Germinated Soybean Protein Products: Chemical and Nutritional Evaluation

H.M. BAU and **G. DEBRY**, University de Nancy, 40, rue Lionnois, 54000, Nancy, France

ABSTRACT

Various protein fractions were prepared from nongerminated and germinated soybeans utilizing a combination of calcium chloride and sodium alginate solutions as extractant. Germination tended to improve the nutritional quality of the protein products as measured by protein efficiency ratios (PER of standard rat bioassay). PER values of proteins containing sodium alginate were reduced significantly, which was attributed to inhibition of pepsin proteolysis. Only 30 percent of the trypsin inhibitor activity in raw soybeans was eliminated during germination for three days. Vitamin C content increased from 0 to 25 mg per 100 g (dry basis) during germination.

INTRODUCTION

Mature soybeans processed into flours, concentrates, and isolates, which in turn are converted into a variety of nonfermented and fermented foods, are a primary international protein resource (1,2). Development of food products from germinated soybeans may be another way to further increase the versatility and utility of soybeans. Legume sprouts constitute a good portion of the total consumption of food legumes in the Far East. Germinated soybeans are receiving attention because of the probability that flavor and nutritional qualities may be improved.

In the present study, various protein fractions were prepared from germinated soybeans, since acidic polymers are effective protein precipitants (3) and improve textural characteristics of protein foodstuffs (4,7). The effect of sodium alginate and calcium chloride on protein yield was investigated. Chemical and biological analyses were also made to provide nutritional data.

MATERIAL AND METHODS

Preparation of Protein Products

The procedure used to prepare protein fractions from germinated soybeans is outlined in Figure 1. Whole soybeans (Société Soja-France) were soaked in water at 50 C for 3 hr, places in a humid, dark chamber, and allowed to germinate for 3 days. The seeds were sprayed daily with distilled water and abnormal seeds were discarded.

After three days, the seed coats were removed by flotation, soaked in a 2% ammonium carbonate solution for 2 hr, drained, washed and ground in hot water (90-95 C), using a polytron PT 10-35 grinder (Kinematica, CH-6005, Luzern, Switzerland).

The ground seedlings were slurried in water, heated for 20 min at 100 C, divided into two portions. One portion was treated with calcium chloride and the other portion with $CaCl_2$ plus a 2% sodium alginate (alginate = soybean w/w dry basis). The coagulated curds were washed, drained, pressed, and freeze dried. Protein fractions corresponding to fractions GPF and GPAF were also prepared from nongerminated soybeans and were designated as PF and PAF, respectively.

Chemical Analyses

Ascorbic acid was determined by the method of Bourgeois and Mainguy (8). A micro Kjeldahl procedure was used to measure nitrogen (9). An 80% ethanol extraction (10) was employed to remove sugars and quantitatively determined according to the method of Loewus (11). Lipids were extracted with a mixture of anhydrous diethyl ether and petroleum ether (12). Calcium was determined by atomic absorption (Perkin-Elmer 400 spectrophotometer, Bodenseewerk GMBH, D-777 Ueberlingen, West Germany). Phosphorus analyses were performed by the Fiske and Subbarow procedure (13).

Enzymatic Analyses

Trypsin inhibitor (TI) activity was determined by the method of Kakade et al. (14). except that an extraction time of 1 hr was employed and centrifuged extracts were used to measure activity. Trypsin (2 x crystallized, salt-free) was purchased from Serva (Heidelberg, Allemagne), ref. 37260.

In vitro digestibility measurements with trypsin and pepsin (Serva Corp., ref. 31820) were carried according to Bau et al. (15). Enzyme/substrate ratios were 2 = 1000 and 2.5 = 1000 for pepsin and trypsin, respectively. Enzymatic hydrolysis was terminated by the addition of 0.8 M trichloacetic acid. The measure in the amount of nitrogen soluble in 0.8 M TCA, referred to as nonprotein nitrogen (NPN), was used to determine extent of enzyme proteolysis.

Amino Acid Analyses

A Technicon TSM autoanalyzer, model II (Irel and Ltf. Swords Co., Dublin, Ireland) was used to determine amino acid content (16). Samples sealed in tubes under nitrogen were hydrolyzed with 6 N HCl at 110 C for 24 hr. Cystine and methionine were determined by the procedure of Schram et al. (17).

Rat Bioassays

Weanling male rats (Wistar strain) at 28 days of age, separated into groups of ten, weighing 55-65 g, were housed in individual cages and fed ad libitum. The rats were weighed twice a week. Protein efficiency ratios (PER) were calcu-

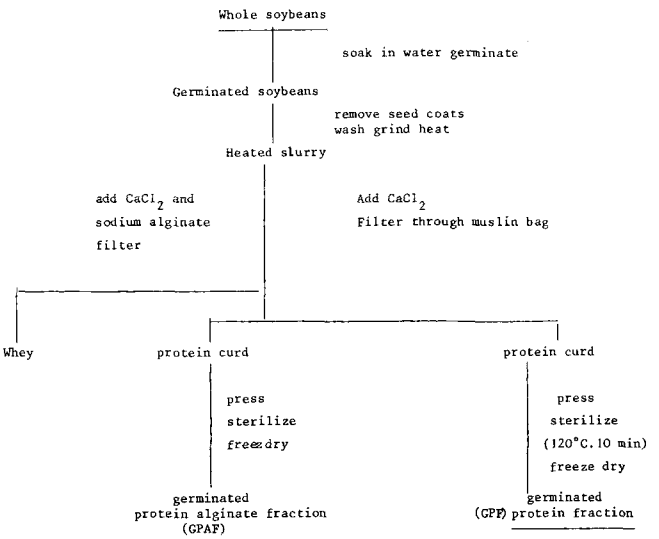

FIG. 1. Preparation of protein fractions from germinated soybeans.

TABLE I

Proximate Content of Protein Fractions from Germinated and Nongerminated Soybeans and Nitrogen Recovery Value (Dry Basis)

Sample[a]	Nitrogen	Fat %	Ethanol-Soluble sugars %	Ash %	Ca %	Dry wt. recovery %	Nitrogen recovery %
RSF[b]	6.69	21.8	12.8	4.7	0.18	---	---
PF	7.65	22.6	0.7	6.8	---	52.1	59.0
PAF	7.38	22	0.8	6.9	---	78.8	87.0
GPF	7.02	19.6	0.3	5.7	2.04	50.2	51.8
GPAF	7.07	20.0	0.3	5.9	2.18	78.1	82.7

[a]See text for details.

[b]RSF = raw soy flour prepared from whole soybeans.

TABLE II

Phosphorus Contents of Soy Protein Fractions and Germinated Soybeans (Dry Basis)

Sample[a]	Total phosphorus (mg/100 g)
RSF	820.0
PF	588.2
PAF	484
GPF	526
GPAF	548.4
GS_1[b]	740.5
GS_2	624.3
GS_3	689.4

[a]See text and Figure 1 for details.

[b]GS_1, GS_2, GS_3 = soybeans germinated 1, 2 and 3 days, respectively.

TABLE III

Essential Amino Acid Composition of Protein Fractions from Germinated Soybeans[a] (mg/16 g nitrogen)

Amino acid	Protein fractions			
	RSF	PF	PAF	GPAF
Methionine	1.7	1.3	1.4	1.6
Cystine	1.0	0.9	0.9	1.0
Lysine	6.0	5.7	6.2	5.6
Threonine	4.0	3.1	4.0	3.7
Valine	4.7	4.4	4.7	4.9
Isoleucine	4.6	4.4	4.8	4.5
Leucine	8.9	7.7	8.8	8.0
Phenylalanine	4.4	5.5	5.5	4.8

[a]See text and Figure 1 for details.

TABLE IV

Soybean Tripsin Inhibitor Activity

Sample[a]	Trypsin units inhibited TIU/mg sample
RSF	107.5
TSF	5.9
PF	1.4
PAF	1.2
GPF	1.2
GPAF	1.1
SS	74.3
GS_1	69.7
GS_2	66.2
GS_3	59.2

[a]RSF- raw full-fat soybean flour –TSF- toasted full-fat soybean flour – SS : whole soybeans soaked in warm water at 50 C for 3 hr – GS_1: one day germinated soybeans – GS_2: two days germinated soybeans – GS_3: three days germinated soybeans. All the samples were freeze dried and ground to powder (100 mesh). See Figure 1 for other details.

TABLE V

Nutritive Value of Soybean Protein Fractions

Diet	PER[a] ±	
	Diet (± SD)	Diet + 0.2% Methionine
Casein	2.5 ± 0.24	---
GPF	2.06 ± 0.10	2.51 ± 0.24
GPAF	1.42 ± 0.19	2.41 ± 0.24
PF	1.85 ± 0.08	2.37 ± 0.21
PAF	1.63 ± 0.13	2.24 ± 0.22

[a]Corrected on a basis of PER = 2.5 for casein.

lated for both experimental and standard diets (9); other diets were supplemented with 0.2% DL-methionine (g methionine/g protein, dry basis).

RESULTS AND DISCUSSION

Composition of Protein Fractions

Proximate analyses and nitrogen recovery values of the various protein fractions are summarized in Table I. Protein content (N x 6.25) for fraction PF, prepared from ungerminated soybeans was somewhat higher than that of the other protein fractions. Fat content also varied slightly. Ethanol soluble carbohydrates were reduced to low levels as a result of processing. The most significant observation was that nitrogen recovery was greatest with protein fraction PAF and GPAF, which involved the use of both calcium chloride and sodium alginate. Apparently, the formation of protein alginate complexes cross-linked to calcium ions resulted in improved protein yields. Germination tended to decrease protein recovery which may be attributed to in-situ proteolysis.

Phosphorus-Vitamin Changes during Germination

Over 90% of the phosphorus in soybeans is in the form of phytic acid; whether phytic acid exists either as phytate salts or phytateprotein mineral complexes, reduced mineral bioavailability depends upon several factors (18). The effect of germination on the phosphorus content of the various protein fractions was evaluated. Results are given in Table II. A net loss of ca. 28-36% phosphorus originally present in raw soy flour (RSF) was observed when soybeans were processed with calcium chloride and sodium alginate; smaller losses occurred during germination. Soybeans are apparently devoid of Vitamin C. After three days of germination, Vitamin C content increased to about 25 mg/100 g dry matter.

Amino Acid Composition

Essential amino acid content of the various protein fractions, compared with that of raw soy flour, is presented in Table III; only small differences were observed.

Nutrition Evaluation

In vitro digestibility. The presence of sodium alginate (PAF and GPAF) greatly inhibited pepsin digestion, whereas trypsin digestion was not inhibited. When comparing the effect of germination, both GPF and TSF were hydrolyzed to the same extent by pepsin, whereas toasted soy flour (TSF) prepared from nongerminated soybeans was poorly digested with trypsin compared with germinated protein fraction (GPF). Therefore, if the combined effect of pepsin and trypsin proteolysis were taken into account, it would appear that germination may not increase protein digestibility significantly.

Trypsin inhibitor activity. TI values are given in Table IV. Compared to raw soy flour, ca. 30% of the TI activity was eliminated by the soaking process (sample SS). Comparable reduction in TI activity was obtained during germination. All of the processed protein fractions contained very little residual TI activity.

Rat Bioassay

The nutritional value of germinated soybean protein fractions, as measured by PER, are given in Table V. Protein fractions, processed with sodium alginate (GPAF and PAF), had the lowest PER values. The growth inhibitory effect of sodium alginate may be attributed to inhibition of pepsin proteolysis. Supplemental methionine increased considerably the values of all diets. The greatest supplemetary effect was obtained with diet containing GPAF and PAF proteins.

These results indicate that sodium alginate form enzyme resistant linakges with methionine residues. In comparing the PER values of diets containing GPF and PF proteins, germination improves slightly the nutritional value of soy protein.

ACKNOWLEDGMENT

The authors express their thanks to Dr. J.J. Rackis (Oilseed Crops Laboratory, Peoria, IL) for assistance in correcting this paper, and the clarity of scientific presentation; Professor M.L. Chapon for the help in phosphorus determinations.

REFERENCES

1. "Proceedings World Soy Protein Conference," JAOCS 51:1 (1974).
2. "Soybean: Chemistry and Technology," Vol. 1, Edited by A.K. Smith and S.J. Circle "Proteins", Avi Publishing, Westport, CT, 1972.
3. Gilberg, L., and B. Tornell, J. Food. Sci. 41:1070 (1976).
4. Luh, N., J.M. Flink, and M. Karel Ibid. 42:976 (1977).
5. Tolstogusow, W.B., Colloid Polym. Sci. 253:109 (1975).
6. Castaigne, F., R.R. Riel, and M. Boulet, J. Inst. Can. Sci. Technol. Aliment. 8:129 (1975).
7. Arima, T., I. Osaka, and Y. Herada, U.S. Patent 3627536, 1968.
8. Bourgeois, C.F., and P.R. Mainguy, Analysis. 2:556 (1973).
9. AOAC, Official Methods of Analysis, Washington, DC, 1970.
10. Bau, H.M., B. Poullain, and G. Debry, Ann. Nutr. Aliment. 32:255 (1978).
11. Loewus, F.A., Anal. Chem. 24:219 (1952).
12. Lees, R., "Laboratory Handbook of Methods of Food Analysis," C.R.C. Press, Cleveland, OH, 1971, p. 110.
13. Fiske, C.H., and Y. Subarrow, J. Biol. Chem. 66:375 (1975).
14. Kakade, M.L., J.J. Rackis, J.E. McGhee, and G. Puski, Cereal Chem. 51:376 (1974).
15. Bau,H.M. B. Poullain, M.J. Beaufrand, and G. Debry, J. Food Sci. 43:106 (1978).
16. Moore, S., D.M. Spackman, and N.H. Stein, Anal. Chem. 30:1185 (1958).
17. Schram, G.S., S. Moore, and E.J. Bigwood, J. Biochem. 57:33 (1954).
18. Rackis, J.J., and R.L. Anderson, Food. Prod. Dev. 11 (10): 38 (1977).

Effects of Soy Proteins Containing Trypsin Inhibitors in Long Term Feeding Studies in Rats

J.J. RACKIS and **J.E. MCGEE**, Northern Regional Research Center, Agricultural Research, SEA/USDA, Peoria, IL 61604, **M.R. GUMBMANN** and **A.N. BOOTH**, Western Regional Research Center, Agricultural Research, SEA/USDA, Berleley, CA USA

ABSTRACT

Pancreatic hypertrophy that occurs in rats fed raw soy flour containing about 1200 mg tripsin inhibitor (TI)/100 g diet was reversed by switching the rats to control diets or to diets containing 30% toasted defatted soy flour. No pancreatic hypertrophy occurs in rats fed commercial, edible grade soy flours, concentrate or isolate from time of weaning to adulthood (ca. 300 days). TI content of the soy diets ranged from 178-420 mg/100 g. Except for pancreas enlargement in rats fed raw soy flour, gross and microscopic examination of pancreata revealed no abnormalities. The gross appearance of heart, kidney, spleen, and liver was normal. Soy flour, protein concentrate, and protein isolate in a formulated corn-soy diet provided optimum growth and maintained body weight only if supplemented with vitamin B-12 in long term feeding studies with rats. In the absence of such supplementation, rats fed soy diets initially grew at a rate equal to or greater than those fed a comparable corn-casein control diet; but, with continued feeding for ca. 300 days, body weight of rats fed the casein control was significantly greater than that of the soy flour-fed rats. Those fed soy isolate ceased to grow; and rats fed soy concentrate lost weight. No significant differences were found in organ weights between groups fed soy products and casein, except for increased kidney, liver, and testes weights relative to body weight with the group fed soy concentrate. Supplementation of the soy diets with vitamin B_{12} stimulated growth to the greatest extent, calcium pantothenate or riboflavin had an intermediate effect, other vitamins had little or no effect; whereas a complete mineral mix was detrimental. Supplementation of the soy diets with vitamin B_{12} stimulated growth to the greatest extent, calcium pantothenate or roboflavin had an intermediate effect, other vitamins had little or no effect; whereas a complete mineral mix was detrimental. Supplementation of the control diet was without effect. The dietary protein level in these diets was 20%, with casein or soy protein representing 75% of total protein. When fed continuously to rats from weaning to adulthood, properly processed soy protein products, when balanced