Effect of Processing Conditions on the Nutritive Value of Isolated Soybean Proteins

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The effect of processing conditions on the protein nutritive value, the methionine and available lysine content, and the antitryptic activity of two types of isolated soybean protein and of intermediate fractions obtained during protein isolation procedure was investigated. Protein efficiency ratios (PER) of the extraction mixture, of the filtered extract, and of the isolated proteins were inferior to those of the original meal. PER of isoelectrically precipitated protein and of calcium salt coagulated protein were practically identical. PER of spray-dried isoelectric proteins was significantly higher than that of the same proteins when freeze-dried. Toasting of the spray-dried proteins did not improve their nutritive value. PER of isolates from unheated and from toasted meal showed identical values.

Isolated soybean protein is a relatively new food ingredient in Western countries. Its high protein content, bland taste, and white color make it far superior to soybean flours for many purposes. Interesting functional properties such as whipping, foaming, gel formation, water and fat retention may be imparted to isolated soybean protein and its derivates (Circle and Johnson, 1958). Lately, fiberlike structures have been prepared by spinning techniques. The use of such structures in simulated meat products seems promising (Buller and Klis, 1965). In the Orient, food products similar to isolated soybean protein have been used for many centuries.

In the U.S., isolated soybean protein is produced in the following manner (Anson and Pader, 1957): Defatted, unheated soybean flakes are extracted in water under alkaline conditions. The extract is clarified. Protein is precipitated by adding acid to pH 4.5 approximately. The precipitate is washed and spray dried. In the Far East, whole soybeans are extracted with water. The extract (milk) is coagulated at boiling temperature in the presence of calcium salts. The best known product prepared in this way is tofu (Watanabe, 1964).

Nutritionally, isolated soybean protein is said to be inferior to the "native" protein in soybean meal or flour (Bloch and Weiss, 1956; Huge, 1961; Rackis *et al.*, 1961). The amino acid composition of the isolated protein is different from that of soybean flour (Huge, 1961; Rackis *et al.*, 1961), the most significant difference being a lower methionine content in the isolated protein. Supplementation of isolated soybean protein with 0.3% methionine brought its PER value above that of casein (Central Soya Co.). Thus, existing experimental evidence supports the claim that the inferiority of isolated soybean protein is due to its lower methionine content, but this explanation may be only partial. The nutritive value of soybean products as protein foods is strongly affected by the presence of antimetabolites such as the soybean trypsin inhibitors. Furthermore, many operations in the process of isolation, such as alkali treatment, heating, and drying may also affect the nutritive value of the product as a protein source.

The object of this work was to determine the effect of such operations on the nutritive value of the final product. Specifically, it was intended to answer what effect the alkali extraction, precipitation, and drying steps have on the nutritive value of soybean protein; what difference is there between the nutritive values of isoelectrically precipitated (modern) and calcium-coagulated (Oriental, traditional) proteins; and what effect the heat treatment history of soybean oil meal has on the nutritive value of isolated protein prepared from it.

EXPERIMENTAL

Preparation of Materials. Unheated meal (U.M.) was prepared by extracting soybean flakes with hexane in an industrial extractor and desolventizing in open air. Eighty-two per cent of the nitrogen of this meal was dispersible in water (Paulsen *et al.*, 1960).

Toasted meal (T.M.), commercial toasted soybean oil meal with a nitrogen dispersibility index of 20.8%, was used.

Extraction Mixture. Twenty kilograms of unheated meal were mixed with 200 liters of 0.03*M* solution of calcium hydroxide and agitated for 30 minutes at 55° C. Final pH was 9.5 to 9.8. The resulting mixture of extract and residue is called extraction mixture. When used in feeding tests, the material was finely ground in a colloid mill, neutralized to pH 6.5 with hydrochloric acid, and spray-dried.

Extract. The extraction mixture was screened through a 60-mesh sieve. The filtrate is called extract. Whenever used in feeding tests, the material was neutralized to pH 6.5 and spray-dried.

Isoelectric soybean protein was prepared by precipitation from the extract at pH 4.5. The precipitate (curd) was washed twice with warm water and dried (spray- or freeze-drying).

Calcium coagulated protein was precipitated from the extract at 90° to 100° C. by admixture of 0.33% calcium chloride, washed twice, and dried (spray- or freeze-drying).

Commercial protein was a sample of commercial soybean protein, "Trate" (Hercules Powder Co., Wilmington, Del.).

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Spray-drying was carried out in a Production Minor unit by Niro, Copenhagen. The air temperature was 200° C. at the inlet and 95° C. at the outlet.

Freeze-drying was done in a Vickers-Armstrong pilot unit. The plate temperature was manually adjusted to keep the product surface temperature below 40° C. at all times.

Subsequent heating was autoclaving—heating in an autoclave under direct steam at 121° C. for 15 minutes; and roasting—heating in an oven at 120° C. for 30 minutes.

Feeding Tests. The procedure for PER determination recommended by A.O.A.C. (Horwitz, 1960) was followed with minor changes as to the source of fat and carbohydrates. Weanling albino rats of the Charles River C.D. strain, age 21 to 24 days, were used. Three males and three females were used for each test material. The animals were housed in individual cages, with food offered *ad libitum*. The test material constituted the sole source of protein in the diet. Its amount was so calculated as to give approximately 10% protein (N × 6.25) in the diet mixture. Test duration was 28 days.

Analytical Procedure. Nitrogen was determined by the macro-Kjeldahl method (Lepper, 1945).

Available lysine was estimated by the dinitrophenyl condensation method as described by Baliga *et al.* (1959).

Methionine was determined microbiologically, according to Bloch and Weiss (1956).

Antitryptic activity was determined according to Kunitz (1947), following the modifications suggested by Laskowski (1955). Results are given as absorbance increase per minute per gram protein; the activity of the raw unheated meal being almost 100 units, the results may be interpreted as percentage activity in the raw meal.

RESULTS AND DISCUSSION

The protein sources tested, group averages of food consumption, weight gain data, and PER values from 28 days' feeding tests are summarized in Table I with the protein, methionine, and available-lysine contents and the antitryptic activity of the protein sources. PER values were compared using the t test, with significance level fixed at $p = 5\%$.

Effect of the Extraction Operation. Comparison of PER values of the original meal, extraction mixture, and extract should furnish information on the nutritional significance of the extraction step. The extraction mixture (3) was definitely inferior to the original meal (1). Apparently, this cannot be attributed to processing damage to the protein during alkali extraction. No considerable

Table I. Description of Soybean Materials, Nutritional Data, and 28 Days' Feeding Test Results

Material No.	Description[a]	Starting Material[b]	Drying Method	Heat[a] Treatment before Feeding	% Protein, N × 6.25	Methionine, G./16 G. N	Avail. Lysine, G./16 G. N	Anti-tryptic Activity Units	Food Consumption, G.	Weight Gain, G.	PER
0	Meal	U.M.	...	...	49.3	1.29	3.51	96.0	...	...	...
1	Meal	U.M.	...	Autoclaving	49.3	1.25	3.06	7.4	498	134	2.49
2	Meal	T.M.	...	...	50.3	1.28	3.07	0.0	455	112	2.48
3	Extraction mixture	U.M.	Spray	Roasting	50.2	1.21	3.23	51	376	75	1.86
4	Extract	U.M.	Spray	Roasting	59.0	1.14	3.55	21	337	62	1.70
5	Extract	T.M.	Spray	Roasting	50.3	1.02	3.25	20	342	68	1.92
6	Isoelectric protein	U.M.	Freeze	...	89.0	1.08	5.10	25	372	75	1.86
7	Isoelectric protein	U.M.	Spray	...	82.7	1.10	4.81	16	360	75	2.06
8	Isoelectric protein	U.M.	Spray	Roasting	82.7	1.02	4.82	13	353	74	1.98
9	Isoelectric protein	T.M.	Spray	...	89.7	0.98	4.52	0.0	355	73	1.98
10	Ca-coagulated protein	U.M.	Freeze	...	84.7	1.10	4.76	4.8	317	64	1.91
11	Ca-coagulated protein	U.M.	Spray	...	83.9	1.05	4.58	5.2	308	65	1.94
12	Ca-coagulated protein	U.M.	Spray	Roasting	83.9	1.05	4.65	3.2	300	61	1.88
13	Ca-coagulated protein	T.M.	Spray	...	84.3	1.03	4.65	0.0	312	67	2.02
14	Commercial	?	?	...	93.8	1.36	5.31	34.2	386	83	1.97
15	Casein				88.3	...	...	...	404	121	2.83

[a] See text for definitions.
[b] U.M. = unheated meal; T.M. = toasted meal.

loss of methionine, and none of available lysine, was observed as a result of this operation. Furthermore, PER values of spray-dried isoelectric protein (7) and the spray-dried and toasted isoelectric protein (8) were significantly higher than that of the extraction mixture from which they had been prepared. The antitryptic activity of the extraction mixture was high, despite spray-drying and subsequent roasting of the material. Apparently, some of the antitryptic activity remains in the extraction residue since the antitryptic activity of the filtered extract (4) was much lower than that of the extraction mixture.

The inferiority of the extraction mixture and filtered extract as a food may be due to antitryptic activity, probably boosted to the presence of toxic nonprotein components retained in the whey. The excellent amino acid composition of soybean whey proteins (Rackis, 1961; Van Etten *et al.*, 1959) on the one hand, and the poor nutritive value of this whey (Rackis, 1961) on the other, seem to indicate an influence of such toxic factors. Hackler *et al.* (1963) reported on the toxic action of soybean whey. Fifty per cent mortality occurred among their soybean whey–fed rats. Adverse effects of soybean whey on calves have also been observed (McKinney, 1964). The only cases of death observed by the present authors were similarly confined to group-fed extraction mixture solids (3) and extract (4).

Thus, feeding experiments with whole extraction mixture or with filtered extract fail to furnish information on the magnitude of direct damage (if any) caused to soybean by the alkali extraction process.

Effect of Precipitation Method. PER values of isoelectrically precipitated proteins (7, 8) were essentially identical with those of calcium salt coagulated proteins (10, 11, 12), provided the former were properly heated, spray-dried, or toasted (see next section). No differences were observed between the samples mentioned in this section with regard to methionine and available lysine. Antitryptic activity was higher in the isoelectric proteins. The high temperature (90° to 100° C.) maintained during precipitation of the calcium-coagulated proteins sufficed to reduce its antitryptic activity.

Effect of Drying and Subsequent Heating. PER value of spray-dried isoelectric protein (7) was significantly higher than that of the same proteins freeze-dried (6). The effect of spray-drying is attributed to depression of the antitryptic activity retained in the curd and to the possible beneficial effect of mild thermal treatment in improving the digestibility of the protein. Further heating (roasting) of the spray-dried powder (8) did not affect PER or antitryptic activity.

Spray-drying and subsequent heating (10,11,12) did not affect the PER of calcium-coagulated protein. This is attributable to the fact that the protein was subjected to thermal treatment during precipitation, which reduced the antitryptic activity to a low level and probably also improved digestibility. Results seem to indicate that spray-drying and toasting did not affect methionine and available lysine in either isoelectric or calcium-coagulated proteins.

Effect of Heat-Treatment of the Meal. Isolated protein obtained from unheated meal did not differ significantly as to PER value from those isolated from toasted meal (8, 9, 12, and 13). However, available lysine and methionine levels were somewhat lower in proteins obtained from toasted meal. Commercial isolation of protein from toasted meal is economically impracticable because of the low yields. Using unheated flakes, 86% of the total nitrogen could be recovered in the curd. In the case of toasted meal, the nitrogen extraction was only 60%, and the nitrogen recovery in the curd was only 52% of the nitrogen originally present in the flakes. The authors' results simply indicate that the nutritive value of the protein fraction obtainable by alkali extraction and precipitated by acid or calcium salts is unaffected by heating the meal.

The sample of commercial soybean protein (14) had a PER value similar to that of the isolated proteins and twice as much antitryptic activity as the isolated proteins (7) prepared in the laboratory. Differences in raw material and in the washing and drying procedures may be the cause.

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