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Effect of Heat, Amylase, and Disulfide Bond Cleavage on the in Vitro Digestibility of Soybean Proteins

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Various protein fractions were prepared from defatted soybean flour based on solubility differences and size on Sephadex G-200. The in vitro digestibility of these fractions by trypsin and by successive pepsin–trypsin treatment was affected by the presence of trypsin inhibitors, native structure of the proteins, and the presence of starch (shown to be present in soybeans). The trypsin inhibitors were destroyed by heating at 100 °C for 30 min at pH 1 but not at neutrality. The native structure of the proteins could be destroyed by heating, particularly at low pH, digestion with pepsin at pH 1, or by cleavage of the disulfide bonds. Cleavage of disulfide bonds increased the in vitro digestibility of the proteins. Prior amylase treatment increased the trypsin digestibility of most of the protein fractions.

With the exception of limiting amounts of methionine and cystine, soybean is an excellent food protein source (Kellor, 1974). However, there are a number of antinutritive compounds in soybeans which may adversely affect the nutritive value. These antinutritive compounds include proteolytic enzyme inhibitors, amylase inhibitors, phytohemagglutinins, phytic acid, flatulents, goitrogenic compounds, saponins, and phenolic compounds (Rackis, 1974). The first three of these can be destroyed by adequate heat treatment and the other compounds are thought not to be of serious nutritional concern, especially in a mixed diet. However, it is of major concern that the biological value and digestibility of soybean proteins are significantly lower than those for egg proteins. Liener (1972) reported the biological value, measured with human subjects, of heat treated full fat soybean flour to be 64 while that of egg was 87. Supplementation of the soybean flour with methionine increased the biological value only to 75. Digestibility values, also measured on human

subjects, were 84 and 97 for soybean flour and eggs, respectively, and the digestibility of soybean flour was not improved by addition of methionine.

The presence of active inhibitors and phytohemagglutinins in improperly heat-treated soybean flour undoubtedly accounts for some of the observed decreased digestibility. Rackis (1974) indicated that phytohemagglutinin could account for 25% inhibition of growth of rats on feeding raw soybean flour. Kakade et al. (1969) showed that both selective removal of the trypsin inhibitors and denaturation of protein by heat increased digestibility of the protein. Excessive heat treatment can also lower the digestibility (Kellor, 1974). However, these are not the total explanation for the lowered digestibility. Jaffé (1972) reported that a variety of red beans which did not contain trypsin or chymotrypsin inhibitors still had only 71% digestibility.

The tertiary structure of the proteins affects digestibility and the structure may not be fully destroyed by heat treatment. Fukushima (1968) suggested that the globular molecules of soybean proteins are compactly folded and have a hydrophobic region in the interior which resists proteolysis. The proteins cannot be fully digested until the interior tertiary structure is destroyed. Seidl et al. (1969) reported that, in in vitro studies, isolated black bean

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Soybean

| Cracked, dehulled
| Ground through 40 mesh sieve
▼

Full fat flour (A)

| 1:5 flour to extraction solvent
▼

Defatted soybean flour (B)

| 1:5 flour to standard buffer, 5 hr,
| centrifuge 3000 X g
▼

precipitate   Supernatant liquid

| standard buffer
| 5 hrs
| centrifuge   cold room overnight

                                centrifuge 5000 X g

precipitate   supernatant ——————→

| wash
| 1:3 distilled water,
| 4 times   precipitate   supernatant

precipitate | dissolve in
                           | 50 ml Std. buffer | dialyzed
| lyophilization | dialyzed and
                           | lyophilized   precipitate   supernatant

(C)   (D) | lyophilization | lyophilization
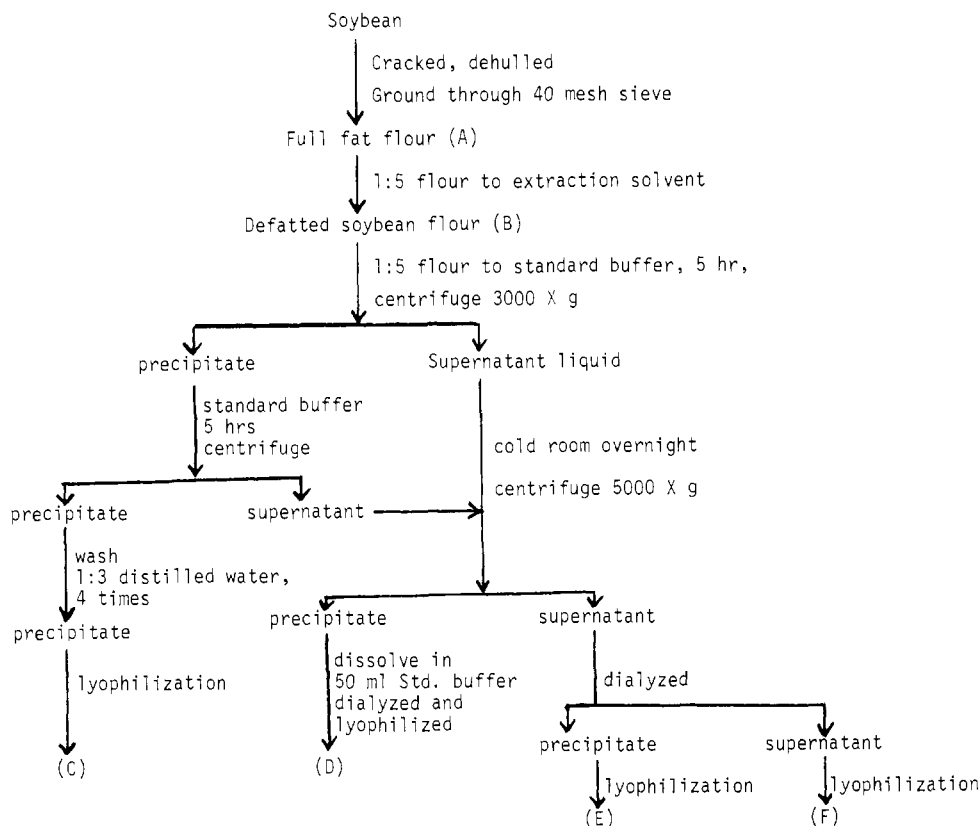   (E)   (F)

Figure 1. Scheme showing the fractionation of soybean flour.

globulin was resistant to proteolytic enzymes even after heat treatment. Kakade et al. (1969) reported that in chick studies the cystine of navy bean trypsin inhibitors was not released by the digestive enzymes.

The proteolytic enzyme inhibitors of beans are known to contain disulfide bonds which contribute to stability of the tertiary structure of the proteins (Kowalski et al., 1974; Ikenaka et al., 1974). The Bowman–Birk inhibitor of mol wt 8000 contains 19.8% sulfur-containing amino acids (Steiner and Frattali, 1969) and has seven disulfide bonds per mole (Ikenaka et al., 1974). The 7S and 11S globulins, two major protein fractions of soybean, have the ability to form insoluble disulfide-linked polymers (Wolf, 1970).

Preliminary studies indicate that starch may contribute to the decreased digestibility of soybean proteins. Kapsiotis (1972) reported that treatment of certain isolated globulin fractions of bean with α-amylase increased their digestibility from 79% for the untreated to 98.5% for the α-amylase treated samples. Heat treatment alone did not cause this improvement. McGinnis (1972) found that mild acid treatment would also overcome the low digestibility.

In the work reported here, we have investigated the effects of heat treatment at neutral and acidic pH, amylase treatment, and breakage of disulfide bonds on the in vitro digestion of various fractions of soybean flour by pepsin and/or trypsin.

EXPERIMENTAL SECTION

Materials. Mature soybeans were purchased from the Health Food Store in Davis. The origin and storage conditions of the beans are unknown. Hog stomach pepsin (3× crystallized, lot V1619) and α-N-benzoyl-L-arginine ethyl ester hydrochloride (lot 5047) were obtained from Mann Research Laboratories, Inc.

Bovine trypsin (2× crystallized, lot 54e-8210), *Bacillus subtilis* α-amylase (4× crystallized, lot 34c-1840), 2,4,6-trinitrobenzenesulfonic acid (lot 13e-2000), and glycine

(ammonia free) were obtained from Sigma Chemical Co. Dithiothreitol (lot 410236) was obtained from Calbiochem. Sephadex G-200 was from Sigma Chemical Co.

All other compounds were reagent grade and deionized water was used.

Methods. *Extraction and Fractionation of Soybean Proteins.* Defatted soybean flour was prepared by the procedure of Schweiger and Muller (1973). After solvent extraction the flour was dried in a vacuum oven for 12 h at 40 °C. Full fat flour was labeled as fraction A and defatted flour as fraction B.

Defatted soybean flour was fractionated as shown in Figure 1. One hundred grams of defatted flour was dispersed in 500 ml of standard buffer (32.5 mM monobasic potassium phosphate, 2.6 mM dibasic potassium phosphate, 0.4 M sodium chloride, 10 mM mercaptoethanol, pH 7.6 and 0.5 ionic strength) at room temperature and stirred continuously for 5 h. After separation of insoluble material by centrifugation at 3000g for 30 min at 20 °C, the insoluble material was again extracted with 500 ml of standard buffer as above, and again separated by centrifugation. The insoluble material was washed four times with 300 ml of deionized water and lyophilized (fraction C).

The two supernatant liquids (containing 52.1% of the total weight of the defatted soy flour) from the previous step were combined, stored in a cold room (~5 °C) overnight, and centrifuged at 5000g for 30 min to remove a precipitate. The precipitate was dissolved in 10 ml of standard buffer, dialyzed exhaustively against deionized water, and lyophilized (fraction D). The supernatant liquid was dialyzed against deionized water in a cold room for 32 h and centrifuged and the supernatant liquid (fraction F) and precipitate (fraction E) were lyophilized.

Fraction F was further separated on a Sephadex G-200 column in a cold room by a modification of the method of Obara and Kimura (1967). Four hundred milligrams
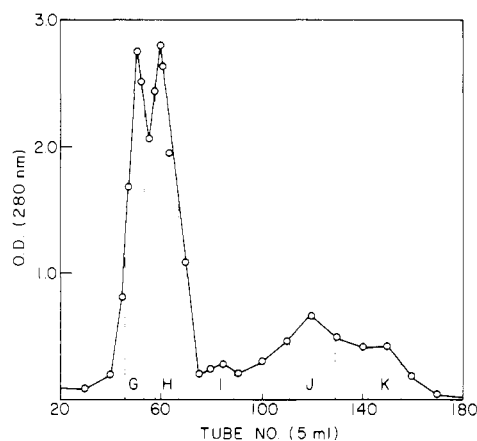
Figure 2. Separation of the proteins of fraction F on a Sephadex G-200 column (3 × 96 cm) by the method of Obara and Kimura (1967). Tubes within the segments indicated by the vertical dashed lines were pooled, dialyzed, and lyophilized to give the indicated fraction.

Table I. Weight, Protein and Moisture Content of Each Protein Fraction Obtained from Soybeans

Sample	Wt,[a] g	Protein content,[a,b] %	Moisture, %
A		39.3 ± 0.9	8.30 ± 0.08
B	100	60.9 ± 0.5	7.21 ± 0.03
C	47.9	46.9 ± 0.0	5.45 ± 0.25
D	0.495	74.4 ± 0.0	8.14 ± 0.09
E	1.79	89.5 ± 0.6	12.5 ± 0.3
F	34.6	85.7 ± 0.2	6.65 ± 0.07
G	9.27	89.4 ± 1.9	5.86 ± 0.03
H	8.54	93.4 ± 3.3	3.72 ± 0.06
I	0.763	75.8 ± 0.7	10.3 ± 0.3
J	4.46	83.5 ± 2 1	16.6 ± 0.9

[a] On a moisture free basis. [b] Based on assumption of 16% nitrogen content.

of lyophilized fraction F was dissolved in 2.5 ml of standard buffer (composition given above) and applied to the top of a column of Sephadex G-200 (3 × 96 cm) equilibrated with the same buffer. Fraction F was separated into five fractions as shown in Figure 2. The collected tubes were pooled as indicated in Figure 2, dialyzed exhaustively against deionized water, and lyophilized. Fraction K was a very small amount of gellike substance which was not investigated further because of its amount. Obara and Kimura (1967) indicated this fraction was not protein. Fractions G, H, I, and J correspond to 15, 11, 7, and 2S compounds of soybeans, respectively (Wolf, 1970), as determined with a calibrated Sephadex G-200 column (Whitaker, 1963).

All lyophilized fractions were stored at 5 °C.

Moisture and Protein Content. The moisture content was determined by loss in weight following drying of a sample in a vacuum oven at 70 °C for 24 h.

For protein determination the samples were digested by the method of Lang (1958) and the ammonia content determined by nesslerization using the procedure of Johnson (1941). A standard curve was prepared using previously dried ammonium sulfate. Percent of protein was calculated by multiplying the percent of nitrogen by 6.25. The results are shown in Table I.

Starch Content. The presence of starch in the soybeans and flour was determined in two ways, by microscopic examination in the absence and presence of iodine and by increase in reducing groups formed on incubation with *B. subtilis* α-amylase (4× crystallized). The reducing groups were measured by the dinitrosalicyclic acid reagent (Bernfeld, 1955) using maltose for the standard curve. Thin slices of soybeans previously soaked for 5 h in distilled water showed several small granules with the structural and iodine staining properties of starch granules. On crushing the tissue on the microscope slide numerous starch granules were readily detected (Whitaker and Sterling, 1976). Starch granules were also readily discernible in the full fat flour fraction. By digestion with α-amylase, 0.52% starch was found. This is a minimum value since it is based on an assumption of 40% hydrolysis of all the glucosidic bonds of all the starch.

Insoluble Organic Solids Content. The insoluble solids content of fractions A, B, and C left after digestion by proteolytic enzymes was determined by the chromic acid method of Johnson (1949). Following proteolytic enzyme digestion, the sample was centrifuged, the supernatant liquid removed, and the precipitate washed three times

with deionized water and then dried in an oven at 85 °C. The organic material was then oxidized with chromic acid. The amount of organic material was determined from a standard curve prepared with sucrose.

Oxidation of Disulfide Bonds. The disulfide bonds of fractions F and J were oxidized with performic acid as described by Hirs (1967). Following oxidation the sample was diluted 25-fold with ice water and lyophilized immediately. The sample was dissolved again in 200 ml of water and again lyophilized.

Reduction of Disulfide Bonds. For reductive cleavage of disulfide bonds, the samples (2 mg weighed accurately on a Cahn Electrobalance) were dissolved in 1.8 ml of water containing 0.56 mM dithiothreitol, covered to eliminate evaporation, and held in a boiling water bath for 30 min. The dithiothreitol was not removed.

Trypsin Inhibitor Activity. Trypsin was incubated with a protein fraction (ratio 10:1 (w/w) protein fraction to trypsin) in 0.1 M phosphate buffer (pH 7.6) for exactly 20 min in a 35.0 °C water bath. An aliquot of the trypsin-protein mixture was used to determine the remaining trypsin activity with 0.5 mM benzoyl-L-arginine ethyl ester at 30.0 °C in 0.1 M phosphate buffer (pH 7.6). The change in absorbance at 253 nm was followed continuously in the thermostated compartment of a Gilford-DU spectrophotometer. The loss of activity due to inhibitors in the protein fractions was compared with the activity of the same amount of trypsin incubated in the absence of added protein fraction. The results are expressed as micrograms of trypsin inhibited per milligram of protein fraction.

Heat Treatment. For heat treatment at neutral pH, the samples (2 mg weighed accurately) were dissolved in 1 ml of deionized water, covered to eliminate evaporation, and held in a boiling water bath for 30 min. For heat treatment at acidic pH, the samples (2 mg weighed accurately) were dissolved in 2 ml of 0.1 M HCl, covered, and held in a boiling water bath for 30 min. Unheated samples were prepared in an identical fashion.

Amylase Treatment. Samples (2 mg weighed accurately) were dissolved in 1.5 ml of water, covered, and held in a boiling water bath for 30 min. After cooling, 0.2 ml of 1 M sodium chloride and 0.1 ml of amylase (a ratio of 40:1 (w/w) of sample to amylase) were added, and the reaction was incubated at 35.0 °C for 24 h.

Proteolytic Digestion. After treatment (heat, amylase, or no heat) as described above, the samples were adjusted to 2 ml and a final concentration of 0.1 M HCl and 1 ml of pepsin (0.04 mg of pepsin/ml in 0.1 M HCl; 50:1 ratio of sample to pepsin (w/w)) was added to each sample. After mixing, the tubes were covered and incubated at 35.0 °C for 24 h. At this time, the acid was neutralized with NaOH, 0.5 ml of 1.0 M phosphate buffer (pH 7.8) was added, followed by 1 ml of trypsin (0.05 mg of trypsin/ml

Table II. Trypsin Inhibitory Activity of the Different Protein Fractions[a]

Treatment	Protein fraction								
	A	B	C	E	F	G	H	I	J
Unheated	32.5 ± 0.0	45.2 ± 1.0	0	25.4 ± 0.3	128 ± 1	0	0	0	774 ± 7
Heated at neutral pH	23.6 ± 0.4	41.0 ± 1.3		12.1 ± 1.0	95.1 ± 0.6				495 ± 2
Heated at acidic pH	0	0		0	0				110 ± 13
Amylase treatment	24.8 ± 1.1	31.7 ± 1.0		19.6 ± 1.9	83.2 ± 4.2				489 ± 15
Performic acid					0				0

[a] The inhibitory activity is expressed as micrograms of trypsin inhibited per milligram of sample.

Table III. Effect of Pepsin and Trypsin Alone and in Combination on Extent of Digestion of Unheated Soybean Proteins[a]

Treatment	Protein fraction, %								
	A	B	C	E	F	G	H	I	J
Pepsin	13.0 ± 0.3	12.6 ± 0.3	15.2 ± 0.3	12.3 ± 0.3	11.6 ± 0.5	11.9 ± 0.1	12.7 ± 0.2	14.4 ± 0.2	9.93 ± 0.12
Trypsin	2.00 ± 0.48	1.83 ± 0.20	6.94 ± 0.02	2.20 ± 0.28	0	8.29 ± 0.71	10.7 ± 0.1	7.01 ± 0.07	0.654 ± 0.090
Pepsin + trypsin	15.8 ± 0.4	16.7 ± 0.1	21.3 ± 0.3	16.5 ± 0.1	15.5 ± 0.3	16.9 ± 0.4	19.0 ± 0.3	18.0 ± 0.4	8.97 ± 0.33

[a] Digestion expressed as percent hydrolysis of the peptide bonds.

Table IV. Effect of Heat Treatment on the Extent of Digestion of Soybean Proteins[a]

Treatment	Protein fraction, %								
	A	B	C	E	F	G	H	I	J
Heated at neutral pH									
Trypsin	4.94 ± 0.43	2.03 ± 0.15	10.9 ± 0.4	9.48 ± 0.02	0.03 ± 0.01	8.53 ± 0.30	11.4 ± 0.6	8.73 ± 0.21	0.439 ± 0.090
Pepsin + trypsin	16.1 ± 0.4	16.4 ± 0.7	21.7 ± 0.4	17.6 ± 0.2	15.7 ± 0.1	17.1 ± 0.2	19.3 ± 0.2	18.5 ± 0.3	9.34 ± 0.29
Heated at acidic pH									
Trypsin	10.3 ± 0.3	8.90 ± 0.70	14.2 ± 0.3	10.3 ± 0.2	8.81 ± 0.18	11.9 ± 0.5	13.6 ± 0.3	12.4 ± 0.2	3.12 ± 0.23
Pepsin + trypsin	16.9 ± 0.4	17.1 ± 0.4	21.8 ± 0.5	18.3 ± 0.6	16.7 ± 0.2	17.6 ± 0.4	19.8 ± 0.5	20.4 ± 0.3	10.8 ± 0.1
Pepsin + heat (low pH) + trypsin	19.8 ± 0.8	18.3 ± 0.1	22.8 ± 0.2	18.9 ± 0.2	18.1 ± 0.2	19.4 ± 0.1	21.9 ± 0.2	21.6 ± 0.2	11.5 ± 0.2

[a] Digestion expressed as percent hydrolysis of the peptide bonds.

in 5 mM HCl; 40:1 ratio of sample to trypsin (w/w)), and the tubes were covered and again incubated at 35.0 °C for 24 h.

Digestibility was also determined with trypsin alone. The procedure was the same as described above except for addition of and incubation with HCl and pepsin.

The extent of hydrolysis was measured by the increase in amino groups as determined by the 2,4,6-trinitrobenzenesulfonic acid method (Fields, 1972). Glycine was used as the standard. Blanks included sample and enzyme incubated separately under the above conditions.

Extent of hydrolysis was calculated as the ratio of increase in amino groups (millimoles) to protein content (millimoles) and expressed as percentage. The protein content, in millimoles, was determined as weight of protein divided by 113, the average amino acid residue weight.

RESULTS AND DISCUSSION

Trypsin Inhibitory Activity. The trypsin inhibitory activities of the different soybean protein fractions are shown in Table II. Protein fractions A, B, E, F, and J contained trypsin inhibitory activity. Fraction J, based on a 1:1 stoichiometric reaction with trypsin and an assumed molecular weight near 20000 (from Sephadex column), was nearly pure inhibitor. Heating of solutions of the samples at 100 °C for 30 min at neutral pH was not sufficient to destroy all the inhibitor. Under these conditions only 10 (fraction B) to 52% (fraction E) of the activity of the inhibitor was destroyed. Amylase (the treatment included a heat step) had no significant effect

on destroying the trypsin inhibitors.

The inhibitor activity was destroyed by heating at 100 °C for 30 min at acidic pH (except for fraction J which still contained 14.2% of the original inhibitor activity) or by cleavage of the disulfide bonds by performic acid.

Digestion of Unheated Soybean Proteins. Pepsin hydrolyzed from 9.93 (fraction J) to 15.2% (fraction C) of the peptide bonds of the different protein fractions (Table III). Trypsin hydrolyzed 6.94 (fraction C) to 10.7% (fraction H) of the peptide bonds of the protein fractions which contained no trypsin inhibitors (fractions C, G, H, and I; Table II) but hydrolyzed a much smaller number of the peptide bonds of those fractions with trypsin inhibitor activities (fractions A, B, E, F, and J). Digestion with pepsin at pH 1 followed by digestion with trypsin at pH 7.8 gave a higher percent hydrolysis than treatment with either enzyme alone as might be expected.

Digestion of Heated Soybean Proteins. Heating solutions of fractions B, F, G, H, and J at 100 °C for 30 min at neutral pH had very little effect on the trypsin digestibility of the proteins (compare Tables III and IV). In the case of fractions B, F, and J this was primarily because the trypsin inhibitors were not completely destroyed (Table II). Heating of solutions of the protein fractions at 100 °C for 30 min at pH 1 improved the trypsin digestibility of all the fractions substantially. With the exception of fraction J which had 3.12% hydrolysis, from 8.81 to 14.2% of the peptide bonds were hydrolyzed by trypsin following heat treatment at pH 1. Heating the samples at neutral or acidic pH prior to the sequential

Table V. Effect of Amylase Treatment on Extent of Digestion of Soybean Proteins[a]

Treatment[b]	Protein fraction, %								
	A	B	C	E	F	G	H	I	J
Trypsin treatment									
Heated	4.94	2.03	10.9	9.48	0.03	8.53	11.4	8.73	0.439
	± 0.43	± 0.15	± 0.4	± 0.02	± 0.01	± 0.30	± 0.6	± 0.21	± 0.090
Amylase	9.81	8.01	11.5	9.22	6.51	11.4	14.8	14.8	0.217
	± 0.28	± 0.19	± 1.0	± 0.34	± 0.79	± 1.0	± 0.1	± 0.2	± 0.090
Pepsin + trypsin treatment									
Heated	16.1	16.4	21.7	17.6	15.7	17.1	19.3	18.5	9.34
	± 0.4	± 0.7	± 0.4	± 0.2	± 0.1	± 0.2	± 0.2	± 0.3	± 0.29
Amylase	17.6	15.3	21.7	17.0	16.3	17.5	19.4	19.2	9.57
	± 0.2	± 0.3	± 0.4	± 0.9	± 0.2	± 0.1	± 0.2	± 0.2	± 0.23

[a] Digestion expressed as percent hydrolysis of the peptide bonds. [b] All treatments followed heating of sample in boiling water bath for 30 min at neutral pH.

Table VI. Effect of Disulfide Bond Cleavage on Extent of Digestion of Soybean Proteins[a]

Treatment[b]	Protein fraction, %								
	A	B	C	E	F	G	H	I	J
Trypsin treatment									
Unheated	2.00	1.83	6.94	2.20	0	8.29	10.7	7.01	0.654
	± 0.48	± 0.20	± 0.02	± 0.28		± 0.71	± 0.1	± 0.07	± 0.090
Heated	4.94	2.03	10.9	9.48	0.03	8.53	11.4	8.73	0.439
	± 0.43	± 0.15	± 0.4	± 0.02	± 0.01	± 0.30	± 0.6	± 0.21	± 0.090
Dithiothreitol	11.1	11.1	12.8	7.59	10.0	11.5	11.7	13.0	0
	± 0.8	± 0.5	± 0.7	± 0.38	± 1.6	± 0.2	± 0.2	± 0.2	
Performic acid					11.4				10.8
					± 0.4				± 0.3
Performic + heated					13.4				12.1
					± 0.4				± 0.7
Pepsin + trypsin treatment									
Unheated	15.8	16.7	21.3	16.5	15.5	16.9	19.0	18.0	8.97
	± 0.4	± 0.1	± 0.3	± 0.1	± 0.3	± 0.4	± 0.3	± 0.4	± 0.33
Heated	16.1	16.4	21.7	17.6	15.7	17.1	19.3	18.5	9.34
	± 0.4	± 0.7	± 0.4	± 0.2	± 0.1	± 0.2	± 0.2	± 0.3	± 0.29
Dithiothreitol	20.7	19.1	22.4	19.8	18.4	18.0	18.2	19.8	12.5
	± 0.8	± 0.9	± 0.5	± 0.2	± 0.0	± 0.5	± 0.1	± 0.7	± 0.3
Performic acid					17.7				15.3
					± 0.0				± 0.4
Performic + heated					17.7				16.7
					± 0.3				± 0.3

[a] Digestion expressed as percent hydrolysis of the peptide bonds. [b] All heat treatments were for 30 min in a boiling water bath at neutral pH.

treatment with pepsin and trypsin had essentially no effect on the extent of hydrolysis of the proteins. It is interesting to note that heat treatment at low pH following pepsin digestion resulted in higher combined digestion than when the heat treatment was prior to pepsin digestion (Table IV).

Effect of Amylase Treatment on Digestion. Amylase treatment appreciably increased the trypsin digestibility of all protein fractions except C, E, and J; this treatment especially increased the digestibility of fractions A, B, and F (Table V). Controls showed there was no proteolysis of the proteins by the amylase alone. These data indicate that starch associated with the protein, either adsorbed or covalently bound (Whelan, 1976), can have a marked effect on the susceptibility of protein to trypsin digestion. Since the samples were heated prior to amylase treatment, the explanation is not that the proteins were more susceptible to heat denaturation following removal of starch. With the exception of fraction A, amylase treatment had no effect on the extent of hydrolysis by sequential action of pepsin and trypsin (Table V).

There is still considerable controversy over whether soybeans contain starch. Smith and Circle (1972) indicate that soybeans do not contain starch. Bailey et al. (1935), as a result of examination of hundreds of samples of soybeans, reported that starchlike substances ranged from 4.65 to 8.97% with a mean value of 5.6%. By microscopic

examination, Mann (1923) found starch in 14 of 17 commercial varieties of soybeans grown in the United States at that time. Wolfe et al. (1942) reported starch contents of 1.91 to 4.17% for four varieties of mature soybeans. Our own work shows the presence of starch in the soybeans used in this work.

Effect of Disulfide Bond Cleavage on Digestibility. Treatment of the soybean protein fractions with dithiothreitol substantially increased the extent of trypsin digestibility of all fractions except E, H, and J. The decrease in digestibility of fraction E in the presence of dithiothreitol was shown to be due to the effect of dithiothreitol on the stability of trypsin. It would appear that additional trypsin hydrolysis of fraction H is not limited by disulfide bonds but perhaps by the presence of starch (Table V). It appears that dithiothreitol is unable to break the disulfide bonds of fraction J under the conditions used here. Dithiothreitol treatment increased the trypsin digestibility of fractions A, B, F, and I over that produced by heating at 100 °C for 30 min at pH 1 (Table IV) although the differences are small.

Performic acid oxidation of disulfide bonds increased the trypsin digestibility of fraction F moderately and had a marked effect on the trypsin digestibility of fraction J. Trypsin digestibility of fraction J following heating at 100 °C for 30 min at pH 1 was 3.12% (Table IV) while it was 10.8% following performic acid oxidation (Table VI).

Table VII. Solubility of Protein Fractions A, B and C after Different Treatments[a]

Treatment	Protein fraction, %		
	A	B	C
Control[b]	64.0 ± 0.4	85.2 ± 0.9	63.4 ± 1.8
Pepsin treatment			
Unheated[c]	65.0 ± 0.6	87.6 ± 3.4	67.2 ± 1.8
Trypsin treatment			
Unheated	80.0 ± 1.4	82.8 ± 0	69.0 ± 1.3
Heated (acidic pH)	83.2 ± 1.1	93.7 ± 0.4	83.0 ± 2.1
Heated (neutral pH)	53.6 ± 1.2	73.8 ± 0.6	62.9 ± 2.1
Amylase	67.5 ± 0.9	70.0 ± 0.5	61.6 ± 2.0
Dithiothreitol	64.5 ± 0.7	71.5 ± 0.7	61.2 ± 0.4
Pepsin + trypsin treatment			
Unheated	73.2 ± 1.5	91.0 ± 1.7	78.8 ± 1.0
Heated (acidic pH)	88.8 ± 2.3	93.8 ± 0.6	83.4 ± 1.0
Heated (neutral pH)	78.5 ± 1.7	85.5 ± 1.7	77.9 ± 1.2
Pepsin (heated at acidic pH)	83.7 ± 1.1	93.0 ± 3.2	82.6 ± 0.5
Amylase	79.8 ± 0.5	84.0 ± 2.6	75.2 ± 2.7
Dithiothreitol	82.8 ± 0.4	90.0 ± 1.2	76.5 ± 0.4

[a] The insoluble organic material was determined by the chromic acid method (Johnson, 1949). [b] Solubility in water at neutral pH. [c] Solubility in 0.1 M HCl.

Heat treatment of the performic acid oxidized fractions F and J further increased their trypsin digestibility.

With the exception of fraction H, dithiothreitol treatment increased the digestibility achieved through the successive action of pepsin and trypsin (Table VI). The extent of digestion was higher than that achieved following heating at 100 °C for 30 min at pH 1 (Table IV). Of special importance is the finding that dithiothreitol treatment increased the digestibility of fractions A and B by successive pepsin and trypsin treatment 28.6 and 16.5%, respectively. This significant improvement in the in vitro digestibility of full fat and defatted soybean flour should be evaluated in in vivo studies.

Performic acid oxidation of disulfide bonds had a significant effect on the pepsin–trypsin digestibility of fraction J but no effect on digestibility of fraction F.

Effect of Trypsin and Pepsin–Trypsin Digestion on Solubility. Fractions A, B, and C were not completely soluble in water (Table VII), fractions A and C being soluble to only 64%. Pepsin digestion at pH 1 did not significantly improve the solubility although there was about 13% of the peptide bonds hydrolyzed (Table III). Trypsin digestion, particularly after heat treatment at pH 1, substantially increased the solubility of all three fractions, increasing the solubility of fraction B to 93.7%. It is interesting that trypsin digestion following either amylase or dithiothreitol treatment did not significantly affect the solubility although there was substantial hydrolysis of peptide bonds (Table VI). The successive action of pepsin and trypsin under all treatment conditions generally led to an increase in solubility of the fractions. As shown in Table I, fractions A, B, and C contained large amounts of compounds other than proteins.

These studies show that the in vitro trypsin digestibility of most soybean proteins can be significantly improved by heat treatment, particularly at pH 1, by amylase treatment, and by disulfide bond cleavage. The effect of heat treatment is in part due to inactivation of trypsin inhibitors and in part to denaturation of the proteins permitting more extensive proteolysis. Improvement following amylase treatment most likely is due to removal of starch associated with the protein in a manner to preclude the effective hydrolysis of proteins. It would be of interest to determine if this improvement by amylase treatment is a function of the storage time and conditions of the bean. Increase in digestibility on cleavage of disulfide bonds is probably due in part to inactivation of the trypsin inhibitors but also to an opening up of the protein to facilitate proteolytic digestion.

There was little or no effect of heat and amylase treatment on increased digestibility achieved by successive digestion by pepsin and trypsin. However, cleavage of disulfide bonds by either dithiothreitol reduction or performic acid oxidation led to increased digestibility by the successive action of pepsin and trypsin.

The presence of lipids in fraction A appeared to have no significant effect on either the trypsin or the successive pepsin–trypsin digestibility (compare fractions A and B).

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