Biological Threshold Levels of Soybean Trypsin Inhibitors by Rat Bioassay[1]

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ABSTRACT

In conventional processing of soy flour by moist heat (toasting) an apparent destruction of at least 95% of the trypsin inhibitor (TI) is observed when assayed by various procedures. A more accurate and reproducible method, particularly suitable for determining TI activity of heat-processed samples, has been used in this study. Toasted soy flour has much greater TI activity when assayed by the modified procedure because it is now possible to measure the activity of insoluble TI. The destruction of TI activity in defatted soy flakes by live steam at 100°C. was determined by the modified procedure. Samples containing graded levels of TI activity were fed to rats. As TI activity decreased, weight gain, protein efficiency ratio, and nitrogen digestibility rapidly increased and pancreatic hypertrophy decreased. The adverse effects of raw meal as measured by these four biological parameters disappeared at different levels of TI activity in the diet. No pancreatic hypertrophy occurred in rats fed soy flour in which only 55 to 69% of TI activity had been destroyed. Maximum body weight, protein efficiency values, and nitrogen digestibility were obtained with rats fed soy samples in which 79 to 87% of the inhibitors were inactivated. Additional heat treatment did not further improve nutritive value. Our data do not support claims that residual TI in properly processed soy flour has antinutritional properties.

Soybeans contain many heat-labile, biologically active components (1). In general, the degree of improvement in nutritive value of soybean meal and the simultaneous inactivation of the trypsin inhibitors (TI) and other undesirable components depend upon temperature, duration of heating, and moisture conditions (2). Soybean TI account for 30 to 50% of the growth inhibitory effect of raw meal and for nearly all of the pancreatic hypertrophic response in rats (3). However, the minimum destruction of TI activity required to obtain maximum nutritive value was not precisely defined in this earlier study (3), in which TI activity was determined by the procedure of Kunitz (4). Questions concerning the accuracy and reproducibility of current TI assay methods led to the development of an improved procedure to determine TI activity in soy protein products, particularly in heat-processed samples (5).

This paper reports on application of an improved procedure for determining TI activity in dehulled, defatted soy flakes processed with live steam at 100°C. Flakes containing graded levels of TI activity were then subjected to biological evaluation in rats. The parameters evaluated were: weight gain, protein efficiency ratio, nitrogen digestibility, and pancreas weight.

MATERIALS AND METHODS

Preparation of Soy Flakes

Dehulled, defatted flakes were prepared at NRRL from certified seed-grade soybeans (Amsoy variety, 1971 crop) according to previously described

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procedures (6). Flakes containing graded levels of TI activity were prepared by placing raw flakes in a shallow tray in an autoclave preheated to 100°C. and immediately treating with live steam at atmospheric pressure for varying lengths of time. Total time in the autoclave was recorded after the temperature returned to 100°C. after closing the autoclave. The heat-treated flakes were dried in a hood for 24 hr. at room temperature and then ground to a flour in an air-cooled Alpine pin mill. Raw dehulled, defatted soy flour designated N-7-B and heat-treated soy flour (Nutrisoy) were obtained from Archer-Daniels-Midland Co., Decatur, Ill.

Analytical

Nitrogen solubility index (NSI), an index of the amount of heat treatment received by the samples, was determined by the NRRL procedure of Smith et al. (7). Residual oil (also referred to as fat) was determined by regrinding and re-

TABLE I. NITROGEN, PROTEIN, RESIDUAL OIL, AND ASH CONTENT OF DEHULLED, DEFATTED SOY FLOURS[1]

Sample	Nitrogen	Protein (N × 6.25)	Residual Oil (Fat)	Ash
Laboratory soy flour	8.74	54.6	0.21	7.1
Commercial flours N-7-B (raw)	9.49	59.3	0.98	7.6
Nutrisoy (toasted)	9.44	59.0	1.10	7.6

[1]Percent of dry weight.

TABLE II. COMPOSITION OF CONTROL DIET

Ingredients[1]	Percent of Diet
Casein	11.62
Corn oil	8.00
Dextrose	49.63
Salts XIV + Zn + Co	4.73[2]
Cellulose	3.00
Vitamix	2.00
Corn starch	20.00
Water	1.02

[1]Soy ingredients were substituted for casein, dextrose, and water to maintain a 10% protein diet (N × 6.25).
[2]Slight adjustment made in the soy diet to compensate for the greater ash content of the soy samples; diets contain 40 p.p.m. of supplemental Zn as $ZnSO_4$ and 2 p.p.m. Co as $CoCl_2$.

extracting with pentane-hexane according to AOCS Method Bc 3-49 (8). TI activity was determined by the procedure of Kakade et al. (5). Nitrogen, protein, residual oil, and ash content of soy flours are given in Table I. Actual lipid content of dehulled, defatted soy flakes is about 3% (9).

Weanling male rats, S-D strain, separated into groups of five according to weight, were housed in individual cages and fed the diets ad libitum. Composition of the control diet is given in Table II. In the experimental diets, soy flour was substituted for casein and dextrose. Adjustments were made in the addition of water to maintain a 10% protein diet (N × 6.25). A 28-day feeding trial was made.

RESULTS

Effect of Heat on TI Activity and NSI

Results showing the inactivation of TI activity and changes in protein solubility as a function of steaming at 100°C. are given in Tables III and IV, respectively. Initially, TI activity decreases rapidly with 79 to 87% of activity destroyed within 10 min. of steaming. Only 92% of the original activity was destroyed after 30 min.

Results shown in Table III are in contrast to the apparent rapid and more complete destruction of TI activity reported previously (2). In the present study, residual TI activity of heat-processed soybean meal is much higher because, with the improved TI procedure, it is possible to measure the activity of insoluble TI (5). To completely destroy TI activity in these soy samples not only would require much more drastic conditions but also would result in marked impairment in

TABLE III. TRYPSIN INHIBITOR (TI) ACTIVITY OF HEAT-TREATED SOY FLOURS

Heat Treatment min.[1]		TI Activity TIU/mg. Sample[2]		Destruction of TI Activity, %	
Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
(A) 0	(G) 0	88.2	96.6	...	...
(B) 2	(H) 1	52.1	74.9	41	23
(C) 4	(I) 3	27.8	45.0	69	54
(D) 7	(J) 6	15.5	28.0	83	71
(E) 10	(K) 9	11.8	20.5	87	79
(F) 20	(L) 20	7.2	10.1	92	90
...	(M) 30	...	8.0	...	92
	(N) 0		80.4		
	(O) ...[3]		15.3		

[1]Live steam at 100°C. Separate preparations of soy flours were used. Sample numbers corresponding to the soy flours used in feeding trials I and II are shown in parentheses.
[2]TIU = trypsin inhibitor units (Ref. 5), as-is basis.
[3]Moist heat, conditions unknown.

nutritive value which accompanies excessive moist heat treatment. The residual TI activity in commercial sample O indicates that the amount of heat treatment received was equivalent to treating the sample with live steam somewhere between 9 and 20 min. at 100°C. Commercial sample O has an NSI value comparable to that of sample M which was treated with live steam for 30 min. at 100°C. (Table IV). In terms of NSI values, protein solubility decreased much more slowly than inactivation of TI.

Biological Threshold Levels of TI

Two feeding trials were conducted to determine the relative capacity of defatted soy flours containing graded levels of TI activity to inhibit growth, reduce protein efficiency ratio (PER), lower nitrogen digestibility, and enlarge the pancreas. Results given in Tables V and VI show that as TI activity in the diet decreased body weight, PER, and nitrogen digestibility values increased to a maximum and then decreased slightly for soy flours that were treated with live steam for an additional 10 min. Pancreatic hypertrophy was eliminated very rapidly as TI activity decreased.

On the basis of Duncan's multiple range test (10) used to evaluate the data, rats fed soy flour in diets 2 through 4 (Table V) were significantly lower in body weight and PER values compared with the maximum values obtained with diet 6. Low nitrogen digestibility values and pancreatic hypertrophy occurred only in rats fed diets 2 and 3.

Comparable results were obtained in trial II (Table VI). Based on body weight, PER, and nitrogen digestibility values, the biological value of soy flour in which only 71% of the TI activity had been destroyed (diet 12) was not statistically different from that of toasted soy flour (diet 13) in which 79% of the TI had been destroyed. Additional heat treatment (diet 14) tended to lower nutritive value based on these parameters. Normal pancreas weights were obtained in rats fed soy flour in which only 54% of the TI activity was destroyed (diet 11), indicating

TABLE IV. NITROGEN SOLUBILITY INDEX (NSI) OF HEAT-TREATED SOY FLOURS[1]

Soy Sample Number	Heat Treatment min.[2]	NSI
G	0	97.2
H	1	78.2
I	3	69.6
J	6	56.5
K	9	51.3
L	20	37.9
M	30	28.2
N	Minimum	93.9
O	...[3]	26.9

[1]Trypsin inhibitor activity values, see data in Table III, trial II.
[2]Live steam at 100°C.
[3]Moist heat, conditions unknown.

TABLE V. EFFECT OF FEEDING DEFATTED SOY FLOUR CONTAINING GRADED LEVELS OF TI ACTIVITY ON BODY WEIGHT, PER,[1] NITROGEN DIGESTIBILITY, AND PANCREAS WEIGHTS OF RATS—TRIAL I

Diet No.	Dietary Protein[2]	TI Content mg./100 g. Diet	Mean Body Weight (g.) ± Standard Deviation	PER		Nitrogen Digestibility[3] %	Pancreas Weight ± Standard Deviation g./100 GBW[4]
				Actual ± Standard Deviation	Corrected		
1	Casein (0)	0	145 ± 19bc[5]	3.35 ± 0.32a	2.50	93	0.55 ± 0.08bc
2	Soy (0)	887	79 ± 10e	1.59 ± 0.25f	1.19	77	0.70 ± 0.08a
3	Soy (2)	532	111 ± 5d	2.37 ± 0.07e	1.77	80	0.58 ± 0.07b
4	Soy (4)	282	126 ± 8cd	2.78 ± 0.12d	2.07	83	0.50 ± 0.03cd
5	Soy (7)	157	134 ± 13bc	2.97 ± 0.11cd	2.22	86	0.49 ± 0.02cd
6	Soy (10)	119	148 ± 9b	3.08 ± 0.18bc	2.30	85	0.47 ± 0.05cd
7	Soy (20)	71	142 ± 9bc	3.03 ± 0.19bcd	2.26	85	0.45 ± 0.06d
LSD[6]			16	0.21			0.06

[1]PER = protein efficiency ratio = weight gain/g. protein intake; initial weight of rats, 49 g.
[2]Time (min.) of heat treatment shown in parentheses (see also Table III).
[3]Digestibility = intake − fecal nitrogen/intake × 100.
[4]GBW = grams body weight.
[5]Letters not in common denote statistical significance ($P < 0.05$), method of Duncan (10).
[6]LSD = Least significant difference at the 95% confidence level.

TABLE VI. EFFECT OF FEEDING DEFATTED SOY FLOUR CONTAINING GRADED LEVELS OF TI ACTIVITY ON BODY WEIGHT, PER, NITROGEN DIGESTIBILITY, AND PANCREAS WEIGHTS OF RATS—TRIAL II

Diet No.	Dietary Protein[1]	TI Content mg./100 g. Diet	Mean Body Weight (g.) ± Standard Deviation	PER[2]		Nitrogen Digestibility %	Pancreas Weight ± Standard Deviation g./100 GBW[2]
				Actual ± Standard Deviation	Corrected		
8	Casein (0)	0	157 ± 16ab[3]	3.51 ± 0.18a	2.50	92	0.48 ± 0.03c
9	Soy (0)	1,001	84 ± 4f	1.59 ± 0.10f	1.13	74	0.68 ± 0.11a
10	Soy (1)	774	94 ± 8ef	1.89 ± 0.26e	1.35	78	0.58 ± 0.01b
11	Soy (3)	464	123 ± 5d	2.46 ± 0.14d	1.75	77	0.51 ± 0.06c
12	Soy (6)	288	141 ± 12c	2.91 ± 0.12bc	2.07	83	0.52 ± 0.04c
13	Soy (9)	212	146 ± 11bc	3.08 ± 0.15b	2.19	84	0.48 ± 0.06c
14	Soy (20)	104	139 ± 13c	2.92 ± 0.10bc	2.08	83	0.49 ± 0.06c
15	Soy (0)	768	82 ± 8f	1.47 ± 0.30f	1.05	75	0.67 ± 0.12a
16	Soy	147	141 ± 3c	2.84 ± 0.09c	2.02	85	0.49 ± 0.05c
LSD			11	0.17			0.06

[1]Time (min.) of heat treatment is given in parentheses (see also Table III).
[2]Initial weight of rats, 54 g., for other details see Table V.
[3]Letters not in common denote statistical significance ($P < 0.05$), method of Duncan (10).

TABLE VII. CALCULATED TOLERANCE LEVELS OF TI ACTIVITY IN RATS

Biological Parameter	LSD Trial I	LSD Trial II	Level of TI Activity[1] mg./100 g. Diet Trial I	Level of TI Activity[1] mg./100 g. Diet Trial II
Body weight	16	11	225	300
PER	0.21	0.17	240	270
Pancreas weight, g./100 GBW	0.06	0.06	340	430

[1]Level of TI activity which will not significantly affect each biological parameter compared to that obtained for diets 6 (Table V) and 13 (Table VI) of feeding Trials I and II, respectively, based on the LSD values for each feeding trial.

that the rat can tolerate relatively high levels of TI activity before the pancreatic hypertrophic properties of the inhibitors can exert a significant effect.

Body weight, nitrogen digestibility, and pancreas weights of rats fed a commercial soy flour (diet 16) were comparable to the laboratory sample (diet 13). However, the PER value of diet 16 was significantly lower compared to diet 13. Varietal content in respect to the limiting essential amino acids, methionine, and cystine, most likely explains these differences in PER (11).

The maximum dietary level of TI activity that can be tolerated by the rat was determined by plotting body weight, PER, and pancreas weight as a function of the TI content of the diet. Such plots were statistically evaluated by calculating the least significant difference (LSD) for each parameter. Results are summarized in Table VII.

In trial I, the LSD value for mean body weight was ±16 g. Based on this value, rats fed a diet containing 225 mg. TI per 100 g. diet would attain a calculated body weight of 132 g. which would not be significantly different from a body weight of 148 g. for rats fed diet 6. Similar calculations for PER and pancreas weight, which would not be significantly different compared to values obtained with diet 6, indicate that the rats used in trial 1 can tolerate 240 and 340 mg. TI per 100 g. diet, respectively.

The rats used in trial II were able to tolerate even higher levels of TI activity in the diets to achieve body weights, PER values, and pancreas weights which were not significantly different compared to diet 13.

DISCUSSION

Comparative feeding tests with purified soybean TI and protein fractions having very high TI activity indicate that the inhibitors account for about 30 to 50% of the growth-inhibitory effect of raw meal and for nearly all of the pancreatic hypertrophic response (3). Based on data obtained in this present study, a reduction of 40 to 50% in TI activity is required to obtain a relatively large increase in rat growth and PER values of diets containing soy flour.

Recently, Societe Industrielle des Oleagineux (12), a French soy processor, has claimed that soy flours conventionally processed with moist heat contain residual free plus "hidden" TI activity in amounts ranging from 1.5 to 5% of the inhibitor activity of raw soybeans and that the hidden inhibitor affects the growth of young calves. A special process was devised whereby the hidden inhibitor was completely inactivated (12,13) as determined by the analytical assay employed in their investigation (14). However, the French product, Soyassim, contains 4.2 to 4.8% of the inhibitor activity of raw dehulled defatted soy flakes when analyzed by the TI procedure used in this study, compared to about 9% residual activity in a regular commercial meal (5). Since the product still contained residual TI, it would appear that increased protein digestibility as a result of alkali treatment may account for the increased nutritional value claimed for this product.

Dogs readily assimilate diets containing 15% raw soybean meal with no deleterious effect on pancreas weights, pancreatic enzyme secretion, and body weight (15,16). There are also differences in tolerance between different strains of rats (3), as well as between species of animals (1), with respect to growth inhibition and pancreas enlargement when either raw meal or soy TI are fed. These differences between species occur at high levels of TI activity.

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Literature Cited

1. RACKIS, J. J. Biologically-active components. In: Soybeans: Chemistry and technology, ed. by Smith and Circle; Vol. 1, p. 158. Avi Pub. Co.: Westport, Conn. (1972).
2. RACKIS, J. J. Soybean trypsin inhibitors: Their inactivation during meal processing. Food Technol. (Chicago) 20: 1482 (1966).
3. RACKIS, J. J. Physiological properties of soybean trypsin inhibitors and their relationship to pancreatic hypertrophy and growth inhibition of rats. Federation Proc. 24: 1488 (1965).
4. KUNITZ, M. Crystalline soybean trypsin inhibitors. J. Gen. Physiol. 29: 149 (1946).
5. KAKADE, M. L., RACKIS, J. J., McGHEE, J. E., and PUSKI, G. A. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem. 51: 376 (1974).
6. SESSA, D. J., HONIG, D. H., and RACKIS, J. J. Lipid oxidation products in full-fat and defatted soybean flakes as related to soybean flavor. Cereal Chem. 46: 675 (1969).
7. SMITH, A. K., RACKIS, J. J., ISNARDI, P., CARTTER, J. L., and KROBER, O. A. Nitrogen solubility index, isolated protein yield and whey protein content of several soybean varieties. Cereal Chem. 43: 261 (1966).
8. AMERICAN OIL CHEMISTS' SOCIETY. Official and tentative methods (3rd ed.). The Society: Chicago, Ill. (1965).
9. HONIG, D. H., SESSA, D. J., HOFFMANN, R. L., and RACKIS, J. J. Lipids of defatted soybean flakes: Extraction and characterization. Food Technol. (Chicago) 23: 803 (1969).
10. DUNCAN, D. B. Multiple range and multiple F tests. Biometrics 11: 1 (1955).
11. KAKADE, M. L., SIMONS, N., LIENER, I. E., and LAMBERT, J. W. Biochemical and nutritional assessment of different varieties of soybeans. J. Agr. Food Chem. 20: 87 (1972).
12. TECHNICAL BULLETINS, Soyassim™ and Flocosoya™. Societe Industrielle des Oleagineux: Saint-Laurent-Blangy, France.
13. RAMBAUD, M. Method of treatment of soya. U.S. Patent 3,220,851 (July 12, 1965).
14. RAMBAUD, M. Cookery of soybeans. French Patent 1,586,122 (Feb. 13, 1970).
15. PATTEN, J. R., RICHARDS, E. A., and POPE, H. The effect of raw soybean on the pancreas of adult dogs. Proc. Soc. Exp. Biol. Med. 137: 56 (1971).
16. PATTEN, J. R., RICHARDS, E. A., and WHEELER, J. The effect of dietary soybean trypsin inhibitor on the histology of dog pancreas. Life Sci. 10(2): 145 (1971).

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