Interactions of Soy Flour Fractions with Wheat Flour Components in Breadmaking[1] [2]

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ABSTRACT

A commercial defatted soy flour was fractionated into water-insoluble residue, water-solubles, isoelectrically precipitated protein and whey (pH 4.5 solubles). The whey was further fractionated by cation- and anion-exchange chromatography. The various fractions were tested individually and in combinations for their effect on loaf volume of bread. The isoelectric protein fraction, when added to wheat flour in the presence of sucrose monotallowate (SMT), produced a loaf volume comparable to that of the control (wheat flour, soy flour, and SMT). The pH 4.5 water-soluble fraction was not beneficial to loaf volume, apparently because it decreased gas retention of the dough. Attempts to characterize the deleterious component(s) were unsuccessful because the fractions were altered on the ion-exchange columns. It was demonstrated by starch-gel electrophoresis that the isoelectric protein fraction, SMT, and the gluten fraction of wheat flour interacted during dough-mixing. Further evidence of the interaction of SMT with gluten was suggested by unusual properties of gluten during washing.

Diets deficient in protein pose a major problem in developing countries. Adding high-biological-value protein to those diets would, of course, improve them. Bread, as an almost universally accepted food item, is a good medium for protein supplementation. In many parts of the developed world, milk is routinely used in breadmaking and materially improves the biological value of bread protein. Because availability of low-cost milk in developing countries is limited, a more readily available substitute is needed. Soy flour's low cost, availability, and high protein content make it a likely milk substitute.

Finney et al. (1) found that soy flours often were deleterious to loaf volume, while soy grits were superior to soy flour for breadmaking. Pomeranz et al. (2,3) found that the deleterious effects of soy flours (up to 16% of wheat flour weight) could be overcome by adding native and synthetic glycolipids to the breadmaking formula. Other surfactants (4,5) also overcome the deleterious effects.

The mode of interaction between soy flour, glycolipids, and wheat flour in dough is obscure. We attempted to determine the effects on loaf volume of various soy-flour fractions in the presence of glycolipids, and to ascertain the nature of the interaction between soy flour, wheat flour, and glycolipids.

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[2] Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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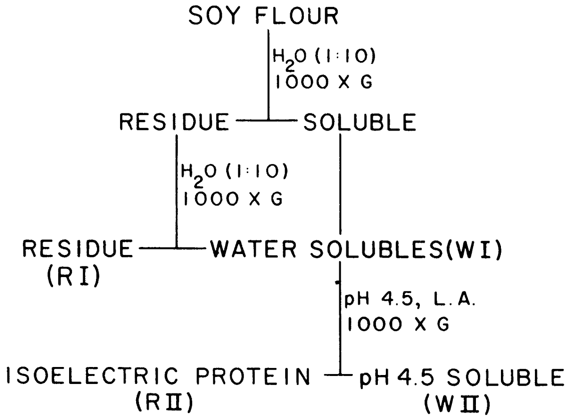

Fig. 1. Scheme for fractionating soy flour.

MATERIALS AND METHODS

Wheat Flour

Wheat flour (RBS-70A) was milled from a composite of many varieties harvested at many locations throughout the U.S. Great Plains in 1969. RBS-70A had a protein content of 12.9%, good loaf volume potential, and a medium mixing time of 3 7/8 min.

Soy Flour

A defatted soy flour (Baker's Nutrisoy, furnished by Archer-Daniels-Midland Company) had a protein content of 49% (N × 6.25).

Fractionating Soy Flour

A slurry of soy flour and water (1:10), was stirred 1 hr. and centrifuged at 1,000 × g for 30 min. (Fig. 1). The residue was re-extracted with water (1:10) and recentrifuged to separate the remaining insoluble residue (RI). The two supernatants were combined to give a water-soluble fraction (WI). The pH of the water-soluble fraction was adjusted to 4.5 with lactic acid (85%), and the precipitate (isoelectric protein, RII) was collected by centrifuging at 1,000 × g for 30 min. and decanting the whey (WII). Thus, four fractions, WI, WII, RI, and RII, were obtained (Fig. 1). Those fractions were lyophilized and ground.

Analytical Procedures

Moisture (air oven) and protein (Kjeldahl N × 5.7 for wheat or 6.25 for soy) were determined by AACC methods (6). Gas production, using 100% water absorption, was a modification of the official AACC method.

Baking Procedure

A straight-dough baking procedure with a rich, optimum-bromate formula was used as described by Shogren et al. (7). The baking formula included 10 g. flour (14% moisture basis), 0.6 g. sugar, 0.15 g. salt, 0.3 g. shortening, 0.2 g. yeast, 0.4 g. nonfat dried milk (NFDM), and 0.05 g. malt syrup (60° L.). Shortening and

TABLE I. LOAF VOLUMES OF BREAD BAKED WITH RBS-70A FLOUR (10 g.) AND ADDED SOY FLOUR OR ITS FRACTIONS[1]

Expt.	Soy Flour Fraction	Weight of Soy Flour Fraction g,	Lipid[2]	Loaf Volume cc.
1	None	0.0	Shortening	82[4]
2	None	0.0	None	66
3	None	0.0	SMT	71
4	Soy flour	1.200	SMT	85
5	Soy flour	1.200	None	65
6	Residue (RI)	0.411	SMT	76
7	pH 4.5 isoelectric (RII)	0.408	SMT	82
8	pH 4.5 soluble (WII)	0.381	SMT	70
9	RI + RII + WII	0.411 + 0.408 + 0.381	SMT	82
10	pH 4.5 isoelectric (RII)	0.408	None	63
11	WII/D (dialyzed)	0.082	SMT	82
12	WII/DS (dialyzate)	...[3]	SMT	74
13	WII/D + WII/DS	...[3]	SMT	73
14	WII/D + WII/DS-NH_4OH	...[3]	SMT	77
15	WII/D + WII/DS-H_2O	...[3]	SMT	62
16	WII/D + WII/DS-NH_4OH + WII/DS-H_2O	...[3]	SMT	63
17	WII/D + WII/DS-H_2O,H_2O	...[3]	SMT	66
18	WII/D + WII/DS-H_2O,HCl	...[3]	SMT	73
19	WII/D + WII/DS-H_2O,H_2O + WII/DS-H_2O,HCl	...[3]	SMT	60

[1] Wheat-flour and soy-flour weights are on a 14% M.B.

[2] Shortening (3%) and SMT, (2.5%) were on the wheat-flour basis.

[3] Added as liquid aliquots.

[4] Loaf also contained 4% nonfat milk solids.

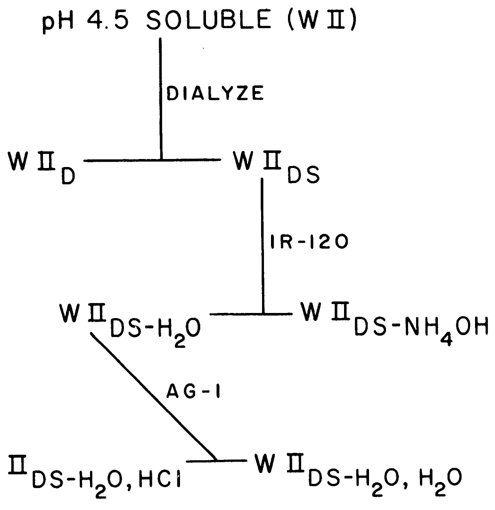

Fig. 2. Scheme for fractionating pH 4.5 soluble fraction (WII) of soy flour by dialysis, cation-exchange column (IR-120), and anion-exchange column (AG-1).

NFDM were omitted in doughs containing soy flour or its fractions. As a source of glycolipids, 2.5% of sucrose monotallowate (SMT) was added. Mixing time, water absorption, and potassium bromate were optimum. Doughs were fermented for 3 hr. and proofed for 55 min. at 30° C. Loaf volume was measured by dwarf rapeseed displacement. The standard deviation for the average of duplicate loaf volumes was 1.75 cc.

Amounts of Soy Flour and Its Fractions Added in Baking

Supplements of soy flour or its fractions per 10 g. RBS-70A wheat flour are given in Table I, unless specifically stated otherwise. Soy-flour weight (1.200 g., 14% moisture basis) is equivalent to 12% of wheat-flour weight. The weight of each fraction is based on its yield from the soy flour, so that a completely reconstituted soy flour would be 12% of the wheat-flour weight. Each reconstituted fraction was equivalent to that fractionated from 1.200 g. of soy flour.

Fractionating pH 4.5 Soluble Fraction (Whey)

Fraction WII was further fractionated by dialyzing for 2 days against a large excess of water at 4° C. (Fig. 2). The two whey fractions WII/D (dialyzed) and WII/DS (dialyzate) were freeze-dried.

Cation-Exchange Column Fractionation of WII/DS

Two-milliliter aliquots containing 0.25 g. of fraction WII/DS were passed through a cation column (0.9 × 12 cm.) of Amberlite IR-120(H^+). Eluting the column first with water yielded an unbound fraction (WII/DS-H_2O), and then eluting with 2N ammonium hydroxide (Fig. 2) yielded a bound fraction (WII/DS-NH_4OH).

The WII/DS-H_2O and WII/DS-NH_4OH fractions were freeze-dried, dissolved in a known amount of water, and stored at −40° C.

Anion-Exchange Column Fractionation

The unbound water-eluted fraction (WII/DS-H_2O) from the cation column, after being freeze-dried and dissolved in 2 ml. water, was passed through an anion column (0.9 × 12 cm.) of AG-1(Cl^-).

The column was first eluted with water (WII/DS-H_2O,H_2O fraction), and then with 1N hydrochloric acid (WII/DS-H_2O, HCl fraction). The two fractions were freeze-dried, dissolved in a known quantity of water, and stored at −40° C. (Fig. 2).

Sucrose Monotallowate

Sucrose monotallowate (SMT), a synthetic glycolipid, was obtained from Colonial Sugars Co., Gramercy, La. Studies in our laboratory have shown that 2.5% SMT produces optimum results with doughs containing 12% soy flour.

Fractionating Dough

Doughs prepared from 100 g. RBS-70A and from 100 g. RBS-70A plus soy-flour fractions (with and without SMT) were fractionated (Fig. 3) by the following method: After being mixed to optimum consistency, each dough was washed out by hand in 250 ml. distilled water. The gluten was rinsed by kneading

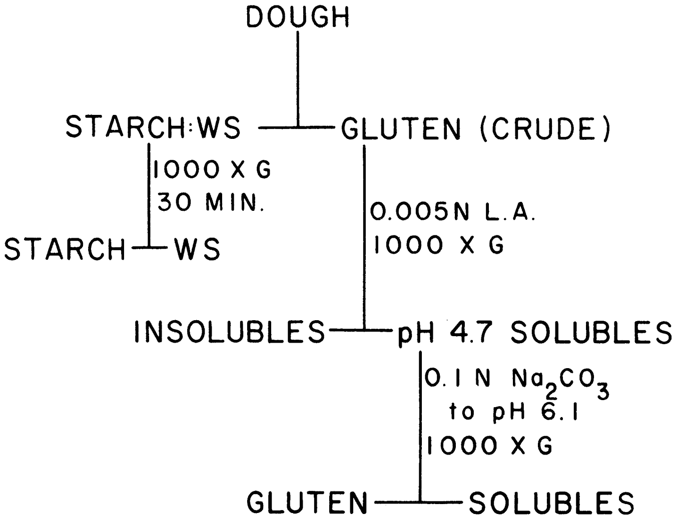

Fig. 3. Scheme for fractionating dough.

in five 25-ml. aliquots of water, and the combined wash water was centrifuged at 1,000 × g for 30 min. to separate water-soluble (WS) and starch fractions.

Crude gluten from each dough was cut into small pieces and stirred in 280 ml. of 0.005N lactic acid. After being stirred 5 hr., the gluten suspension was centrifuged at 1,000 × g for 30 min. to remove the insoluble fraction. The supernatant (pH 4.7 soluble fraction) was adjusted to pH 6.1 with 0.1N sodium carbonate and then centrifuged at 1,000 × g for 30 min. The supernatant (pH 6.1 soluble fraction) and the centrifugate (pH 6.1 insoluble gluten) were lyophilized.

Starch Gel Electrophoresis

Starch-gel electrophoretic technique was a modification (8) of the procedure described by Woychik et al. (9).

TABLE II. ANALYTICAL DATA FOR SOY FLOUR LAND ITS FRACTIONS

Fraction	Yield %	Protein Content %	Protein per 100 g. Flour g.	% of Total Flour Protein
Soy flour	100	49.0	49.0	100
Residue (RI)	34	46.8	15.91	32.4
pH 4.5 isoelectric fraction (RII)	33	84.0	27.72	56.6
pH 4.5 soluble (WII)	30	11.1	3.33	6.8

RESULTS AND DISCUSSION

Fractionating Soy Flour

When soy flour was fractionated by the scheme given in Fig. 1, about 63% (RII + WII) of the total soy flour protein was extracted with water (Table II). The isoelectrically-precipitated fraction (RII, 84% protein) contained 56.6% of the total soy flour protein.

The pH 4.5 soluble fraction (WII) contained the smallest portion (6.8%) of the total soy flour protein. The insoluble fraction (RI) contained 32.4% of the total soy-flour protein. The protein contents of the insoluble fraction (RI), isoelectric fraction (RII), and pH 4.5 water-soluble fraction (WII) were 46.8, 84.0, and 11.1%, respectively.

Baking Properties of Soy Flour and Its Fractions

When baked alone (no milk or shortening), RBS-70A produced a loaf volume of only 66 cc. (Table I). Adding 2.5% SMT improved loaf volume to 71 cc., a value materially less than the 82 cc. for wheat flour + shortening + milk.

Supplementing RBS wheat flour (no milk, no lipid) with 12% soy flour produced a loaf volume of 65 cc., essentially equal to RBS-70A alone (66 cc.). Adding 2.5% SMT to the formula increased loaf volume to 85 cc. Thus, the loaf-volume depressing effect reported earlier (2) is attributable to the absence of lipids in the formula.

Supplementing the RBS-70A with the three soy flour fractions, RI, RII, and WII, together with SMT, produced loaf volumes of 76, 82, and 70 cc., respectively. Thus, only the RII fraction produced a loaf volume comparable to the complete soy flour. However, without SMT, adding RII produced a loaf volume of only 63 cc. Reconstitution of the soy fractions (RI, RII, and WII) and adding to the RBS-70A flour produced a loaf volume (82 cc.) comparable to that of the unfractionated soy flour. Thus, the fractionation scheme did not alter the baking characteristics of the soy flour fractions.

From the foregoing, it is evident that to replace milk in the baking formula with soy flour (12%) or its isoelectric protein fraction, SMT is essential for normal loaf volume. Amount of each soy flour fraction added was equivalent to

TABLE III. LOAF VOLUME OF RBS-70A WHEAT FLOUR SUPPLEMENTED WITH DECREASING AMOUNTS OF ISOELECTRIC-PROTEIN FRACTION (RII) FROM SOY FLOUR

RII Added g.	Percent of Original Conc.	Loaf Volume cc.
0.408[1]	100.0	82
0.245	60.0	83
0.082	20.0	80
0.041	10.0	80
0.021	5.0	78
0.010	2.5	77

[1] From 1.200 g. soy flour (14% m.b.).

TABLE IV. GAS PRODUCTION DATA FOR RBS-70A FLOUR (10 g.) PLUS SOY FLOUR OR ITS FRACTIONS

Soy Flour or Fraction	Pressure Reading
None	440
Soy flour	450
pH 4.5 soluble (WII)	455
Residue (RI)	430

that in 12% soy flour. We also determined the minimum amount of isoelectric protein fraction necessary to replace milk in the baking formula. As the quantity of isoelectric protein fraction was progressively reduced (Table III), loaf volume changed little. For example, reducing the quantity of isoelectric-protein fraction in the dough to 10% of the original did not significantly reduce loaf volume. Reductions below 10% of the original gave somewhat lower loaf volume.

Fractionating the pH 4.5 Soluble Fraction (WII)

The doughs containing the pH 4.5 soluble fraction (WII) of soy flour appeared "dead" and had low proof heights. Gas production data (after 4 hr., Table IV) indicated that the fraction was not deleterious to yeast fermentation. Thus, it was assumed that the pH 4.5 soluble fraction (WII) had a deleterious effect on gas retention.

In an effort to identify the substance deleterious to gas retention, we further fractionated the pH 4.5 water-soluble fraction (WII) by dialysis. When the resultant two fractions, WII/D (dialyzed) and WII/DS (dialyzate), were added to RBS-70A + SMT singly and in combination, and baked into bread (Table I), the WII/DS fraction produced a significantly lower loaf volume (74 cc.) than the volume of the WII/D fraction (82 cc.).

Reconstituted WII/D and WII/DS fractions produced a loaf volume (73 cc.) comparable to that of the unfractionated pH 4.5 soluble fraction (70 cc.). Therefore, dialysis did not alter the WII/D or WII/DS fractions. The WII/D fraction produced a volume nearly equivalent to the complete soy flour and equal to the RII fraction. Thus, the substance deleterious to gas retention appears to be in the WII/DS fraction. It is also apparent that something in the total soy flour overcomes that deleterious effect.

After WII/DS was fractionated further on a cation exchange column, the WII/DS-H_2O (unbound) and WII/DS-NH_4OH (bound) fractions of the WII/DS fraction were reconstituted with WII/D, added to RBS-70A + SMT, and baked into bread. The loaf containing WII/D + WII/DS-NH_4OH had a loaf volume of 77 cc., 7 cc. above that of the control loaf containing WII (Table I). The loaf containing WII/D + WII/DS-H_2O had a loaf volume 8 cc. lower than that of the loaf containing WII (70 cc.). Thus, the substance most deleterious to loaf volume seemed to be in the WII/DS-H_2O fraction.

When the WII/D, WII/DS-NH_4OH, and WII/DS-H_2O fractions were reconstituted and added to RBS-70A wheat flour + SMT, the resultant loaf had a volume of only 63 cc., 7 cc. below the control loaf containing WII. Apparently at least one of the fractions was altered on the cation-exchange column.

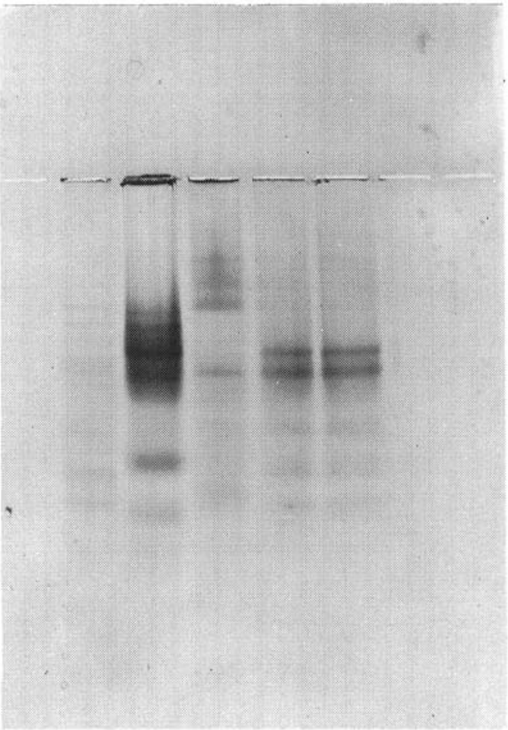

Fig. 4. Starch-gel electrophoretic patterns of (left to right) insoluble fraction from the dough containing RBS alone, isoelectric-protein fraction (RII) of soy flour, pH 6.1 insoluble gluten fraction from dough containing RBS wheat flour alone, and two patterns of the insoluble fraction isolated from the dough containing RBS wheat flour and isoelectric protein fraction of soy flour.

Because the WII/DS-H_2O fraction was more deleterious to loaf volume than WII/DS-NH_4OH, the WII/DS-H_2O fraction was further fractionated by being passed through an anion-exchange column.

When the two anion-exchange column fractions, WII/DS-H_2O,H_2O unbound and WII/DS-H_2O,HCl bound, of the WII/DS-H_2O fraction were reconstituted with the WII/D fraction, added to RBS-70A + SMT, and baked into bread, volume of the loaf containing the WII/DS-H_2O,H_2O unbound fraction was 66 cc. and that of the WII/DS-H_2O,HCl bound fraction was 73 cc., equal to that for WII/D + WII/DS. Thus, the WII/DS-H_2O,H_2O accounted for the deleterious effect of the WII/DS-H_2O fraction. When WII/DS-H_2O,H_2O, WII/DS-H_2O,HCl, and WII/D fractions were reconstituted and added to RBS-70A + SMT and baked into bread, loaf volume was 60 cc. (equal to that of the control WII/D + WII/DS-H_2O). Thus, the fractions from the anion column were not altered. However, because at least one of the fractions from the cation column was altered, there was no conclusion about the nature or identity of the deleterious substance.

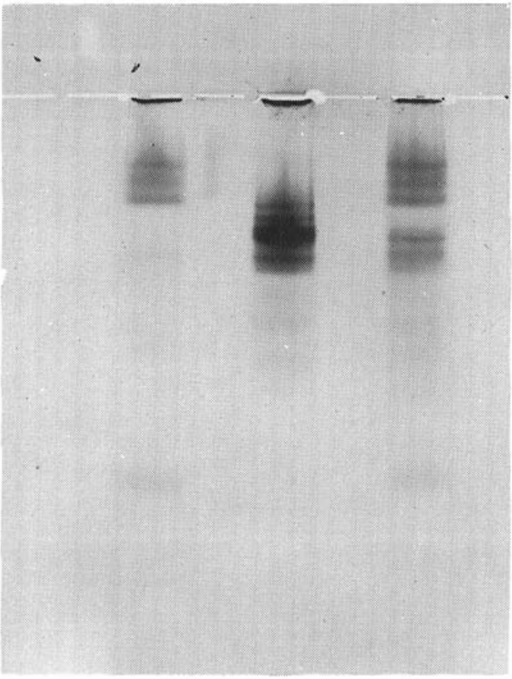

Fig. 5. Starch-gel electrophoretic patterns of pH 6.1 insoluble gluten fraction from the dough containing RBS wheat flour and isoelectric protein fraction (RII) of soy flour (left), isoelectric-protein fraction (RII) of soy flour (center), and the pH 6.1 insoluble gluten fraction from the dough containing RBS wheat flour, SMT, and isoelectric protein fraction (RII) of soy flour (right).

Starch-Gel Electrophoresis

Because of the large response of the wheat- and soy-flour mixture to SMT, an interaction of their proteins was assumed. Starch-gel electrophoretic pattern of the isoelectric protein fraction was quite different from those of the insoluble and pH 6.1 insoluble gluten fractions, and was distinguishable in their mixtures (Fig. 4).

Starch-gel electrophoretic patterns of the protein fractions from dough of RBS wheat flour plus isoelectric protein (no SMT) showed that the soy protein (insoluble at pH 4.5 to 4.7) was in the insoluble fraction (Fig. 4), where it would be expected if there was no interaction. So, no interaction was apparent between the isoelectric-soy-protein fraction and the wheat-flour proteins.

Starch-gel patterns of the protein fractions from the dough of RBS wheat flour, isoelectric soy protein, and SMT showed that the isoelectric protein fraction was clearly in the pH 6.1 insoluble gluten fraction (Fig. 5), apparently because it interacted with the pH 6.1 insoluble gluten fraction (Fig. 5), apparently because it interacted with the pH 6.1 insoluble gluten fraction of flour in the presence of SMT. Thus, the large loaf volume response of soy flour to SMT is attributable to an interaction of soy proteins with wheat proteins. Additional evidence of the interaction of SMT with wheat-flour gluten was noted while the fractions were being prepared. For example, when the dough contained 2.5%

SMT, a gluten ball was not formed, and the small pieces of gluten had to be collected on a stainless-steel screen.

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