Effect of Heating Soybean Proteins in the Autoclave on the Liberation of Cystine and Methionine by Several Digestion Procedures

ROBERT JOHN EVANS, SELMA L. BANDEMER, and DORIS H. BAUER

Department of Biochemistry, Michigan State University, East Lansing, Mich.

Part of the cystine in total soybean protein appears to be heat labile and is destroyed in the preparation of Alpha protein. Enzymatic digestion liberated but a portion of the cystine from soybean proteins in a form available for growth of *L. mesenteroides* P-60. More cystine and methionine were liberated from raw protein than from autoclaved proteins except for those raw proteins that contained the trypsin inhibitor. Partial acid hydrolysis of soybean proteins released less cystine from autoclaved than from raw proteins, but similar amounts of methionine were released. Complete acid hydrolysis resulted in some destruction of cystine. Methionine peptides released by trypsin digestion were more stimulatory for microbiological growth than were more completely hydrolyzed peptides.

THE PROTEIN NUTRITIVE VALUE of soybean meal for growing chicks was reduced by overheating (*7*), and the heat damage was corrected by supplementing the ration with lysine, methionine, and cystine but not by any one alone (*12*). Evans and Butts (*5*) observed that although no methionine was destroyed when soybean meal was heated in the autoclave, 32% less methionine was liberated by enzymatic digestion from autoclaved meal. There was no destruction or inactivation of methionine when soybean (Alpha) protein was autoclaved, but when the protein plus 20% of glucose or sucrose was autoclaved, the methionine released by enzymatic digestion was 45 to 65% less than that from the unautoclaved mixture (*5*). Evans *et al.* (*6*) observed that 31% of the cystine was destroyed when soybean meal was heated in the autoclave, but that no destruction occurred when soybean (Alpha) protein was autoclaved by itself or mixed with 20% of sucrose, glucose, dextrin, agar-agar, gum arabic, or soybean oil.

At least two possibilities exist as to why cystine was destroyed when soybean meal was autoclaved but not when Alpha protein or protein plus various carbohydrates or oil, which are constituents of the meal, were autoclaved. One possibility was that the microbiological procedure was not determining all the cystine in Alpha protein. To study this, other methods of cystine determination were used. A second possibility was that part of the cystine of soybean proteins is more resistant to heat destruction than the rest and that the more labile cystine has been destroyed in the preparation of Alpha protein. Alpha protein contains less than half as much cystine as does the total protein of soybean meal, which indicates a cystine loss in preparation of Alpha protein. Smith (*15*) observed losses of hydrogen sulfide and ammonia in the preparation of industrial proteins such as Alpha protein. Several other commercially isolated soybean proteins, which contain a greater proportion of the total cystine than Alpha protein, were compared with the latter.

Since some cystine is destroyed during prolonged acid hydrolysis of proteins in the presence of carbohydrate, short hydrolysis times were studied. The chromatographic procedure of Schram *et al.* (*14*), where cystine is converted to more stable cysteic acid before hydrolysis, was also used.

The following experiments were designed to study the liberation of cystine and methionine from soybean proteins by different digestion procedures and to investigate the destruction of cystine that occurs when soybean meal is drastically heated in the autoclave.

Experimental

A commercial soybean meal and Alpha protein (Glidden Co., now Central Soya Co.—14.39% nitrogen) were used as controls because they had been used in previous studies. Four other commercially isolated soybean proteins which contained over 1.0% of cystine (*3*) were also used. They were Amisoy (Glidden Co.—13.51% nitrogen), Buckeye protein (Buckeye Cellulose Corp.—14.32% nitrogen), C-1 assay protein (Dracket Co., now Archer-Daniels-Midland Co.—13.75% nitrogen), and Protosoy (Mann Research Laboratories—13.64% nitrogen). Casein (13.46% nitrogen), zein (14.96% nitrogen), gluten (14.08% nitrogen), lactalbumin (11.79% nitrogen), and finely ground, dried egg white (13.25% nitrogen) were used in some of the preliminary experiments.

Soybean meal, isolated proteins, or proteins plus sucrose at a 20% level were autoclaved at 15 pounds pressure (121° C.) for 4 hours. Samples were well mixed before being autoclaved or analyzed.

Acid hydrolyses were carried out by autoclaving the samples with 20% of hydrochloric acid for 30 minutes or 6 hours or by allowing them to stand in concentrated hydrochloric acid at 40° C. for the indicated number of days. Enzymatic digestions were performed as described by Evans (*2*). Methionine was determined microbiologically by assay with *Leuconostoc mesenteroides* P-60 using the oxidized peptone medium of Lyman *et al.* (*11*) and chromatographically by the procedure of Moore and Stein (*13*) including methionine sulfoxide with methionine. Cystine was determined either by the chromatographic procedure of Schram *et al.* (*14*) as modified by Bandemer and Evans (*1*) or microbiologically by assay with *L. mesenteroides* P-60 using the oxidized peptone medium of Lyman *et al.* (*11*).

Results and Discussion

Heat Destruction of Cystine Determined by Chromatography. Since the earlier work of Evans *et al.* (*6*) on the heat inactivation of cystine, two new methods have appeared in the literature. In the chromatographic procedure (*14*), cystine is converted to cysteic acid before the protein is hydrolyzed. Cysteic acid is not destroyed by prolonged hydrolysis with carbohydrate as cystine is (*10*).

Table I. Destruction of Cystine by Heating Soybean Meal or Proteins in an Autoclave

(Calculated on a basis of 16% nitrogen)

	Chromatographic		Microbiological: 6-Hr. Hydrolysis		Microbiological: 30-Min. Hydrolysis	
	% Cystine[a]	% Loss[b]	% Cystine[a]	% Loss[b]	% Cystine[a]	% Loss[b]
Soybean meal	1.54	45	1.14	47	1.60	49
Alpha protein	0.69	6	0.24	17	0.29	10
Alpha protein + sucrose	0.59	0	0.17	0	0.30	13
Buckeye protein	1.24	35	0.85	21	1.38	28
Buckeye protein + sucrose	1.22	22	0.88	17	1.37	40
Casein	0.37	16	0.33	15		
Casein + sucrose	0.32	22	0.47	6		
Zein	0.49	14	0.49	22		
Zein + sucrose	0.45	20	0.38	11		
Egg white	2.61	...	2.38	67		
Gluten	2.18	11	1.58	32		
Lactalbumin	3.47	9	2.80	13		

[a] Per cent cystine in unheated protein.
[b] Per cent less cystine in autoclaved than in unheated protein.

Table II. Destruction of Cystine by Autoclaving[a]

(Calculated on a basis of 16% nitrogen)

Autocloving Time	% Cystine Content[b]
Buckeye Protein	
None	1.39
15 min.	1.49
30 min.	1.55
1 hour	1.43
2 hours	1.25
4 hours	0.99
C-1 Assay Protein	
None	1.18
30 min.	1.23
4 hours	1.01

[a] Proteins were autoclaved at 121° C. for 4 hours.
[b] Microbiological assay using oxidized peptone medium. Proteins were hydrolyzed by heating with 20% hydrochloric acid for 30 minutes at 121° C.

Table III. Release of Cystine from Soybean Alpha Protein in a Microbiological Available Form by Acid Hydrolysis

(Calculated on a basis of 16% nitrogen)

Hydrolysis Time	Cystine Content[a]: % in Unheated	% in Autoclaved[b]	% Unavailable
Concd. HCl, 40° C.			
8 hr.	0.04	0.02	50
24 hr.	0.09	0.06	33
72 hr.	0.34	0.17	50
7 days	0.40	0.24	40
20% HCl, 121° C.			
15 min.	0.30	0.23	23
30 min.	0.36	0.29	19
1 hr.	0.38	0.32	16
2 hr.	0.34	0.30	12
4 hr.	0.33	0.29	12
6 hr.	0.31	0.29	6
8 hr.	0.29	0.29	0

[a] Microbiological assay using oxidized peptone medium.
[b] Protein was autoclaved at 121° C. for 4 hours.

The effect of heating soybean meal, Alpha protein, Buckeye protein, casein, zein, and each of these proteins with sucrose on cystine destruction was determined by the chromatographic method (Table I). Very little cystine was destroyed by heating Alpha protein, which contained less than half as much cystine as total soybean proteins. Buckeye protein contained 80% as much cystine as the total protein of soybean meal, and 35% of the cystine was destroyed by heating in the autoclave for 4 hours compared to 45% destruction of that in soybean meal. The addition of sucrose did not increase the amount of destruction. Comparable destruction did not occur when casein or zein were heated in the autoclave.

Although microbiological assay after 6 hours hydrolysis in the autoclave gave lower values for cystine than chromatographic assay did, the two methods gave similar percentages of cystine destroyed when soybean meal was heated.

Determined by Microbiological Assay after Partial Hydrolysis. Evans and coworkers (*3*) hydrolyzed proteins in the autoclave for 30 minutes with 20% hydrochloric acid for the microbiological assay of cystine using the oxidized peptone medium. Cystine contents of soybean meal and Buckeye protein determined by this procedure were comparable to those determined chromatographically (Table I). However, less cystine destruction in autoclaved Buckeye protein occurred with the microbiological procedure. Buckeye protein was heated in the autoclave at 15 pounds pressure for various times from 0 to 4 hours (Table II). Autoclaving for 4 hours decreased the cystine content by 36%, which is comparable to that indicated by the chromatographic method.

Higher cystine values were obtained by microbiological assay using the lanthionized casein medium of Horn and Blum (*9*) than by any of the other procedures. However, Evans *et al.* (*3*) obtained erratic results by this method and it was not used here. Evans and St. John (*8*) observed that cystine peptides exerted a greater stimulating action on growth of *Lactobacillus arabinosis* than did free cystine. A similar stimulatory action by cystine peptides on *L. mesenteroides* may have occurred when the lanthionized casein medium was used.

Cause of Cystine Destruction When Soybean Meal Is Autoclaved. Data obtained by the chromatographic procedure and microbiological assay after a 30-minute hydrolysis period indicate that cystine destruction in heated soybean meal is caused by the action of heat on protein-bound cystine. When Buckeye protein was heated, almost as high a percentage of cystine was destroyed. The protein of soybean meal contained 1.54% cystine, and 45% of that was destroyed by heating in the autoclave. Buckeye protein contained 1.24% cystine, and 35% was destroyed by heat. Alpha protein contained 0.69% cystine, and 6% was destroyed. Cystine remaining after heating was then about 0.85% in soybean meal protein, 0.81% in Buckeye protein, and 0.65% in Alpha protein. Part of the cystine in total soybean protein appears to be more heat labile than the rest and most of this was destroyed in the preparation of Alpha protein.

Liberation of Cystine from Soybean Proteins by Acid Hydrolysis. Hydrolysis of soybean proteins with concentrated hydrochloric acid at 40° C. has been used to differentiate between linkages of lysine that are easily broken and those that are more difficult to break (*4*). The possibility that some of the cystine and methionine linkages are more easily broken than others was investigated. Short time hydrolysis with 20% hydrochloric acid at 121° C. was compared with other hydrolysis methods.

A preliminary study was conducted using unheated and autoclaved Alpha proteins. Cystine was assayed with *L. mesenteroides*. Results are given in Table III. The highest cystine values in the unheated protein were obtained after 7 days of hydrolysis with concentrated hydrochloric acid at 40° C., but only 60% as much cystine was released from the heated protein by this method as from the unheated protein. Hydrolysis with 20% hydrochloric acid at 121° C. for 30 minutes or 1 hour released the most cystine from the heated protein. Longer hydrolysis than 1 hour with 20% hydrochloric acid resulted in destruction of cystine. Hydrolysis and cystine destruction proceeded more rapidly with the unheated than with

Table IV. Liberation of Cystine and Methionine from Soybean Meal and Soybean Proteins

(Calculated on a basis of 16% nitrogen)

Method of Digestion	Soybean Meal		Alpha Protein		Amisoy		Buckeye Protein		C-1 Assay Protein[a]	
	% in Raw	% in Auto.	% in Raw	% in Auto.	% in Raw	% in Auto.	% in Raw	% in Auto.	% in Raw	% in Auto.
					Cystine					
Chromatographic	1.53	0.85	0.69	0.65	1.30	1.27	1.24	0.82	1.22	1.12
Pepsin	0	0	0	0	0	0	0	0	0	0
Trypsin	0.08	0	0.16	0.07	0.17	0.14	0.02	0.08	0.21	0.13
Trypsin + erepsin	0.12	0	0.19	0.09	0.24	0.28	0.01	0.11	0.41	0.12
Pepsin, trypsin, erepsin	0.45	0	0.18	0.20	0.34	0.44	0.38	0.26	0.48	0.35
Concd. HCl, 40° C., 3 days	0.75	0.33	0.21	0.14	0.96	0.79	0.93	0.65	0.74	0.61
Concd. HCl, 40° C., 7 days	1.50	0.68	0.41	0.28	1.79	1.44	1.54	1.26	1.51	1.18
Concd. HCl, 40° C., 14 days	1.24	0.58	0.36	0.27	1.60	1.29	1.46	1.13	1.28	1.00
20% HCl, 121° C., 30 min.	1.72	0.82	0.31	0.26	1.37	1.28	1.53	1.08	1.14	0.99
20% HCl, 121° C., 8 hrs.	0.97	0.56	0.30	0.26	0.78	0.78	0.87	0.69	0.72	0.68
					Methionine					
Chromatographic	0.87	0.87	1.47	1.33						
Pepsin	0.18	0	0.12	0.07	0.14	0.08	0.15	0.07	0.16	0.07
Trypsin	1.07	0.82	1.06	0.99	0.98	1.25	0.58	1.01	1.23	1.04
Trypsin, erepsin	0.40	0.16	0.61	0.40	0.52	0.56	0.23	0.46	0.65	0.37
Pepsin, trypsin, erepsin	0.95	0.27	0.61	0.65	0.80	0.81	0.83	0.70	0.80	0.77
Concd. HCl, 40° C., 3 days	0.61	0.54	0.60	0.60	0.65	0.70	0.68	0.66	0.77	0.72
Concd. HCl, 40° C., 7 days	0.71	0.70	0.79	0.76	0.78	0.88	0.79	0.84	0.87	0.86
Concd. HCl, 40° C., 14 days	0.73	0.72	0.80	0.75	0.88	0.90	0.83	0.80	0.91	0.92
20% HCl, 121° C., 30 min.	1.01	0.99	1.05	1.05	1.02	1.06	0.99	0.95	1.04	0.95
20% HCl, 121° C., 8 hrs.	0.77	0.76	1.09	1.02	1.01	1.13	1.04	1.09	1.18	1.13

[a] Values for C-1 Assay Protein and Protosoy were similar, and only the C-1 Assay Protein data are presented here.

the heated protein. After 15 minutes digestion 23% less cystine was released from the heated protein than from the unheated one. Equal amounts were liberated after 8 hours digestion.

Liberation of Cystine and Methionine from Soybean Proteins by Different Methods of Hydrolysis. Unheated and autoclaved soybean meal and five soybean proteins were hydrolyzed with enzymes, concentrated hydrochloric acid at 40° C., or 20% hydrochloric acid at 121° C., as shown in Table IV, and the cystine and methionine values obtained by microbiological assay were compared with chromatographic values. No cystine was released from autoclaved soybean meal by any of the enzymes or combinations used. Very little cystine was released from unheated Buckeye protein by digestion with trypsin or with trypsin and erepsin, but combination of pepsin, trypsin, and erepsin liberated more. More cystine and methionine were liberated from the heated than from the unheated Buckeye protein by trypsin or a combination of trypsin and erepsin, but not by the combination of pepsin, trypsin, and erepsin. Buckeye protein, therefore, appears to contain the soybean trypsin inhibitor, which was destroyed by heat treatment.

Digestion with concentrated hydrochloric acid at 40° C. released more cystine but not methionine or lysine (*4*) from the soybean proteins than did enzymatic digestion with pepsin, trypsin, and erepsin. About half as much cystine was released from heated soybean meal as from unheated, and 20 to 30% as much from heated isolated proteins as from unheated.

Digestion for 30 minutes with 20% hydrochloric acid at 121° C. liberated as much cystine in a microbiologically available form as did digestion at 40° C. with concentrated hydrochloric acid for 7 or 14 days. Further hydrolysis for 8 hours with 20% hydrochloric acid reduced the apparent cystine content of the meal and protein, because of cystine destruction.

With soybean meal and Buckeye protein, more than 20% destruction of cystine for the heated material occurred when 20% hydrochloric acid at 121° C. was used for hydrolysis. Heating the soybean proteins caused cystine losses of 20 to 30% when concentrated hydrochloric acid hydrolysis at 40° C. was used.

Pepsin digestion released very little methionine from soybean meal or any of the soybean proteins (Table IV). Trypsin digestion appeared to release peptides that had a greater growth stimulatory effect on *L. mesenteroides* P-60 than did free methionine, because subsequent digestion with erepsin decreased apparent methionine content in all cases. Heating soybean meal or soybean proteins in the autoclave decreased the release of available methionine by all enzymes except for Amisoy and Buckeye protein, where trypsin and a combination of trypsin and erepsin released more available methionine from the autoclaved than from the unheated proteins. Acid hydrolysis released as much available methionine from autoclaved soybean meal or proteins as from the unheated ones. Most of the methionine appeared to be liberated by hydrolysis with 20% hydrochloric acid for 30 minutes at 121° C. Hydrolysis for 8 hours caused a decrease of 24% in the amount of methionine liberated from soybean meal.

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