overheated flour (diet of group F) did not increase much when supplemented with mixture of lysine, arginine, histidine, tryptophan, and serine (group G). In the group H (group G diet plus cystine), the NER increased, but did not reach that of properly heated flour. From these results on rat growth, it is obvious that supplemental addition of cystine and amino acid mixture lost during heating were found to be effective at 1.6% nitrogen levels, but those amino acids mixture plus histidine, except cystine, had no beneficial effect at levels of 3.2% nitrogen in the diet.

It has been recognized that excessive heating damages the nutritive value of soybean, and that this loss is restored by supplementation of the diet with lysine and methionine⁹⁻¹¹⁾ or lysine, methionine, and cystine.¹²⁾ At levels of 10% dietary protein (1.6% nitrogen levels), Iriarte *et al.*¹³⁾ reported that cystine supplementation to overheated soybean products promoted growth of rats very much, but addition of lysine, threonine, histidine, and tryptophan either with methionine or with cystine, did not improve nutritive quality above that obtained with methionine or cystine alone. The results presented in this paper show that addition of mixture of lysine, arginine, tryptophan, and serine did improve the nutritive quality of overheated soybean flour. This may be due to the effect of additional arginine and serine, which were not used in the Iriarte's experiment, or to supplementation of lysine, threonine, histidine,

TABLE VI. EFFECT OF AMINO ACID SUPPLEMENTATION UPON THE NITROGEN QUALITY AND NITROGEN BALANCE OF UNHEATED AND HEATED SOYBEAN FLOUR DIET

Groups	Nitrogen in diet	Nitrogen quality					Nitrogen balance					
		Number of rats	Weight gain (a)	Nitrogen intake (b)	Nitrogen efficiency ratio (NER) $\left(\frac{a-a'}{b}\right)$	Nitrogen retention $\left(\frac{a-a'}{b}\right)$	Intaken food nitrogen mg/100 g Body weight	Fecal nitrogen mg/100 g Body weight	Absorbed food nitrogen (c) mg/100 g Body weight	Urinary nitrogen mg/100 g Body weight	Retained food nitrogen (d) mg/100 g Body weight	Biological value $\left(\frac{d}{c} \times 100\right)$
%			g	g								
A Diet*	1.60	4 (0)**	52.5 ± 8.35***	5.49	9.54 ± 1.12***							
B Diet	1.60	4 (0)	4.0 ± 8.76	4.24	0.83 ± 1.99							
C Diet	1.60	4 (0)	10.8 ± 3.10	4.29	2.48 ± 0.68							
D Diet	1.60	4 (0)	16.5 ± 5.26	5.71	2.95 ± 1.13							
E Diet	3.20	6 (0)	106.2 ± 15.4	12.1	8.74 ± 1.06	10.5	1090	211	927	403	562	60.6
F Diet	3.20	6 (0)	46.8 ± 12.3	9.6	4.85 ± 0.92	7.01	1510	403	1160	242	956	82.4
G Diet	3.20	6 (0)	47.8 ± 7.0	10.1	4.71 ± 0.40	6.76	1610	381	1280	331	987	77.1
H Diet	3.20	6 (0)	70.7 ± 23.2	11.5	6.15 ± 1.04	7.93	1360	309	1100	249	889	80.8
I Diet	3.20	6 (0)	-20.5 ± 5.1a'	0			48.4			37.9		

* The composition of diets A-I was shown in Table II.

** Figures in () show the number of rats died during the experiment.

*** Standard error of mean.

and tryptophan, individually, along with large quantity of cystine (0.3%) used in the diets of their experiment. It is considered that the quantity of added cystine (0.3%) was far more than that destroyed during overheating of soybean flour. Supplementation of large quantity of cystine, the principal limiting amino acid (sulphur containing amino acid) in soybean, led to over-grow of the rat owing to the restoration of the decreased nutritive value. Consequently, the supplemental effect of amino acids except cystine could not be detected in the Iriarte's work. On the other hand, in our results, the additional effect of mixture of lysine, arginine, histidine, tryptophan, and serine on NER was not found in the dietary nitrogen levels of 3.2%, and the NER showed the same ratio as that of overheated soybean flour, but conspicuous increasing of NER was found on the cystine supplemented diet. It is evident that the supplementation of lost amount of lysine, arginine, histidine, tryptophan, and serine except cystine was not necessary for rat to get further growth on the overheated soybean flour at the nitrogen levels of 3.2%.

b. Nitrogen balance. The nitrogen balance was evaluated from the difference of nitrogen intake in food and that excreted in feces and urine, when the dietary nitrogen level was 3.2%. The excretory nitrogen increased considerably in the feces when overheated flour was used in the diet, but when the lost amino acids were supplemented, the fecal excretion decreased gradually. On the other hand, urinary excretion did not show any tendency for correction even when the lost amino acids in the overheated flour were supplemented in the diet. The increased fecal nitrogen excretion indicates that the overheated flour decreased absorption and lowered the digestibility and that this can be corrected by supplementing the diet with the lost amino acids. Continued lower values for urinary nitrogen under further supplementation of amino acids may

be due to endogenous factors. Same phenomenon was also observed in the biological value. Therefore, evaluation of nutritive value of heated samples can not be based on biological value. It can be concluded that the disagreement of tendency between biological value and rat growth *i.e.* NER is due to the variation in the digestive release of amino acids and absorption which is related to the quantity and quality of dietary protein.

The influence of feeding of the heated soybean flour on rat growth tested by incorporating the amino acid mixture similar to those released by enzymatic digestion of heated and unheated soybean flour

The results of determination of nitrogen quality and nitrogen balance are given in Table VII.

a. Nitrogen quality. Weight gain in rats of group A' fed with unheated soybean flour and of group C' fed with overheated flour was lower than those in group B' fed with properly heated flour. The same trend was observed in group G', H', and I' where the diets were prepared from amino acid mixtures based on the amino acids released during 120 hr enzymatic digest of unheated, properly heated, and overheated soybean flour, but the growth was lower when compared with those of A', B', and C'. The data obtained in the case of diets D', E', and F' (the amino acid patterns of 6 hr enzymatic digestion period) were different and above-mentioned phenomena, which were observed in the group G', H', and I', were not found.

The nutritive value of protein must obviously depend firstly on its digestibility. In evaluating protein quality of foods and feeds, the *in vitro* test is based on the pattern of amino acids released during enzymatic digestion of the protein. Numerous methods are available to carry out the *in vitro* enzymatic digestion. Akesson and Stahmann¹⁹⁾ devised a pepsin-pancreatin digest index based on the

TABLE VII. THE NUTRITIVE VALUE OF DEFATTED SOYBEAN FLOUR AND AMINO ACID MIXTURE

Groups	Nitrogen in diet	Number of rats	Nitrogen quality				Nitrogen balance					Biological value $\left(\frac{d}{c} \times 100\right)$
			Weight gain (a)	Nitrogen intake (b)	Nitrogen efficiency ratio (NER) $\left(\frac{a-a'}{b}\right)$	Nitrogen retention $\left(\frac{a-a'}{b}\right)$	Intaken food nitrogen mg/100 g Body weight	Fecal nitrogen mg/100 g Body weight	Absorbed food nitrogen (c) mg/100 g Body weight	Urinary nitrogen mg/100 g Body weight	Retained food nitrogen (d) mg/100 g Body weight	
A' Diet*	3.20	6 (0)**	60.6 ± 10.7***	12.0	5.05 ± 1.13***	6.09	1300	270	1070	327	791	73.9
B' Diet	3.20	6 (0)	93.3 ± 8.95	11.8	7.91 ± 1.20	8.97	1110	204	947	309	686	72.4
C' Diet	3.20	6 (0)	55.1 ± 20.9	10.1	5.46 ± 1.03	6.69	1140	381	800	219	629	78.6
D' Diet	3.20	6 (1)	52.6 ± 8.34	9.44	5.57 ± 1.55	6.90	1420	79.2	1380	614	814	59.0
E' Diet	3.20	6 (1)	45.8 ± 16.8	9.39	4.88 ± 1.23	6.21	1420	58.8	1400	512	936	66.9
F' Diet	3.20	6 (3)	45.6 ± 7.97	10.1	4.51 ± 0.35	5.75	1240	84.8	1200	507	741	61.8
G' Diet	3.20	6 (5)	56.8	9.12	6.23	7.60	1350	81.4	1310	623	735	56.1
H' Diet	3.20	6 (1)	71.4 ± 8.95	10.8	6.61 ± 1.10	7.77	1310	84.4	1270	640	678	53.4
I' Diet	3.20	6 (1)	62.9 ± 10.1	11.8	5.33 ± 1.03	6.39	1390	74.2	1360	482	926	68.1
J' Diet	3.20	6 (0)	-12.5 ± 4.33 ^{a'}	0				40.7		47.5		

* The composition of diets A'-J' was shown in Table V.

** Figures in () show the number of rats died during the experiment.

*** Standard error of mean.

TABLE VIII. SELECTED COMPARISON OF THE DATA FROM TABLE III EXPRESSED AS PERCENTAGE CHANGES IN AMINO ACID LIBERATION BY ENZYMATIC DIGESTION (%)

Digestion period (hr)		The effect of heat treatment											
		$\frac{B-A^{1)}}{A} \times 100$						$\frac{C-B^{2)}}{B} \times 100$					
Amino acids		0	6	18	24	48	120	0	6	18	24	48	120
	Glycine	0	33	82	136	293	248	100	239	145	55	16	6
	Alanine	200	-26	-26	33	32	43	83	9	0	-22	-22	10
	Valine	14	-11	61	65	98	75	63	153	76	61	31	5
	Isoleucine	0	21	28	65	141	78	40	59	68	8	-7	-11
	Leucine	67	78	177	172	139	162	100	238	172	122	70	17
	Aspartic acid	17	-14	106	148	167	142	79	204	14	-9	-8	-16
	Glutamic acid	-10	32	82	126	33	59	33	97	69	53	59	43
	Lysine	67	167	170	128	196	147	7	178	20	14	-18	-8
	Arginine	50	83	239	429	573	533	219	311	89	55	25	5
	Histidine	25	20	69	76	111	66	50	128	50	15	4	-22
	Phenylalanine	20	86	183	193	80	95	175	195	68	47	30	3
	Tyrosine	14	-13	-11	55	73	75	100	55	102	23	36	-4
	Proline	0	25	85	172	97	157	67	130	96	18	23	-5
	Tryptophan	100	-29	-9	25	90	118	-33	95	77	12	9	-14
	Methionine	0	25	63	67	73	121	50	150	108	50	42	2
	Cystine	10	-38	39	73	124	228	-45	96	79	16	6	-44
	Serine	71	86	186	283	322	260	92	169	112	69	25	16
	Threonine	88	58	23	20	36	27	147	90	4	0	-5	-11

1) Properly heated (108°C, 1 hr) defatted soybean flour (B) vs. unheated defatted soybean flour (A).

2) Overheated (126°C, 4 hr) defatted soybean flour (C) vs. properly heated (108°C, 1 hr) defatted soybean flour (B).

amino acids released during *in vitro* digestion estimated by chromatographic procedure. This index showed better correlation with the biological value for rat growth than that with the essential amino acid index which gives higher values or with the chemical score where the values are lower. Some authors^{3,14,16,18,23)} prefer enzymatic hydrolysis with pancreatin only. Melnick and Oser¹⁴⁾ have reported that in evaluating nutritive value of products improved or impaired by heat-processing and exhibiting no change in amino acid composition or degree of digestibility, the rate of enzymatic digestion is critical. They are also of the view that for optimal utilization of food proteins, all essential amino acids must

not only be available for absorption but also must be liberated during *in vitro* digestion at rates permitting mutual supplementation. In this experiment, the proportions of amino acid mixtures were based upon the released amino acid pattern. As compared properly heated flour with unheated flour, or overheated flour with properly heated flour in liberated amino acid percentage, it was observed that the extent of the release of each of various amino acids by enzymes increased by proper heating, but decreased by prolonged heat treatment of the protein, and the rate of release of the amino acid varied widely (Table VIII).

It was also observed that pattern of released amino acids differed during different stages of digestion (Table VIII). Between 6 and 18 hr reaction period, digestibility of overheated soybean flour was higher than that of the

23) E. Menden and H.D. Cremer, *Nutr. Dieta.*, 8, 188 (1966).

properly heated flour. Moreover, the pattern of released amino acids also varied in the two treatments. There was variation in glycine, valine, arginine, histidine, phenylalanine, proline, methionine, and serine contents of the hydrolysates obtained after 6 hr digestion whereas the 120 hr hydrolysate differed in leucine, aspartic acid, glutamic acid, histidine, tryptophan, cystine, and serine contents. It is clear that heating causes denaturation of protein and that this has a profound influence on the biological value. The results obtained in these experiments clearly show that there is agreement between the growth of rats fed on unheated soybean flour and those fed on a diet based on the amino acid mixtures released during 120 hr enzymatic digestion.

Deshpande *et al.*²⁴⁾ agreed that the results of *in vitro* studies can not be used as an index of the availability of the amino acids in animal studies because the digestive products and their absorption affect the release of amino acids *in vitro*. Recently, Szmclman and Guggenheim²⁵⁾ have reported that *in vitro* assay of available amino acids serves only as a guide to the quantity of amino acids which will be released by enzymatic digestion in the intestines. In the present studies, *in vitro* evaluation of protein quality by pancreatic digestion has been found useful for assessing the quality of unheated, properly heated, and overheated soybean flour. The reduction in the nutritive value of overheated soybean flour may be due to the irregular release of amino acids and availability of amino acid after absorption.

b. Nitrogen balance. Metabolism trials indicate that overheated defatted soybean flour excretes more fecal nitrogen when compared with properly heated flour (Table VII). Same results were observed in group D', E', and F'

in which the similar amount of amino acid mixture to the pattern released by 6 hr digestion was added to their diets. But no significant difference in fecal nitrogen was observed in the diet groups G', H', and I'. From these data, it would be possible to conclude that diets D', E', and F' showed more fecal nitrogen excretion due to the irregular amino acid pattern.

Amino acid mixture diets also show lower fecal nitrogen losses when compared with heated and unheated soybean flour diets. It is assumed that the nitrogen absorption is greater when the amino acid mixture diets are used. On the contrary, urinary nitrogen losses of unheated or heated soybean flour were small when compared with amino acid diets. From these data, it may be surmised that the utilization of the amino acid mixture diets may be less than that of the defatted soybean flour because of the increased excretion of nitrogen in urine.

Biological value of unheated, properly heated, and overheated defatted soybean flour diets did not agree with rat growth: the superiority of properly heated soybean flour diet (B') in rat growth was not observed in biological value. This finding is in agreement with the results of amino acid supplemental test presented in Table VI. Mitchell and Hamilton²⁶⁾ have reported that the biological values of protein increased with decreasing protein content of the diet. The biological value also varies with increase in the level of soybean oil meal in the feed.²⁷⁾ Further, Forbes *et al.*²⁸⁾ have reported that the biological value decreased linearly for three different proteins as the protein concentration increased, and the rate of change in calculated biological value also varied with increasing feed levels

24) P. D. Deshpande, A. E. Harper, M. Collins and C. A. Elvehjem, *Arch. Biochem. Biophys.*, **67**, 341 (1957).

25) S. Szmclman and K. Guggenheim, *J. Sci. Food Agric.*, **18**, 347 (1967).

26) H. H. Mitchell and T. S. Hamilton, "The Biochemistry of the Amino Acids". Little and Ives, New York, 1929.

27) R. M. Forbes and M. Yohe, *J. Nutr.*, **55**, 499 (1955).

28) R. M. Forbes, M. Yohe and L. Vaughan, *Federation Proc.*, **15**, 551 (1956).

of each protein. The similar observation was made when the amino acid mixture diets were used. The biological value of amino acid diets was lower than that of defatted soybean flour, and this finding coincides with that reported by Murlin and his associates.²⁹⁾

According to the data presented in this paper, inaccuracy of biological values of defatted soybean flour may have been caused

by a change in the property of protein (*i.e.* amino acid destruction or decrease in amino acids released by enzymatic digestion), and these problems raise the question of the validity with respect to nutritional evaluation of biological value for heat damaged defatted soybean flour samples.

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²⁹⁾ J. R. Murlin, L. E. Edwards, E. E. Hawley and L. C. Clark, *J. Nutr.*, **31**, 555 (1946).