THE USE OF ISOLATED SOYBEAN PROTEINS IN BREAD[1]

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ABSTRACT

The effect of including isolated soybean proteins (isoelectric and Ca-coagulated) in wheat bread on its baking characteristics, acceptance, and nutritive value has been investigated. Admixture of soybean proteins was found to increase the water-absorption capacity of the flour. Loaf volume decreased proportionately with the level of protein addition. However, when 1% lecithin was included in mixes containing less than 6% soybean protein, the decrease in loaf volume could be counteracted. Isolated protein at levels up to 8% did not affect the taste significantly. The nutritive value of the bread samples, measured as protein efficiency ratio (PER), increased with the percentage of soybean protein. The increase in PER was in linear relationship with the lysine content of the bread. It is concluded that isolated soybean protein may be admixed with wheat flour up to the level of 6% as a means of successful nutritional supplementation without significant impairment of over-all acceptability.

Bread, in one form or another, is undoubtedly one of the most widely used food items. It is quite natural, therefore, that whenever improvement of the nutritional status of a bread-eating population is contemplated, bread is a convenient vehicle for introducing the desired factors into the daily diet.

The nutritional deficiencies of wheat proteins have often been demonstrated. In a recent survey, Diser (1) points out that lysine and valine are the first two limiting amino acids of wheat proteins. Since soybean is relatively rich in these two amino acids, the use of soybean flour and other preparations as a means of supplementation has received considerable attention (2,3). However, admixture of soybean flour in bread presents many technological problems. Soybean flour can be used in bread, at a level of 6% or slightly more, without serious impairment of flavor, texture, or loaf volume (4–9), but some alterations in the dough formula or baking procedure are necessary to obtain the best results. These include the use of higher amounts of fats and milk solids (5,6), adjustment of oxidation level (7,8), and inclusion of lecithin (10). When higher amounts of soybean flour are used, however, the bread becomes decidedly less acceptable (5).

Isolated soybean protein presents interesting possibilities as a supplementation medium for wheat bread. Being practically tasteless and odorless, it may be expected to affect the organoleptic characteristics of bread less than soybean flour. Furthermore, isolated protein contains twice as much protein as soybean flour, and therefore the same supplementation effect can be obtained with a smaller admixture.

The object of the present study was to investigate the effect of isolated soybean protein admixture on the baking quality, organoleptic characteristics, and nutritive value of wheat bread.

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Materials and Methods

Commercial Wheat Flour. The wheat flour was commercially milled from a mixture of 60% hard winter, 15% Manitoba, and 25% locally grown soft wheat. The extraction rate was 58%.

Soybean Protein Materials. Soybean proteins were prepared on pilot-plant scale as follows: Soybean oil meal was extracted with 0.03 molar solution of calcium hydroxide at 55°C. for 30 min. The extract was filtered first through a rotary screen (1.5-mm. aperture diam.) and then through successive sieves of mesh sizes 20, 40, 60, and 80.

Two types of proteins were precipitated from the filtered extract: (a) isoelectric soybean protein (ISP), precipitated at room temperature by adjusting the pH of the extract to pH 4.5 with hydrochloric acid, and (b) Ca-coagulated protein (CCP), precipitated at 90°–100°C. by addition of $CaCl_2$ (0.33 w./v.). Both types were separated from the whey by decantation, washed several times with water, resuspended in water, homogenized, and spray-dried at 100°–105°C.

The soybean flour used in feeding tests was a commercial product used by the baking industry for bread supplementation. It is prepared by grinding soybean meal from untoasted or very lightly toasted nondehulled beans and contains calcium carbonate and riboflavin admixtures.

Analytical data for commercial wheat flour, isolated soybean proteins, and commercial soybean flour are as follows:

Sample	*Protein (N × 5.7)*	*Moisture*	*Ash*
	%	%	%
Commercial wheat flour	11.5	12.1	0.37
Isoelectric-precipitated soybean protein (ISP)	78.7	10.0	2.10
Ca-salt-coagulated soybean protein (CCP)	76.0	....	8.80
Commercial soybean flour	38.5	....	....

Semifluid, unbleached commercial soybean lecithin (acetone-insoluble matter 67%) was used.

Analytical Procedures. Nitrogen was determined by the AOAC macro-Kjeldahl method (11). Ash, calcium, and moisture were determined according to *Cereal Laboratory Methods* (12).

Lysine was determined according to the microbiological assay procedure of Barton-Wright (13).

Physical Dough Tests. The effect of admixture of soybean proteins, at levels of 2, 4, 6, 8, and 10%, on water absorption of wheat flour was evaluated by farinograph tests in the usual manner (12). Farinograms were obtained by mixing 50 g. of blend in a small bowl (64-r.p.m. drive) for 15 min. with distilled water, to give the maximum consistency around 500 B.U. The farinograph absorption reported is the amount of water added to the blend to yield the mentioned consistency.

Experimental Baking Tests. These tests were carried out according to the standard formula and straight-dough baking procedure described in *Cereal Laboratory Methods* (12) except for fermentation, proofing, and baking times, which were modified according to suggestions by Adler and Pomeranz (10). The formula and procedures used in these tests are given below.

Baking Formula
% of wheat flour, 14% m. b.

	%
Flour (blend)	100.0
Yeast	3.0
Sugar	5.0
Salt	2.0
Water	variable

Procedure

Mixing time (Kenwood "Chef" mixer) (min.)	2
Bulk fermentation (min.)	120
Fermentation at (°C.)	30
and r.h. (%)	90
Proofing time (min.)	75
Baking time at 220°C. (min.)	30

Pilot-Plant Baking Procedure. Relatively large quantities of bread were needed for taste panels and animal feeding tests. These were baked at the experimental bakery of the Ministry of Commerce and Industry according to the formula and procedures given below. Lecithin was added only to the formula containing soybean material.

Baking Formula

	g.
Flour (blend)	800
Yeast	24
Sugar	40
Salt	16
Lecithin	8
Water	variable

Baking Procedure

Mixing time (Hobart A-200) (min.)	5
Bulk fermentation (min.)	120
Fermentation at (°C.)	30
and r.h. (%)	90
Proofing time (min.)	75–55
Baking time at 220°C. (min.)	40

Duplicate pan loaves were baked from each mix. Loaves were scaled at 580 g. and baked so as to weigh 500 g. Volume was measured with a National Loaf Volumeter, 1 hr. after baking, and loaves were examined after 24 hr. for other characteristics.

"Baking absorption" refers to the amount of water which had to be added to the flour to yield the highest loaf volume, all other conditions remaining unchanged.

Evaluation of Organoleptic Characteristics. The loaves were sliced 24 hr. after baking and offered to a 19-member panel for evaluation, according to the following scale: excellent, +2; good, +1; acceptable, 0; poor, —1; very poor, —2.

Each of the following factors was considered separately: taste, texture, and odor. The slices were cut to uniform size and presented to the panel in a partly darkened room, to eliminate any influence of color and loaf volume on the rating.

Feeding Tests. Albino rats (Charles River C.D. strain) at the age of 21 days (weight range 45–52 g.) were used. Randomized groups of eight animals (four males and four females) were formed. Each group was fed a different diet. The animals were housed in individual cages in an air-conditioned room (24°C., 50–60% r.h.). Food and water were offered *ad libitum*. The animals were weighed and their food consumption was measured weekly. The total test period was 28 days.

The procedure for PER determination recommended by AOAC (11) was followed, with minor changes in the diet (corn oil replaced with soybean oil, corn starch used instead of sugar).

The material under test was fed as the sole source of protein at the 10% protein level. The diet was composed as follows to give protein as indicated (approximate; see Table V).

	%
Protein source (bread)	10
Soybean oil	8
Cellulose	1
Minerals mixture USP XVI	5
Vitamin mixture	1
Corn starch, to make	100

"Vitamin-free" casein was employed as the protein source in the control diet. The bread used for the tests was sliced, dried in a current of air at 45°C. to a final moisture of 8–10%, and ground. Both crust and crumb were included in the diets.

At the end of the feeding period, three male and three female animals from each diet group were dissected and their livers weighed. The livers were dried in a vacuum oven at 70°C. for determination of their dry-matter content, and then extracted with diethyl ether for evaluation of ether-extractable matter.

Results

Baking Properties. (a) *Water absorption.* Admixture of soybean proteins

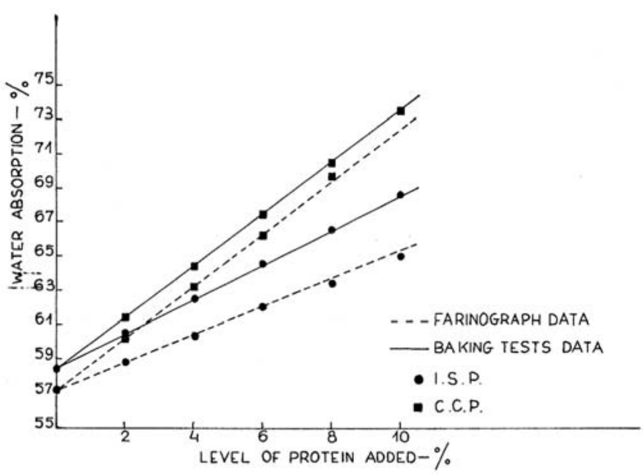

Fig. 1. Water absorption of blends.

caused an increase in the water absorption capacity of the blends. Water absorption values, determined by baking and farinograph tests, are shown graphically in relation to the level of protein added (Fig. 1).

The increase in water absorption per 1% added protein was 1% for ISP, which is quite similar to the increase in baking absorption reported elsewhere for soybean flour (5–8). Admixture of CCP resulted in a higher rate of increase in absorption capacity: 1.5% per 1% added protein.

(b) *Loaf volume: effect of bromate and lecithin.* Inclusion of soybean protein in the baking formula reduced loaf volume considerably. This effect could not be counteracted by inclusion of potassium bromate, since all the blends containing soybean proteins seem to possess a "negative bromate requirement" (Fig. 2).

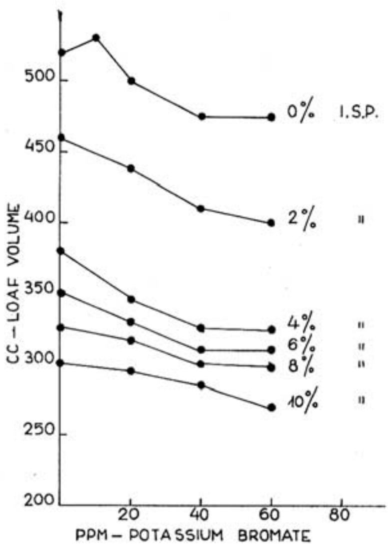

Fig. 2. Response of loaf volume to bromate in breads containing ISP.

Inclusion of lecithin up to a level of 1% resulted in a significant increase in loaf volume. Higher levels of lecithin failed to improve the volume and imparted an objectionable flavor to the bread.

Results of laboratory-scale baking tests are shown in Fig. 3. Data obtained from pilot-plant scale runs are given in Table I.

Organoleptic Characteristics. Average scores assigned by the panel to breads containing both types of isolated soybean protein at different levels are diagrammatically shown in Fig. 4. Shaded rectangles represent the samples found to be significantly different from the control, at 95% confidence level.

Crumb color was not measured quantitatively, but qualitatively it can be stated that admixture of both types of proteins resulted in darkening. This effect increased with increasing levels of protein admixture. The color im-

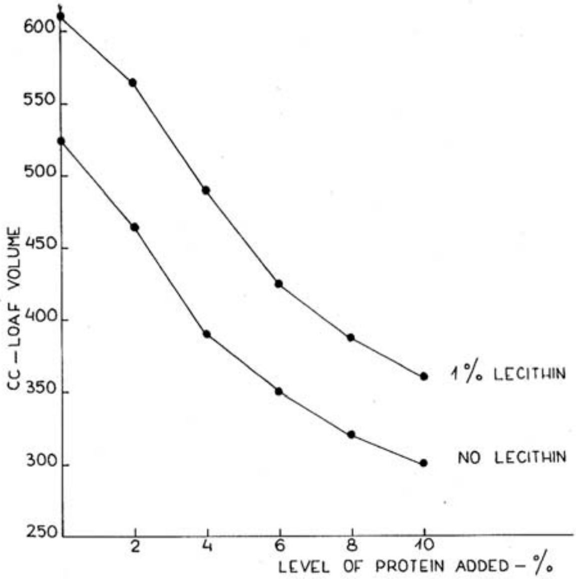

Fig. 3. Response of loaf volume to lecithin in breads containing ISP.

TABLE I
BAKING DATA FROM PILOT-SCALE RUNS

Level of Protein Added	Baking Absorption	Lecithin	Loaf Volume[a]	Volume
%	%	%	cc.	*% of control*
0 (control)	58.5	0	2,150	(100.0)
0 + lecithin	58.5	1	2,520	117
ISP 2 + lecithin	60.5	1	2,275	106
4 + lecithin	62.5	1	2,025	94.5
6 + lecithin	64.5	1	1,575	73.5
8 + lecithin	66.5	1	1,325	61.5
10 + lecithin	68.5	1	1,137	53.0
0 (control)	58.5	0	2,125	(100.0)
CCP 2 + lecithin	61.5	1	2,175	102.0
4 + lecithin	64.5	1	1,965	92.5
6 + lecithin	67.5	1	1,775	83.5
8 + lecithin	70.5	1	1,600	75.0
10 + lecithin	73.5	1	1,375	64.5

[a] Mean of duplicates. The standard error of the mean computed for all sets of duplicate values was 35 cc. A minimum difference at 197 cc. between the means is required to be significant at 95% confidence level.

parted can be described as creamy-gray for ISP and gray for CCP.

Feeding Tests. Protein and lysine contents of the different breads and diets, together with the average PER values, weight gains, liver weight, and other data are given in Table II. Level of L-lysine per g. nitrogen plotted against PER is shown in Fig. 5. Protein efficiency ratio is defined as the ratio of the gain in body weight to the amount of protein ingested by the animal during the test period.

TABLE II
Feeding Test Data

Soybean Protein in Bread: Type	Soybean Protein in Bread: Level	Protein in Bread[a] N × 5.7	Lysine[b] in Bread	Bread in Diet	Protein in Diet N × 5.7	Lysine[c] in Diet	Average Weight Gain per Animal	PER[d] 0 to 28 Days	Weight of Fresh Liver per 100 g. Body Weight[e]	Dry Matter in Liver[f]	Ether-Extractable Matter in Liver (Dry Basis)[g]
	%	%	%	%	%	%	g.		g.	%	%
ISP	0	11.5	0.28	87	9.67	0.24	36.1	1.46	4.60	28.2	13.1
	2	12.8	0.37	78	10.54	0.29	54.5	1.80	4.31	28.0	13.8
	4	14.0	0.44	71	10.29	0.32	60.1	1.91	4.65	28.6	14.4
	6	15.0	0.51	67	10.56	0.34	71.0	2.02	4.26	28.5	14.0
	8	16.5	0.60	61	10.05	0.37	70.0	2.12	4.53	27.7	14.6
	10	17.7	0.69	56	10.17	0.39	85.7	2.26	4.38	27.9	14.5
CCP	2	12.8	0.35	78	10.18	0.28	45.4	1.78	4.99	27.6	12.0
	4	13.7	0.44	73	10.56	0.32	53.5	1.91	5.00	26.9	13.6
	6	15.1	0.51	66	10.25	0.33	60.2	1.98	5.02	27.4	13.3
	8	16.1	0.59	62	10.43	0.37	77.4	2.12	4.96	26.9	13.6
	10	17.1	0.65	58	10.45	0.38	100.5	2.25	4.47	27.1	15.2
Soybean flour	5	12.9	0.38	77	11.60	0.29	55.3	1.62	5.18	26.4	10.6
	15	14.7	0.55	68	10.50	0.37	66.7	1.89	5.32	26.4	11.3
Casein (control)		....	....	....	N×6.38 10.23	....	111.7	2.98	4.03	28.5	9.6

[a] "Bread" means dried bread powder containing 9% moisture.
[b] Determined as free L-lysine.
[c] Calculated from lysine.
[d] Std. error of means computed for all sets of measurements was 0.06.
[e] Std. error, 0.505.
[f] Std. error, 0.204.
[g] Std. error, 1.130.

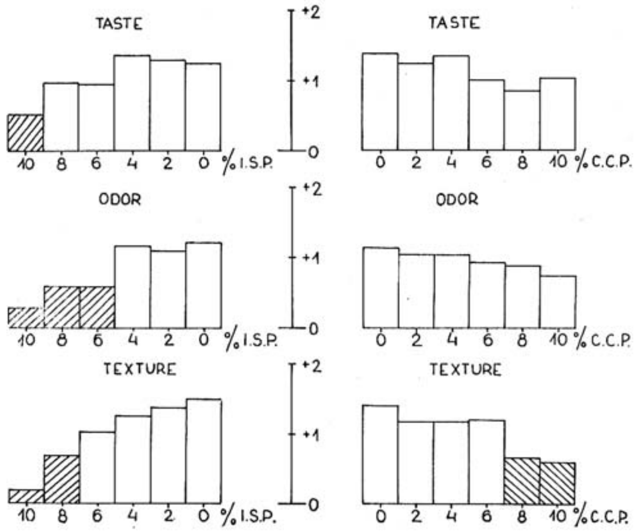

Fig. 4. Average scores given by the panel to breads.

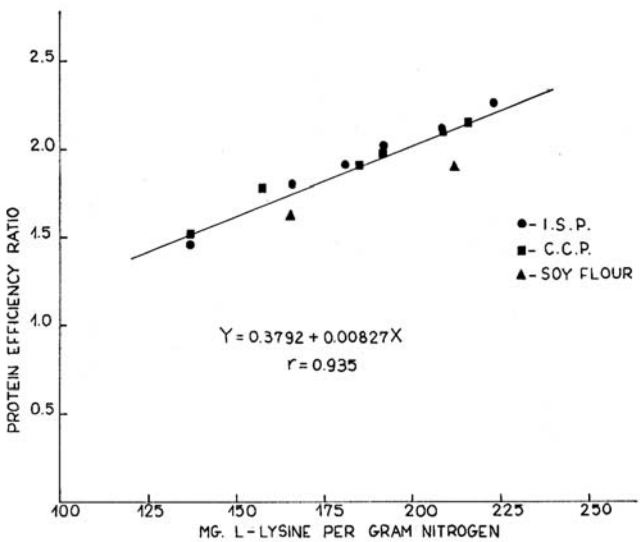

Fig. 5. Effect of lysine content of bread diets on PER.

Discussion

Baking Characteristics and Acceptability. The possibility of using isolated soybean protein in bread without serious impairment of loaf volume, taste, color, and odor is made evident by the results.

Inclusion of 1% of lecithin in the mix permits admixture of soybean protein of either of the types investigated, without significant reduction in loaf volume, provided the level of protein added is less than 6%. For the same range of admixture, the crumb texture remains unaffected. From the point of view of taste and odor, even higher levels can be achieved without undesirable effects (8% for ISP, 10% for CCP).

One may conclude, therefore, that the over-all acceptability of bread is not impaired when up to 6% of the flour is replaced with isolated soybean protein. To achieve the same protein supplementation effect, one would have to use 10–12% defatted soybean flour or 14–16% full-fat soybean flour. Such high amounts are known to affect the quality of bread considerably.

It is interesting to compare these results with data reported by Finney *et al.* (5). These investigators used hard winter wheat flour and different soybean flour samples. Loaf volume and crumb texture were not affected by admixture of soyflour up to 8%; however, the palatability was impaired significantly, especially when full-fat soybean flour was used.

A group of investigators at the Northern Regional Research Laboratory, reporting on the baking behavior of commercial full-fat (7) and defatted (8) soybean flour, stated that neither type, when used with the proper amount of oxidation, impaired loaf volume, crumb properties, color, or dough properties.

In an earlier paper (9), investigators from the same laboratory reported that the flavor of bread containing defatted soybean flour could not be differentiated by the tasting panel from that of bread baked with milk solids. However, in all these investigations, soybean flour (full-fat or defatted) was used at only 5%, or the equivalent of 2–3% isolated soybean protein.

Paulsen and Horan (14) recently reported on the properties of heat-treated and chemically treated soybean flours. They found that loaf volume, texture, and odor were affected by some of the materials but not by the chemically treated flours. Here again, however, the level of admixture was only 3%.

Dough-handling characteristics are not impaired by inclusion of soybean protein. The negative response to bromate which was observed here is in accordance with previous publications (6,15,16) on the use of isolated soybean protein in bread. Interestingly, the oxidation requirement is increased when soybean flours are included (7,8). CCP was superior to ISP from the viewpoint of working characteristics (stickiness, mixing tolerance) of the dough. This, combined with its higher water-absorption-increasing effect and its totally bland taste, makes CCP a better material for supplementation of bread.

Nutritional Considerations. Isolated soybean protein has been repeatedly reported to be inferior to soybean meal when used as a sole source of protein (17,18,19). The explanation most commonly offered is its lower methionine content (18). In fact, during the isolation process, a higher proportion of methionine remains with the protein fractions in the whey (20).

When soybean material is used to supplement wheat protein, the lysine content may be expected to be the most important factor. Data on fractional distribution of lysine during isolation of soybean protein are somewhat contradictory. According to Rackis *et al.* (20), the lysine content of isolated protein is only 83% of that of soybean meal. Huge (18) reported essentially equal lysine content in soybean grits and commercial isolated protein. More recent investigations by Yaron (21) also failed to show a difference in lysine content between isolated soybean protein and soybean flour.

Results from our feeding tests showed no significant difference between the PER of bread containing isolated soybean protein and that of bread supplemented with soybean flour at isonitrogenic levels.

PER values of bread containing 2% isolated protein are already significantly higher than those of bread made of wheat flour only. Above 6% and up to 10%, the increase in PER with increasing soybean protein content was not significant. Nutritionally, no difference could be detected between ISP and CCP in their behavior as protein supplementation media. The increase in PER caused by supplementation with soybean protein was proportional to the lysine content of bread. Regression analysis of the results showed linear correlation between the lysine content and the PER (Fig. 5); this is in agreement with Brown *et al.* (3), who found a similar correlation in breads enriched with lysine, milk solids, soybean, and lactalbumin.

Deposition of excess fat in the liver was shown to be caused by amino acid imbalance in low-protein diets (22,23). Brown and co-workers utilized this technique in their investigation with high-protein breads (3). They found that the livers of rats on casein diet contained significantly less fat than those of diet groups fed on bread, but no correlation could be established between liver fat and the lysine content of the bread. Our results seem to confirm this finding. Only the liver fat content of the control group was significantly different from that of other groups. The livers of rats fed on a diet of wheat bread or soybean-supplemented bread showed no significant difference in fat content when the data were analyzed at 95% confidence level. Under the conditions of the present investigation, no correlation was found between PER and liver fat content for the different bread diets. Nor could we find significant differences (at 95% confidence level) between the fresh weight and dry matter contents of the livers for all diet groups (including the control group).

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