SOYFLOUR FRACTIONS IN BREADMAKING AND THEIR INTERACTION WITH WHEAT FLOUR COMPONENTS

by

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Introduction

Protein difficiency is a major problem of lesser developed countries. Food scientists and nutritionists, from all over the world, have emphasized that addition of high biological value protein to the diet would greatly relieve that problem.

Bread is an almost universally accepted food item and therefore a good medium for protein supplementation. In the developed world, milk, a good source of high biological protein, is used routinely in breadmaking. In practice dried, nonfat, milk solids are added to the bread formula. Since the availability of milk is restricted in lesser developed countries, an easily available, low cost substitute, is needed. Soybean flour merits consideration as that substitute because of its low cost, availability, and high nutritionally-balanced protein content.

For bread to be acceptable, certain standards must be met regarding the crumb texture, taste, loaf volume, and nutrient content. Thus, it is the responsibility of a food scientist to see that a bread meeting all those requirements is prepared with as much ease and economy as possible.

When soyflour was used as a substitute for milk in the bread formula it proved deleterious to loaf volume. But this limitation was overcome by adding glycolipids along with the soyflour to the bread formula (1, 2). The loaf volume of bread baked with soyflour and glycolipids, was equal to that of bread baked with nonfat dry milk. Thus glycolipids were essential to get the maximum beneficial effect of soybean flour in breadmaking.

The mode of interaction between soybean flour glycolipids and wheat flour in dough is obscure. The purpose of this study was to determine the

effect of various soy fractions on loaf volume in the presence of glycolipids and the mode of interaction between soyflour, wheat flour, and glycolipids; and to attempt to isolate substances in soyflour that are beneficial or deleterious to loaf volume.

Literature Review

Fractionation of Soybean Flour. Soybean protein have been dispersed with many solvents. Smith et al. (3) found sodium carbonate to be a better extracting agent than potassium or sodium hydroxide for soybean oil cake. Wolf et al. (4) extracted soy protein at room temperature from dehulled, hexane defatted soybean flakes with potassium phosphate and sodium chloride buffer, pH 7.6. After centrifuging, the insoluble was twice re-extracted with the same buffer. The three extracts were combined and concentrated by pre-evaporation and equilibrated against the extraction buffer by dialysis prior to ultracentrifugal analysis.

Nagel et al. (5) extracted flaked, defatted (petroleum ether), ground, (ball mill to pass 100 mesh sieve) soybean with alcohol-water solution at room temperature for 30 minutes. The suspension was centrifuged and the supernatant liquid was decanted into a digestion flask. The nitrogen was determined by Kjeldahl-Gunning-Arnold method. However, the amount of protein thus extracted was not above 50 percent of the total soyflour protein.

The general factors influencing dispersion of soyflour protein in water, such as time, temperature, method and time of stirring and pH value of the solution, emphasize the emperical nature of the determination and the need of selecting a standard procedure to suit a given need. Smith et al. (6)

adopted a procedure using a 30 minute water extraction, room temperature, and soyflour-solvent ratio of 1:40.

Smith and Wolf (7) found that the commercial isolation of soy protein has a number of variations. The two main steps, however, are extraction of dehulled and undenatured soybean flakes with water or dilute alkali solution; and precipitation of about 88 percent of extracted nitrogen by adjusting the pH to 4.5. Laboratory yield of acid precipitated protein may be as high as 70 percent of the nitrogen present in the defatted soyflour.

Different NSI (Nitrogen Solubility Index) procedures were developed both at Northern Utilization Regional Research Laboratories and Southern Utilization Research Laboratories to determine the factors that affect nitrogen solubility (8). Soyflour was ground in a hammer mill to pass a 100 mesh screen to minimize sampling errors. Grinding in a hammer mill had little, if any, effect on NSI. The pH of distilled water extracts varied between 6.52 to 6.85 depending on the strain. The experiments were based on the work of Smith and Circle, who earlier reported that highest solubility of nitrogen occurs at pH 7.2.

In the Southern Utilization Research Laboratories' method, a 2 gm. sample of dehulled, defatted flakes was placed in a 250 ml. centrifuge bottle and distilled water was added to give 125 ml. total volume. The sample was stirred at about 1000 r.p.m. for one hour, by means of a one-inch teflon coated magnet. After centrifuging at 1,900 x g. for 15 minutes, the extract was decanted into a 250 ml. volumetric flask and the residue was re-extracted with 100 ml. of water for one hour. The combined extracts were made up to volume. Kjeldahl analysis was made on 50 ml. aliquots of the extraction. The results showed that as much as 60 percent of the total soyflour protein could be extracted.

In the same laboratories crude, acid-precipitated soybean protein was prepared by extracting hexane defatted flakes twice with water, first 10:1 solvent-meal ratio and then 5:1. After centrifuging, the supernatants of the two solutions were combined and adjusted to pH 4.5 with hydrochloric acid precipitated curd was centrifuged, freeze dried and ground in a hammer mill using a 100 mesh screen.

Eldridge et al. (9) also prepared purified, acid-precipitated protein. Crude acid-precipitated soybean protein was dissolved in 10 percent sodium chloride-potassium phosphate buffer, pH 7.6, and dialyzed at 4°C for one week, against several changes of buffer. The second week the protein was dialyzed against water. The protein then was precipitated with acid at pH 4.5, and the curd was thoroughly washed and freeze-dried.

Water extractable soybean protein was obtained by Wolf and Sly (10) by stirring defatted soyflour with water, 1:10 meal-solvent ratio, at 25°C for 30 minutes and centrifuging for 10 minutes at 1200 x g. The clarified extract was dialyzed in the cold for several days against the ultracentrifuge buffer to remove low-molecular-weight ultraviolet absorbing material, sugar, and other nonprotein contents. Mercaptoethanol in the buffer depolarized the disulphide polymers of 7^S and 11^S ultracentrifugal components present in the aqueous extraction.

Soybean globulin was prepared by making a water extract, 1:10 soy-flour water ratio, followed by a 1:5 re-extraction. The combined extracts were adjusted to pH 4.5 with 1N hydrochloric acid, and the globulins were separated from the whey by centrifuging. The globulin and whey protein fractions were then dialyzed against the centrifuge buffer.

Eldridge et al. (11) extracted defatted soybean flour twice with water, first with a 1:10 soyflour solvent ratio and then with a 1:5 ratio in second extraction. Supernatants were combined and the pH was lowered to 4.4 - 4.6 with hydrochloric acid. The curd was collected by centrifuging, and then freeze dried. The dried protein was ground in a hammer mill and washed with 86 percent aqueous ethyl alcohol at room temperature. The curd was dried for several hours in vaccum at 50°C to remove ethyl alcohol and then dispersed in water and adjusted to pH 7.5. The insolubles were removed by centrifuging and the supernatant was freeze-dried. The yield varied from 22 to 29 percent of the total soyflour protein.

Soybean flakes were extracted in an industrial extractor by Cogan et al. (12). About 82 percent of the nitrogen of the soyflour was dispersible. In this procedure, 20 kg. of unheated soyflour was mixed with 200 liters of 0.03 M solution of calcium hydroxide to give a pH of 9.5 - 9.8 and agitated for 30 minutes at 55°C. The resulting mixture was screened through a 60 mesh sieve and the isoelectric protein was precipitated at pH 4.5.

Smith and Circle (13) extracted oil-free soybean flour with water and alkaline solutions. The protein was precipitated from those dispersions by various acids and electrolysis. The methods were compared with respect to yield and ease of preparation. There was no advantage of electrolysis through parchment. Tannic acid was used to recover protein not precipitated by sulfuric acid.

Wolf et al. (14) studied the physical properties of alcohol extracted soybean protein. Isolated protein contained phospholipid like materials with were extractable with aqueous alcohols. Since alcohols were protein denaturants, their effects on the physical properties of soybean protein

were investigated. Solubility of freeze-dried isoelectric precipitated soybean protein in pH 7.6, 0.5 ionic strength phosphate buffer was 59 percent. In the presence of 0.01 M mercaptoethanol, solubility in buffer increased to 80 percent as a result of depolymerization of disulfide polymers of the 7^S and 11^S ultracentrifugal components. Extraction of the protein for two hours at 25°C with 94 percent methanol and 83 percent ethanol, or 77 percent isopropanol, decreased solubility to 25 percent.

Effects of Various Soy Products on Bread Characteristics. Present day commercial soyflour had been greatly improved by "chemical and physical treatment." The chemical treatment not only brings about a marked flavor improvement, but also leaves the fundamental functional properties and especially the absorption characteristics, of soyflour unchanged (15). Sipos and Turro used three different soyflours in breadmaking namely, special process, chemically treated, and regular. They used a conventional sponge-dough method and observed the bread characteristics when the soyflour was added either to the sponge or to the dough. The soyflour was used as a replacement, complete and partial, for nonfat dry milk. Doughs containing soyflour had lower gas production than doughs containing milk.

Soy Product Variables Affecting Breadmaking. Finney et al. (16) found that bread characteristics were affected by a number of variables in the manufacture and composition of soyflour. Finely powdered soyflour was more deleterious than soy grits. Heat treatment affected the breadmaking potentialities of the soyflour. Fat and lecithin had a beneficial effect on breadmaking characteristics. Water-dispersible protein and 70 percent protein concentrates from soyflour had a deleterious effect on breadmaking. Toasted grits produced appetizing breads, whereas, finely powdered soyflour produced loaves with an objectionable brown color.

Syliva Mizrahi et al. (17) added soy protein isolates to wheat flour and studied acceptance and nutritive value. Admixture of protein was found to increase the water absorption capacity of the dough. Loaf volume decreased proportionately with the level of soy protein addition. However, when 1 percent lecithin was added in mixes containing less than 6 percent of the soybean protein fraction, the decrease in loaf volume could be counteracted. Isolated protein added at levels up to 8 percent did not affect the taste significantly. The nutritive value of the bread samples measured as protein-efficiency ratio increased with the percentage of soybean protein.

Glycolipids in Breadmaking. Glycolipids are complexes of carbohydrates and lipids. They combine the hydrophilic features of the polyols with the lipophilic behavior of long aliphatic chains and are nonionic detergents. Thus some glycolipids, while showing considerable solubility in lipid solvents, also form aqueous solutions.

Native wheat flour lipids contain significant quantities of monogalactosyl and digalactosyl diglycerides that were first isolated from a benzene extract of wheat flour by Carter et al. (18). Those glycolipids have been shown to be necessary for a flour's normal breadmaking characteristics (19).

Together with other complex lipids, the glycolipids may be responsible for interactions between lipid micelles and proteins in the chloroplast (1).

Audidier and Lapape (20) studied the effects of several synthetic sucroglycerides on rheological properties of dough and starch pastes. Water absorption was increased, softness was increased and crumb grain was improved.

Wheat flour fortified with several protein-rich additives such as fish protein concentrates, soyflour, defatted cottonseed meal, wheat gluten, wheat germ etc., had substantially lowered loaf volume and impaired crumb

grain. With all those additives, loaf volume was increased and crumb grain improved by adding sucrose monotallowate (21, 22). The deleterious effect of soy was minimized by adding shortening and completely overcome by sucrose monotallowate. The loaf volume of bread containing tallowate plus 12 percent soyflour was above that of the control containing no soyflour.

Pomeranz et al. (23) found that when lecithin was added to doughs containing shortening, there was no improvement in the loaf volume. Hydrogenated lecithin was not a good substitute for free flour lipids. Alcohol soluble phosphatides were effective in improving loaf volume and crumb grain of bread baked from unheated and petroleum-extracted flour without shortening. Adding alcohol soluble phosphatides lowered the gelatinization temperature and hot-paste viscosity peak of a flour-water slurry. Hydroxylated phosphatides increased farinograph dough development time, valorimeter values, and mixogram time.

The water-saturated butanol extract of flour was fractionated by silicic acid-column chromatography into polar and nonpolar lipids and the fractions were analyzed for phosphorus content. A small decrease in the ratio of polar and nonpolar lipids and an increase in the phosphorus content of the polar fractions caused profound changes in rheological properties and breadmaking characteristics of wheat flours supplemented with those lipid fractions (24).

Protein Lipid Interaction. Recently Chapman (25) emphasized that there are many ways in which lipids and proteins can interact. Even for a given type of protein and lipid, interaction may vary depending on the pH, ionic strength, temperature, and other variables in the system. The possible binding forces involved in the interaction of lipids and proteins are:

(A) Co-valent

(B) Ionic

(C) Hydrogen

(D) Van Der Waals

(E) Hydrophobic bonds

No hydrophobic bonds can be produced without free water. The question arises, is there enough free water in a dough to form hydrophobic bonds? According to Tole·o et al. (26) only about 50 percent of the water in dough is bound, consequently, hydrophobic bonds are possible. Pomeranz (2), in his presentation of lipid-protein interaction, indicates that the formation of lipoprotein may involve glycolipids. The chemical structure of interacting components, and the mechanism of interaction are related to breadmaking potential.

Role of Lipids and Shortening in Breadmaking. Pomeranz et al. (19) extracted free lipids from the flours milled from two hard red winter wheats, one hard red spring, and one soft red winter, with petroleum ether. The original and the defatted flours were baked with a rich formula with and without 3.0 percent vegetable shortening. A substantial improvement in the loaf volume and crumb grain was noticed when shortening was added to the original flour, whereas the same shortening when added to defatted flours, impaired the loaf volume and crumb grain. Loaf volumes of bread baked from defatted good quality wheat flours were decreased and from defatted poor quality flours were increased by adding shortening to the dough formula.

Daftary et al. (27) extracted free and bound lipids, from flours from four wheat varieties that varied widely in breadmaking potentialities. The lipids were fractionated by means of silicic acid column-chromatography and by acetone-precipitation. Free polar lipids increased loaf volume

considerably more than did bound polar lipids. There was no significant difference in the performance of lipid fractions isolated from various sources.

Breadmaking Properties of Wheat Flour. According to Finney (28) a flour of good quality for bread baking should have a high water absorption, a medium to medium-long mixing requirement, satisfactory mixing tolerance and good loaf volume potentialities (considering protein content), and should yield a loaf having good internal crumb grain and color.

Materials and Methods

Wheat-Flours. The RBS-70A wheat flour used in this study was a composite of many wheat varieties grown at many locations throughout the great plains in 1969. The wheat flour had a protein content of 12.9 percent, good loaf volume potential, and a medium mixing requirement of $3\frac{7}{8}$ min.

Soyflours. The soyflours, Ardex 550 and Baker's Nutrisoy, were used. Ardex-550 is a treated, defatted commercial soyflour. It has a protein content of 50 percent (N x 5.7). It was obtained from Archer Daniel Midland Co.

Baker's Nutrisoy is a defatted soyflour with a minimum treatment. It has a protein content of 49 percent (N x 5.7) and was obtained from Archer Daniel Midland Co.

Fractionation of Ardex-550. Various methods were used to fractionate Ardex-550.

(A) Soyflour was slurried with water (1:10), stirred for one hour and centrifuged for 30 minutes at

1000 x g. The residue was again slurried with water (1:10) and the pH adjusted to 7.5 with sodium carbonate, after stirring for one hour the slurry was centrifuged at 1000 x g. for 30 minutes. The supernatants of the two washings were combined and analyzed for protein content by the Kjeldahl method (Fig. 1).

(B) A series of soyflour-water slurries (1:10) was adjusted to pH 8 with sodium carbonate. These slurries were stirred for $\frac{1}{2}$ hr., 1 hr., $2\frac{1}{2}$ hr., and 5 hrs., respectively. After centrifugation at 1000 x g. for 30 minutes. The supernatants were analyzed individually for protein content.

(C) A series of slurries of soyflour and water (1:10) was adjusted to pH 7, 8, 9, 10, and 10.5 respectively, stirred for one hour, and centrifuged at 1000 x g. for 30 minutes. The supernatants were analyzed for protein content.

Fractionation of Baker's Nutrisoy. A slurry of soyflour and water (1:10) was stirred for one hour and centrifuged at 1000 x g. for 30 minutes. The residue was again extracted with water (1:10) and centrifuged. The two supernatants were combined to give a water-soluble fraction (W I). The residue was collected as R I. The pH of the water soluble fraction was adjusted to 4.5 with lactic acid (85%) and the precipitate (isoelectric protein) was collected by centrifuging at 1000 x g. for 30 minutes. Thus

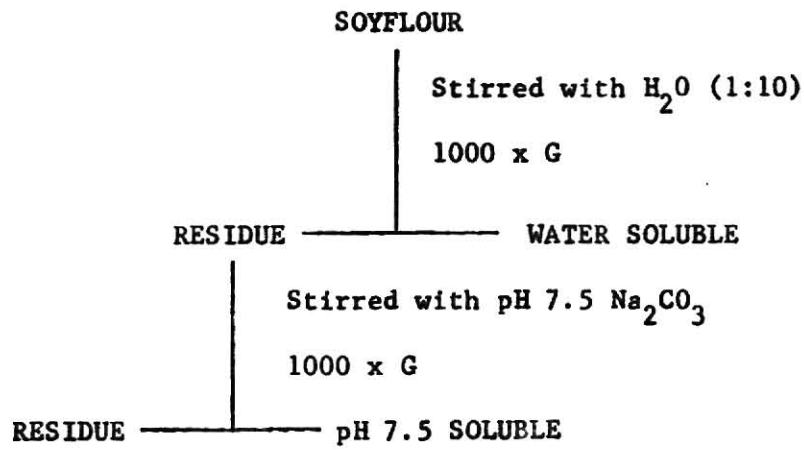

Fig. 1. Fractionation scheme for solubilizing protein from Ardex-550.

the fractions i.e. residue (R I), 4.5 isoelectric protein (R II) and 4.5 soluble (W II) were obtained (Fig. 2). These fractions were then lyopholized and ground.

Analytical Procedures. For protein estimation of all flours and their fractions, Kjeldahl method was used, N x 5.7 conversion factor (29). For gas production studies a modified pressure meter-method was employed using 100 percent absorption (29). Air oven method was used to determine moisture (29).

Baking Procedure. The micro baking procedure employed was as described by Shogren, Finney, and Hoseney (30). The 10 gm. baking formula included the following ingredients:

(A)	Wheat flour	10 gm. (14% M.B.)
(B)	Sugar	0.6 gm.
(C)	Salt	0.15 gm.
(D)	Shortening	0.3 gm.
(E)	Yeast	0.2 gm.
(F)	Nonfat dry milk	0.4 gm.
(G)	60°L malt syrup	0.05 gm.

Shortening and nonfat dry milk solids were excluded in doughs containing soyflours or their fractions. Sucrose monotallowate (2.5%) was added as a source of glycolipids.

The method employed optimum mixing time, water absorption and potassium bromate (10 to 60 p.p.m.). Fermentation time was three hours and the doughs were proofed for 55 minutes at 30°C. Loaf volume was measured by rape seed displacement.

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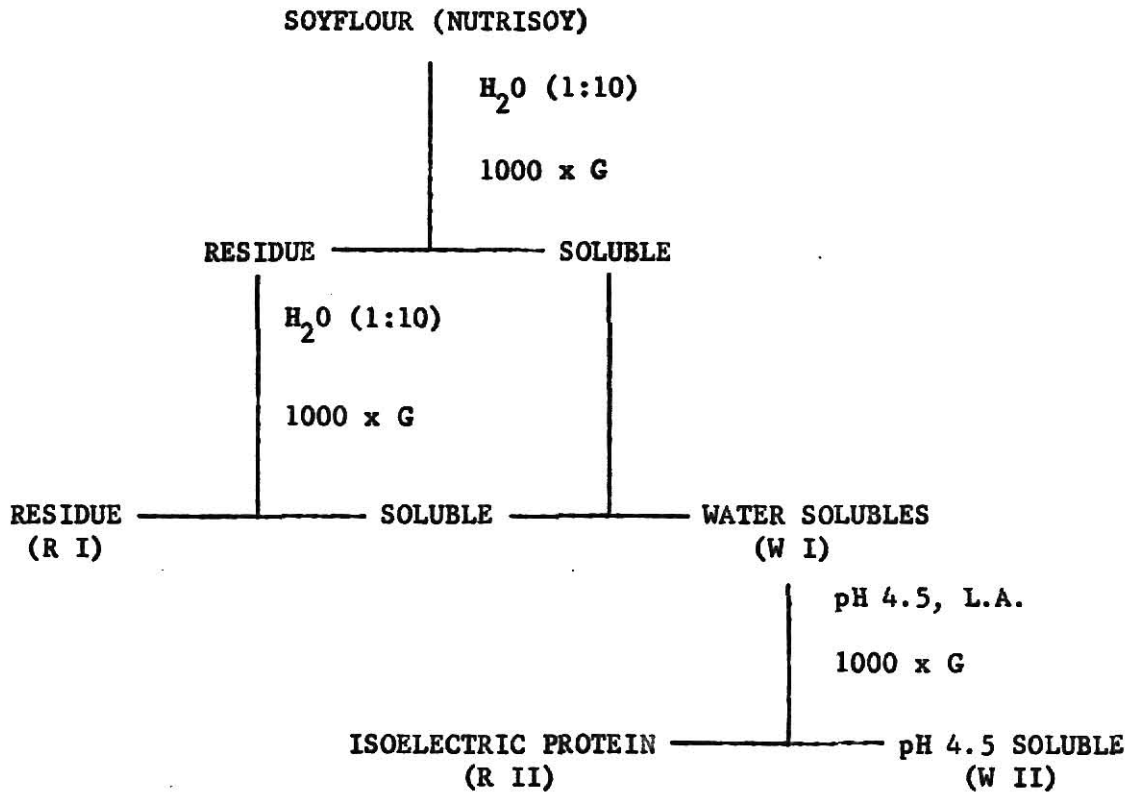

Fig. 2. Fractionation scheme for solubilizing protein from Baker's Nutrisoy.

Amount of the Soyflours and Their Fractions Added in Baking. When 10 g. of RBS wheat flour was supplemented with soyflours or their fractions for various baking tests the following weight of the soyflours or fractions was used, unless specifically stated otherwise. The "as is" weight given equals a 12 percent addition based on the flour weight and a 14 percent moisture basis. The weights of the fractions are based on their yield from the soyflour such that a completely reconstituted soyflour would be 12 percent of the wheat flour weight. With the fractions marked liquid, an aliquot of sufficient size was used to reconstitute the fraction at the level it was obtained and to give a reconstituted soyflour equivalent to 12 percent soyflour based on the weight of the wheat flour.

Soyflour or Fraction	As is Weight
Ardex-550	1.12 gms.
Baker's Nutrisoy	1.11 gms.
Nutrisoy residue	0.377 gms.
Nutrisoy pH 4.5 isoelectric protein	0.374 gms.
Nutrisoy pH 4.5 soluble	0.349 gms.
Nutrisoy W II_D	0.075 gms.
Nutrisoy W II_{DS}	Liquid
Nutrisoy W II_{DS-H_2O}	"
Nutrisoy W II_{DS-NH_4OH}	"
Nutrisoy W $II_{DS-H_2O,\ H_2O}$	"
Nutrisoy W $II_{DS-H_2O,\ HCl}$	"

Fractionation of pH 4.5 Soluble Fraction. The pH 4.5 soluble fraction of nutrisoy was fractionated further by dialysis (Fig. 3). The two fractions thus obtained were W II_D (dialyzed) and W II_{DS} (dialyzate).

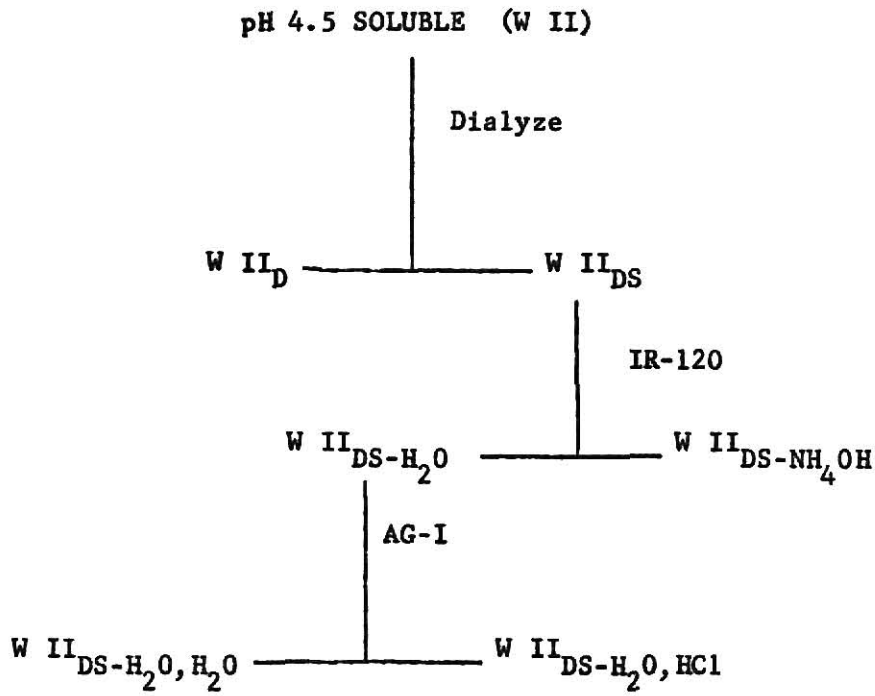

Fig. 3. Scheme showing the fractionation of pH 4.5 soluble fraction (W II) of Baker's Nutrisoy by dialysis, cation exchange column (IR-120), an anion exchange column (AG-I).

The 4.5 soluble was dialyzed for two days against a large excess of water at 4°C. The two fractions (W II_D and W II_{DS}) were vaccum-dried.

Cation Exchange Column Fractionation of W II_{DS}. Two milliliters aliquots containing 0.25 g. of the W II_{DS} fraction were passed through a cation column (0.9 x 12 cm.) of Amberlite IR-120H^+.

The sample was eluted with water and 2 N ammonium hydroxide (Fig. 3). Thus two fractions were obtained, an unbound, water eluted fraction (W II_{DS-H_2O}), and a bound fraction eluted with 2 N ammonium hydroxide (W II_{DS-NH_4OH}).

The W II_{DS-H_2O} and W II_{DS-NH_4OH} fractions, were vaccum dried, dissolved in a known amount of water stored at -40°C.

Anion Exchange Column Fractionation. The unbound water-eluted fraction (W II_{DS-H_2O}) from the cation column, was vaccum dried, dissolved in 2 ml. of water, and passed through an anion column (0.9 x 12 cm.) of AG-I(Cl^-).

The column was eluted with water (W II_{DS-H_2O,H_2O} fraction), and with 1 N hydrochloric acid (W $II_{DS-H_2O,HCl}$ fraction). The two fractions were vaccum-dried, dissolved in a known quantity of water and stored at -40°C (Fig. 3).

Paper Chromatography. The constituents of fractions from the cation and anion columns were characterized by paper chromatography. Each fraction was chromatographed on Whatman No. 4 paper and developed for 16 hours with the top layer of butanol, acetic acid and water (63:10:27).

Sugars were detected by dipping in silver nitrate in acetone followed by dipping in 0.1 N potassium hydroxide in methanol, Linko et al. (31).

Phosphates were detected by spraying with 0.1 percent feric chloride in 80 percent ethanol followed by spraying with 1 percent sulphosalicylicacid in 80 percent ethanol (32). Amino acids and peptides were detected by spraying with 2.0 percent ninhydrin in 95 percent ethyl alcohol and heating at 95°C for 5 minutes (33).

Fractionation of Dough. Doughs prepared from 100 g. wheat-flour or 100 gm. wheat flour plus soy fractions (Page 15), were fractionated by the following method. The dough was mixed to optimum consistency and washed out by hand in 250 ml. of distilled water. The gluten was rinsed 5 times (25 ml. each) and the combined wash water was centrifuged at 1000 x g. for 30 minutes to separate water soluble and starch fractions (Fig. 4).

The gluten thus obtained from each dough was cut into small pieces and stirred for 5 hours in 280 ml. of 0.005 N acetic acid. The gluten suspension was centrifuged at 1000 x g. for 30 minutes to remove the insoluble fraction. The supernatant (pH 4.7 soluble fraction) was adjusted to pH 6.1 with 0.1 N sodium carbonate and centrifuged at 1000 x g. for 30 minutes. The resultant fractions, the supernatant (pH 6.1 soluble) and the centrifugate (pH 4.7 gluten) were dried by lyophilization.

The following fractions were collected from each dough (Fig. 4):

(A) starch

(B) water soluble

(C) insoluble fraction

(D) pH 4.7 gluten fraction

(E) pH 6.1 soluble fraction

Starch Gel Electrophoresis. The starch gel electrophoretic technique employed was a modification of the procedure described by Woychik et al. (34).

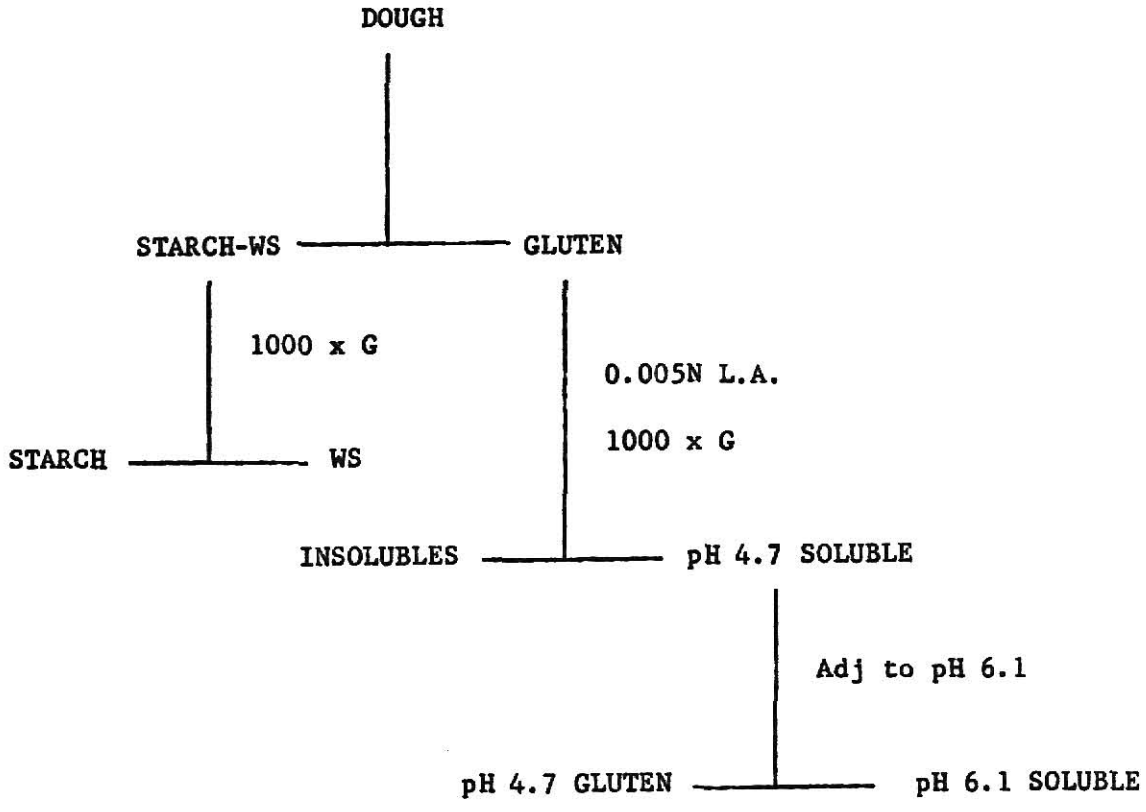

Fig. 4 Scheme showing the procedure adopted for fractionating dough.

The hydrolyzed starch used was obtained from The Connaught Medical Research Laboratories, Toronto, Canada; through Fisher & Co. U.S.A.

The pH 3.2 buffer used, contained 10 ml. lactic acid, 180 gm. urea and 1.925 gm. aluminum lactate per liter.

Forty five gm. of the hydrolyzed starch was suspended in 400 ml. of buffer, heated until a drop in viscosity after gelatinization is noted. Air bubbles are allowed to rise to the surface and skimmed off; and the gel was poured in the mold. The gels are kept in the cold room (4°C) for two or three days before use. The starch gels were run for six hours at 350 volts and 30 milliamps.

After cutting the gels to reduce its thickness by one-half, it was stained with aqueous 0.1 percent Amido Black overnight and repeatedly washed with distilled water for about 3 days. The starch gels were photographed using transmitted light.

Sucrose Monotallowate. Sucrose monotallowate (SMT) was obtained from Colonial Sugars, Co: Gramercy, La. It was a commercial synthetic glycolipid. Preliminary work in our laboratory showed that 2.5 percent SMT produced optimum results with dough containing 12 percent soyflour.

Results and Discussion

Fractionation of Ardex-550. Extracting Ardex-550 with water (1:10) followed by extracting with sodium carbonate (pH 7.5) solubilized less than 38 percent of the total soyflour protein. Increasing the stirring time while keeping the pH constant at 8 did not increase the amount of soluble-protein above 38 percent of total soyflour protein (Fig. 5). After $2\frac{1}{2}$ hours stirring the curve flattened out and there was no further increase in the amount of solubilized protein.

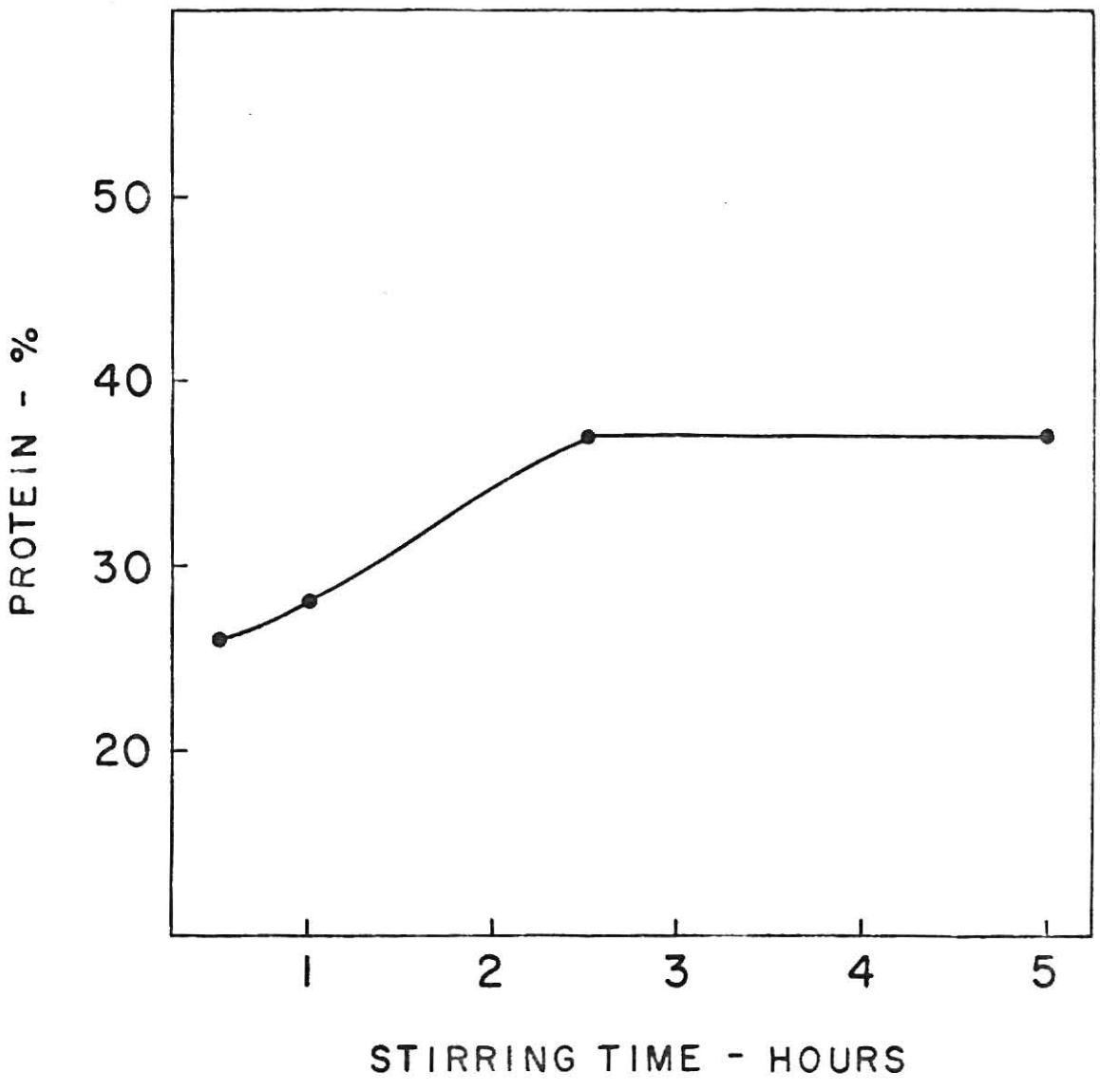

Fig. 5. Fractionation of Ardex-550. Effect of increasing stirring time, at pH 8.0 on percent protein solubilized.

Varying the pH, while holding the stirring time constant at 1 hour, similarly did not increase the amount of solubilized protein above 38 percent of the total soyflour protein (Fig. 6). The amount of protein solubilized increased with increased pH up to pH 10, and thereafter decreased slightly.

Since Ardex-550 did not give the fractions necessary for our study, a new soyflour, Baker's Nutrisoy, was obtained for fractionation.

Fractionation of Baker's Nutrisoy. Baker's Nutrisoy was fractionated by the scheme given in Fig. 2. These fractions were obtained, an insoluble fraction (R I), an isoelectric precipitated fraction (R II) and a soluble fraction (W II).

About 70 percent of the total soyflour protein was extracted with water from this sample. The isoelectric precipitated fraction (R II) contained the greatest quantity of total soyflour protein (60.6%). The protein content of the ieoelectric precipitated protein fraction (R II) was 80 percent.

The water soluble fraction (W II) contained the smallest quantity of the total soyflour protein (6.8%). The insoluble fraction (R I) contained 32.4 percent of the total soyflour protein (Table 1). The protein contents of the insoluble fraction (R I) and water soluble fraction (W II) were 42 and 12 percent respectively.

Baking. The baking results shown in Table 2 indicate that there is no significant difference between the loaf volume of breads baked with wheat flour + Ardex-550 + SMT, and wheat flour + Baker's Nutrisoy + SMT. Therefore, both of these soyflours were used as controls in baking.

The 4.5 isoelectric protein fraction (R II) of Baker's Nutrisoy produced as good a loaf volume as Ardex-550 and Baker's Nutrisoy. It also

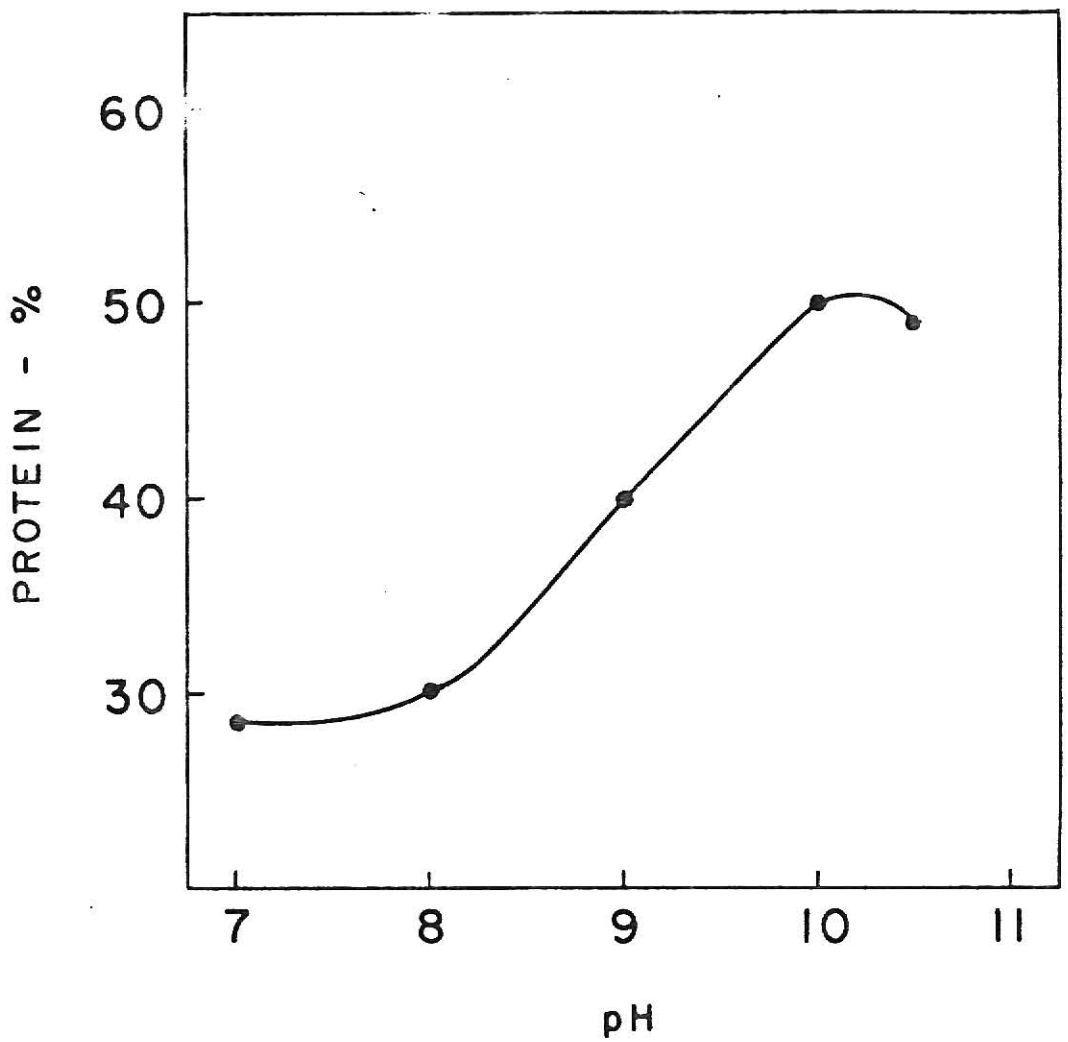

Fig. 6. Fractionation of Ardex-550. Effect of increasing pH, stirring time held constant at one hour, on percent protein solubilized.

Table 1. Analytical data for Fractions Obtained from Baker's Nutrisoy Flour.

Fraction	Yield	Protein Content	Protein Per 100 g Flour	% of Total Flour Protein
	g.	%	g.	
Soyflour	100	49.0	49.0	100
Residue (R I)	34	46.8	15.9	32.4
Isoelectric fraction (R II)	33	84.0	29.7	60.6
pH 4.5 soluble (W II)	30	11.1	3.3	6.8

Table 2. Loaf Volumes of Loaves Baked with RBS Flour (10 g, 14% M.B.) with Various Soyflours or Nutrisoy Fraction Added.

Soymeal Fraction[a]	Lipid[b]	Loaf Volume
		cc.
None	Shortening	82[c]
None	None	66
None	SMT	71
Ardex	SMT	85
Nutrisoy	SMT	85
Nutrisoy	None	65
Residue (R I)	SMT	76
Isoelectric (R II)	SMT	82
pH 4.5 Soluble (W II)	SMT	70
R I + R II + W II	SMT	82
Isoelectric (R II)	None	63
W II_D	SMT	82
W II_{DS}	SMT	74
W II_D + W II_{DS}	SMT	73
W II_D + W $II_{DS\text{-}NH_4OH}$	SMT	77
W II_D + W $II_{DS\text{-}H_2O}$	SMT	62
W II_D + W $II_{DS\text{-}H_2O,H_2O}$	SMT	66
W II_D + W $II_{DS\text{-}H_2O,HCl}$	SMT	73
W II_D + W $II_{DS\text{-}H_2O,H_2O}$ + W $II_{DS\text{-}H_2O,HCl}$	SMT	60
W II_D + W $II_{DS\text{-}NH_4OH}$ + W $II_{DS\text{-}H_2O}$	SMT	63

a/ Weight of fraction given on page 15.

b/ Shortening added at 3% of wheat flour weight, SMT added at 2.5% of wheat-flour weight.

c/ Loaf also contained 4% nonfat dried milk solids.

produced a better crumb grain and texture than that of the loaf baked with Baker's Nutrisoy (Table 2). The pH 4.5 soluble fraction of Baker's Nutrisoy proved deleterious to loaf volume.

The three fractions, pH 4.5 ieoelectric protein, pH 4.5 water soluble, and insoluble were reconstituted in their original porportions. RBS flour supplemented with 12 percent of the reconstituted soyflour produced a loaf volume nearly comparable to RBS flour supplemented with 12 percent unfractionated Baker's Nutrisoy. Thus, the fractions were not significantly altered by the fractionation procedure.

Gassing Power. The doughs containing pH 4.5 water soluble fraction (W II) of Baker's Nutrisoy appeared "dead" and had a low proof height. Therefore, the influence of that fraction on gas production was investigated. The results (Table 3) show no significant difference between the gas production of the doughs containing the pH 4.5 water soluble-fraction and the dough containing the two soyflours, Ardex-550 and Baker's Nutrisoy.

Since there was no difference between the gas production of the controls and the pH 4.5 water soluble fraction, it was assumed that the pH 4.5 soluble fraction had a deleterious effect on gas retention rather than on gas production.

Fractionation of the pH 4.5 Soluble Fraction. In an effort to identify the substance deleterious to gas retention, the pH 4.5 water-soluble fraction was fractionated further by dialysis. The resultant two fractions, W II_D and W II_{DS}, of the pH 4.5 water soluble fraction, were added to RBS + SMT singly and in combination, and baked into bread (Table 2). The W II_{DS} fraction produced a slightly lower loaf volume than did the W II_D fraction.

Table 3. Gas Production Data for RBS Flour (10 g.) Plus Various Soyflours and Fractions from Baker's Nutrisoy.

Flour	Soy Fraction	Pressure Reading
RBS	----	440
RBS	Ardex-550	460
RBS	Nutrisoy	450
RBS	pH 4.5 soluble (W II)	455
RBS	Residue (R I)	430

The loaves containing the reconstituted W II_D and W II_{DS} fractions produced a loaf volume comparable to the loaves containing the unfractionated pH 4.5 water soluble fraction (Table 2). Therefore dialysis did not damage the W II_D and W II_{DS} fractions.

The W II_{DS} was fractionated further on a cation exchange column. The W II_{DS-H_2O}(unbound) and W II_{DS-NH_4OH} (bound) fractions, of the W II_{DS} fraction were reconstituted with W II_D added to RBS + SMT and baked into bread. The loaf containing W II_D + W II_{DS-NH_4OH} + SMT had a loaf volume of 77 cc. compared to 82 cc. for the loaf containing W II_D + RBS + SMT (Table 2). The loaf containing W II_{DS-H_2O} + W II_D + SMT had a loaf volume 8 cc. lower than the loaf containing W II_D + RBS + SMT.

Thus the substance deleterious to loaf volume appears to be in the W II_{DS-H_2O} fraction. Therefore, the W II_{DS-H_2O} fraction was further fractionated by passing it through an anion exchange column.

The two anion exchange column-fractions, W II_{DS-H_2O,H_2O} unbound, and W $II_{DS-H_2O,HCl}$ bound, of the W II_{DS-H_2O} fraction were reconstituted with the W II_D fraction, added to RBS wheat flour + SMT, and baked into bread. The loaf containing the W II_{DS-H_2O,H_2O} unbound fraction had loaf volume of 66 cc. and the W $II_{DS-H_2O,HCl}$ bound fraction had a loaf volume of 73 cc., both of which were considerably below the loaf volume of bread containing the W II_D fraction (82 cc.).

The W II_{DS-H_2O,H_2O}, W $II_{DS-H_2O,HCl}$, and W II_D fraction were reconstituted and added to RBS wheat flour + SMT and the resultant bread had a loaf volume of 60 cc., which was 10 cc. lower than the control loaf of W II + RBS + SMT. Therefore, it appears that something in the fractionation scheme has altered one or more of the fractions.

The W II_{DS-H_2O,H_2O}, W $II_{DS-H_2O,HCl}$, and W II_D fractions were reconstituted and added to RBS wheat flour + SMT the resultant loaf had a volume of 63 cc., some 7 cc. below the control loaf of W II + RBS wheat flour + SMT. Thus it appears the cation exchange column may be responsible for changing the fractions. Nevertheless, it was felt that since the fractions were changed no meaningful conclusions concerning the nature or identity of the deleterious substance can be drawn.

Baking Properties of the 4.5 Isoelectric Fraction. When baked alone (no milk or shortening), RBS wheat flour produced a very poor loaf volume (Table 2). Adding 2.5 percent SMT improved the loaf volume to some extent, but it was still not comparable to controls, (RBS wheat flour + shortening + milk).

Supplementing RBS wheat flour (no milk, no shortening) with 12 percent Baker's Nutrisoy produced a poor loaf volume (65 cc.). Addition of 2.5 percent SMT to the formula brought the loaf volume up to 85 cc. (Table 2).

Supplementing the RBS wheat flour (no milk, no shortening) with the pH 4.5 isoelectric protein fraction produced a poor loaf volume (Table 2). However, addition of 2.5 percent SMT improved the loaf volume to a level comparable to the controls, Ardex-550 and Baker's Nutrisoy.

From the foregoing discussion it is evident that to replace milk in the baking formula with soyflour or its 4.5 isoelectric protein fraction, SMT is essential to maintain a good loaf volume.

Since one of the objectives of the study was to improve the nutritive value of bread, 12 percent soyflour was used in the baking studies. In baking studies involving soyflour fraction, the fractions were added at a level equivalent to the level present when 12 percent soyflour was added

to the doughs. However, it was also of interest to determine the minimum amount of pH 4.5 isoelectric protein fraction necessary to replace milk in the baking formula. Therefore, the quantity of pH 4.5 isoelectric protein fraction was reduced in a series of loaves and the baking data (Table 4) shows that there was little change in the loaf volume.

Reducing the quantity of the pH 4.5 isoelectric protein fraction in the dough to 0.5 percent of the original level had no significant effect. Further reductions in the level of pH 4.5 isoelectric protein fraction in the dough gave lower loaf volumes (Table 4).

Starch Gel Electrophoresis. Since 4.5 isoelectric protein-fraction from Nutrisoy produced a loaf volume equal to the controls (Ardex-550, and Baker's Nutrisoy) in the presence of SMT, it was assumed that the various components (pH 4.5 isoelectric protein, SMT and RBS wheat flour) interacted. Starch gel electrophoresis was used in an attempt to demonstrate that interaction.

Doughs were prepared and fractionated by the scheme shown in Fig. 4. The fractions obtained were starch, water soluble, insoluble, pH 6.1 soluble and pH 4.7 gluten fraction. Starch gel electrophoretic patterns of the insoluble + pH 4.7 gluten fraction from wheat-flour are shown in Fig. 7. The 4.5 isoelectric protein fraction (Fig. 8) was quite different from the flour fractions and thus should be distinquishable in mixtures of wheat-flour protein and pH 4.5 isoelectric protein fraction.

The starch gel electrophoretic patterns of the fractions from dough that contained only RBS wheat flour and pH 4.5 isoelectric protein appeared in the insoluble fraction. Since the soy protein is insoluble at pH 4.7, the pH 4.5 isoelectric protein fraction would be expected to appear in the insoluble fraction if there was no interaction. Therefore, there appears

Table 4. Loaf Volume of RBS Wheat Flour Supplemented with Decreasing Amounts of Isoelectric Protein Fraction (R II) from Baker's Nutrisoy.

Flour	Weight of R II	Loaf Volume
	g.	cc.
RBS	0.374	82
RBS	0.225	83
RBS	0.075	80
RBS	0.0375	80
RBS	0.0188	78
RBS	0.0094	77

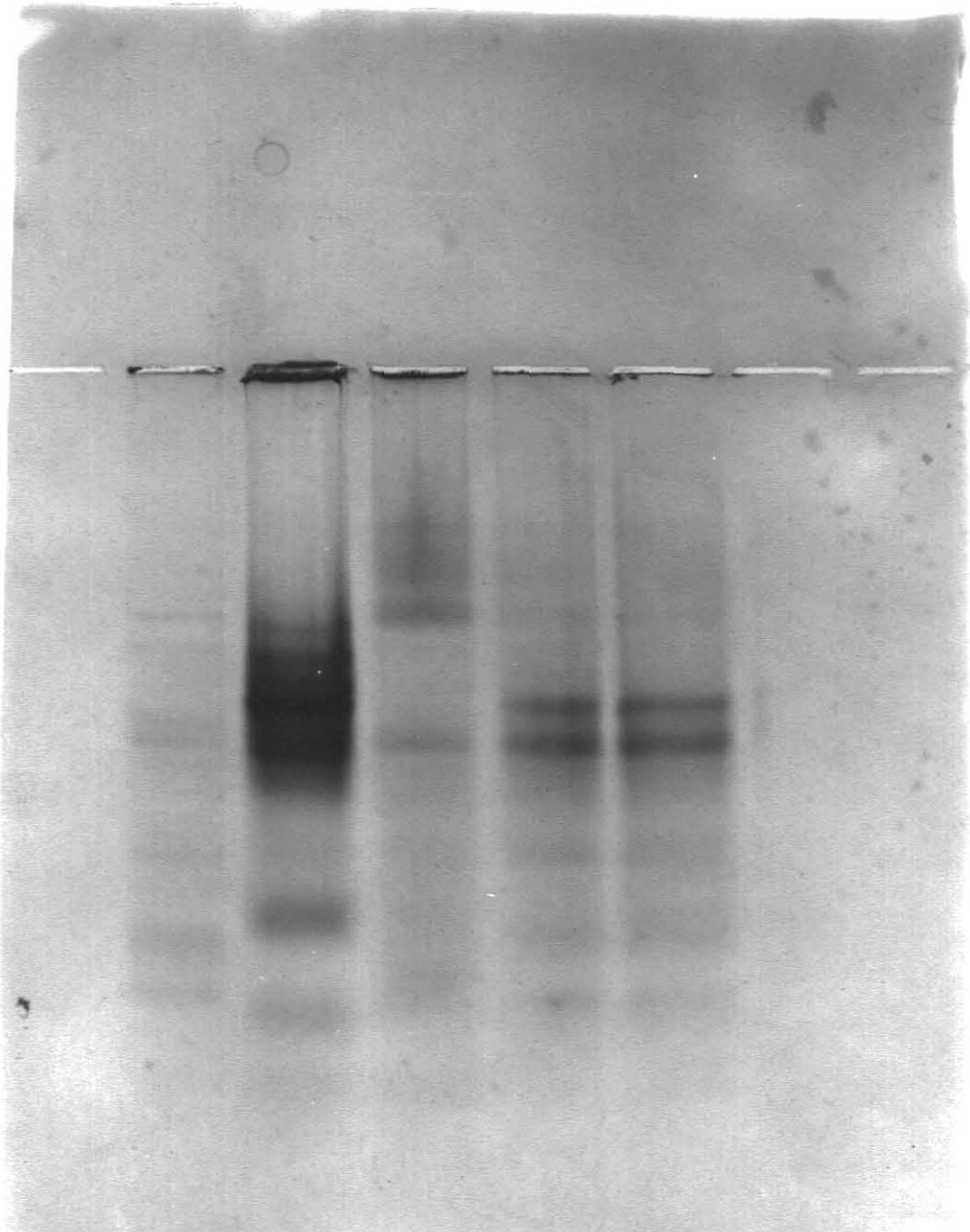

Fig. 7. Starch gel electrophoretic patterns of (left to right) insoluble fraction from the dough containing RBS alone, pH 4.5 isoelectric protein fraction (R II) of Baker's Nutrisoy, pH 4.7 gluten fraction from the dough containing RBS wheat flour alone, and two patterns of the insoluble fraction from the dough containing RBS wheat flour and pH 4.5 isoelectric protein fraction of Nutrisoy.

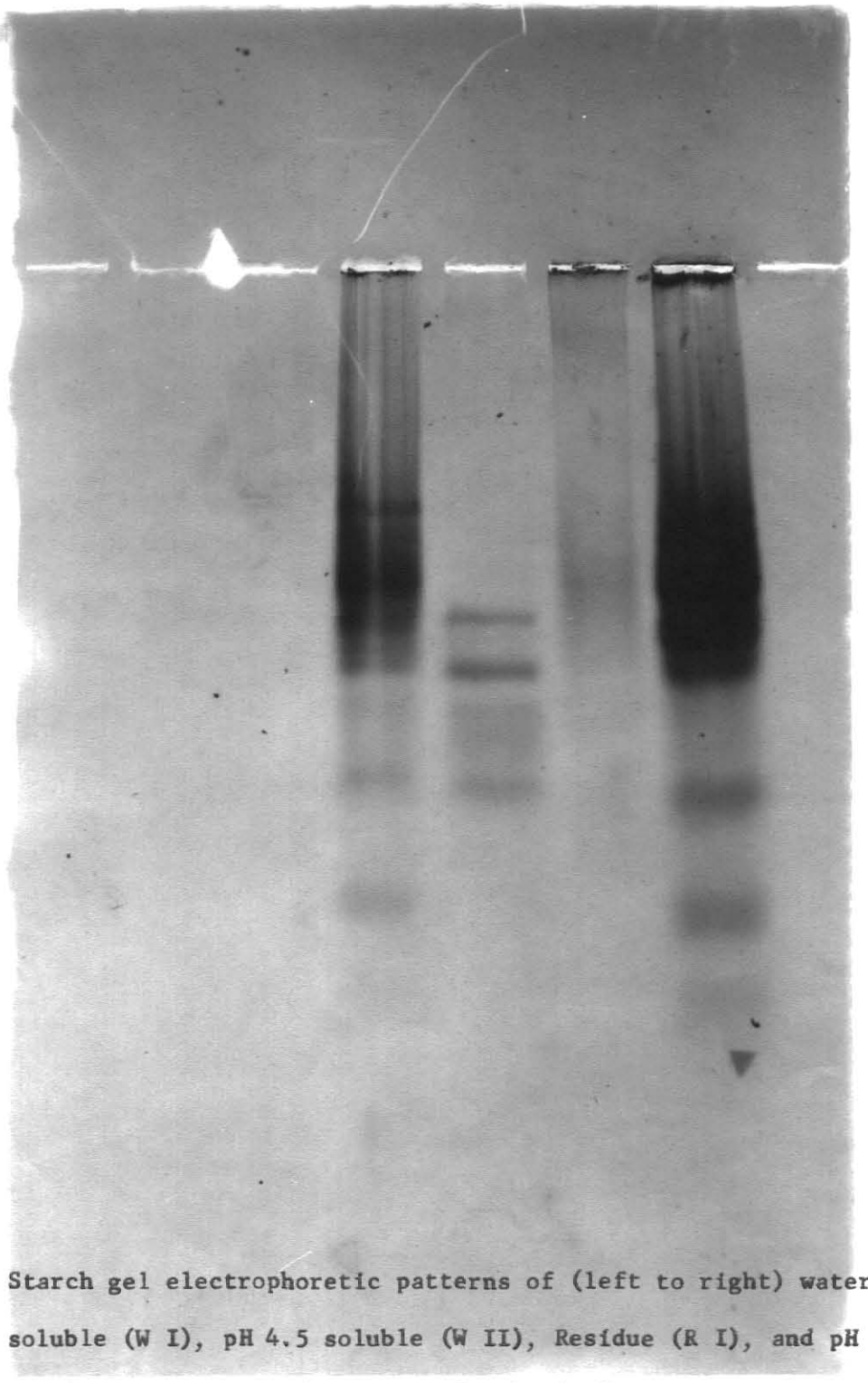

Fig. 8. Starch gel electrophoretic patterns of (left to right) water soluble (W I), pH 4.5 soluble (W II), Residue (R I), and pH 4.5 isoelectric protein fractions of Baker's Nutrisoy.

to be no interaction between the pH 4.5 isoelectric protein fraction and the wheat-flour components.

Starch gel patterns of the fractions from the dough that contained RBS wheat flour, pH 4.5 isoelectric protein and SMT are shown in Fig. 9. The pH 4.5 isoelectric protein fraction was clearly in the pH 4.7 gluten fraction. Therefore, the pH 4.5 isoelectric-protein fraction appears to interact with the pH 4.7 gluten fraction of flour in the presence of SMT. Additional evidence of the interaction of SMT with wheat flour gluten was noted during the preparation of the fractions. When the dough contained 2.5 percent SMT a normal gluten ball was not obtained, instead the gluten broke up into small pieces that had to be collected off the screen.

Paper Chromatography of Ion-Exchange Column Fractions of W II_{DS}.

Paper chromatography of the ion-exchange column fractions confirmed that the bound fraction eluted with 2 N ammonium hydroxide (W II_{DS-NH_4OH}) obtained from cation exchange column contained amino acids and peptides. The other cation exchange column fraction, unbound water eluted (W II_{DS-H_2O}) contained sugars and phosphates.

The unbound anion column fraction (W II_{DS-H_2O,H_2O}) was shown by paper chromatography to contain sugars. The bound, hydrochloric acid eluted fraction (W $II_{DS-H_2O,HCl}$) contained phosphates.

Summary

Ardex 550 soyflour is not suitable for fractionation because of its solubility characteristics.

Baker's Nutrisoy which had baking characteristics similar to Ardex-550 when added to wheat flour in the presence of SMT, also had suitable solubility characteristics.

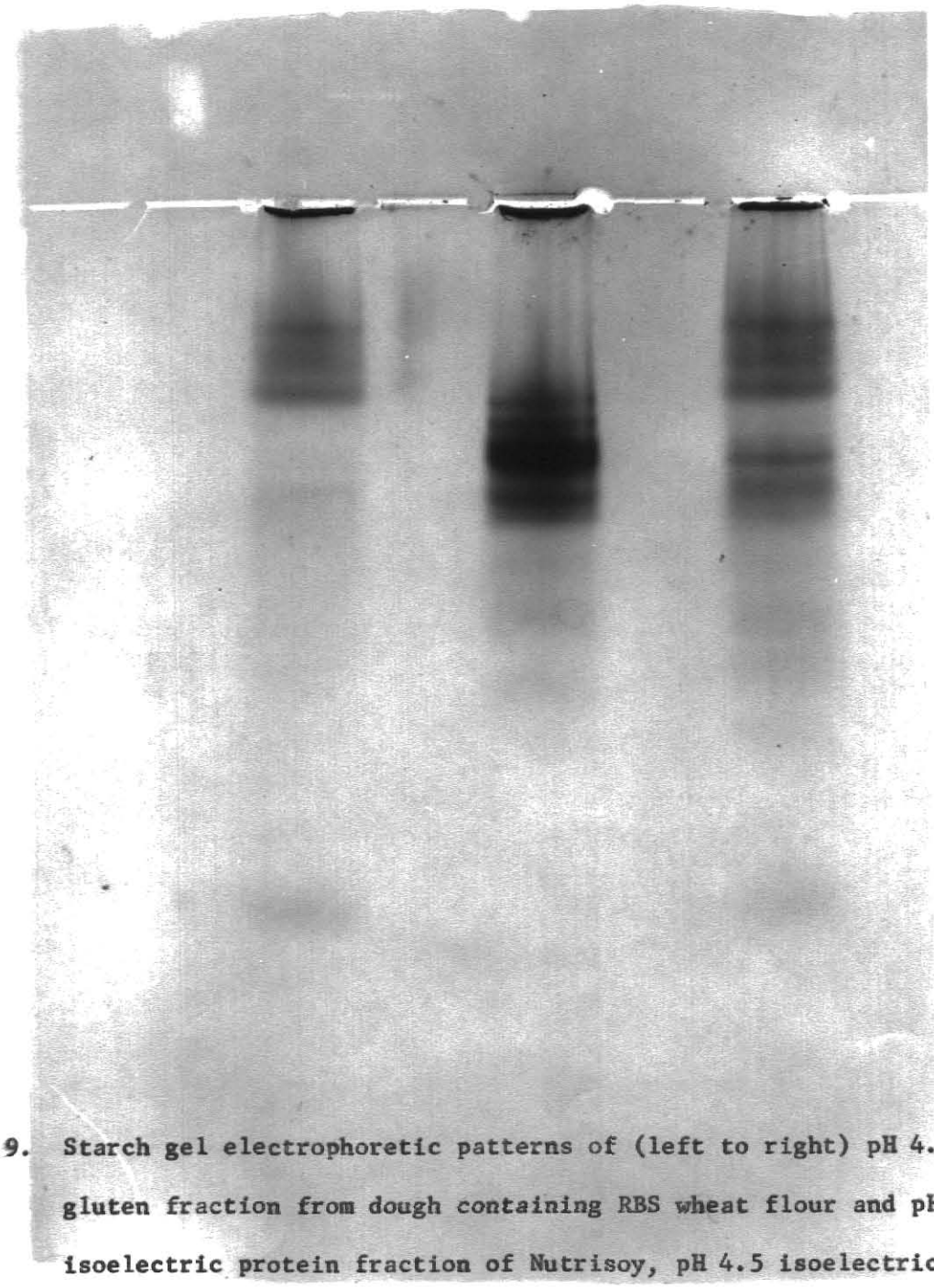

Fig. 9. Starch gel electrophoretic patterns of (left to right) pH 4.7 gluten fraction from dough containing RBS wheat flour and pH 4.5 isoelectric protein fraction of Nutrisoy, pH 4.5 isoelectric protein fraction (R II) of Nutrisoy, and pH 4.7 gluten fraction from the dough containing RBS wheat flour, sucrose monotallowate and pH 4.5 isoelectric protein fraction of Nutrisoy.

About 70 percent of the total soyflour protein could be solubilized. The pH 4.5 water soluble fraction (W II) of Baker's Nutrisoy was deleterious to loaf volume and it appeared that the effect was on the gas retention of the dough.

Attempts to isolate the substance deleterious to loaf volume by dialysis and ion-exchange columns (cation and anion) were unsuccessful since the fractions were altered during the fractionation.

The 4.5 isoelectric protein fraction (R II) of Baker's Nutrisoy produced a loaf volume as good as the controls, Ardex-550 and Baker's Nutrisoy, in the presence of SMT.

An interaction between RBS wheat flour, SMT and soy 4.5 isoelectric protein-fraction was demonstrated by starch gel electrophoresis. Thus, in the presence of SMT the pH 4.5 isoelectric protein fraction interact with the 4.7 gluten fraction of wheat flour. Further evidence of the interaction of SMT with gluten was suggested by its unusual gluten washing characteristics. Only 0.0375 g. of the 4.5 isoelectric-protein fraction was necessary to replace 0.4 g. milk in the baking formula.

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SOYFLOUR FRACTIONS IN BREADMAKING AND THEIR INTERACTION WITH WHEAT FLOUR COMPONENTS

by

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B. Sc. Osmania University (India) 1960

AN ABSTRACT OF A THESIS

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Two defatted commercial soyflours, Ardex-550 and Baker's Nutrisoy, had similar bread-baking characteristics when added to wheat flour and supplemented with sucrose monotallowate. Baker's Nutrisoy proved better than Ardex-550 for fractionation studies because of its increased solubility in water.

About 70 percent of the total soyflour protein could be solubilized from Baker's Nutrisoy with water. The bulk of the protein was precipitated from the solution by adjusting the pH to 4.5 with lactic acid. Three fractions were thus obtained, a pH 4.5 water-soluble, a pH 4.5 isoelectric protein, and an insoluble residue. The pH 4.5 water soluble fraction represented 30 percent of the soyflour and contained 6.8 percent of the total soyflour protein. The pH 4.5 isoelectric protein represented 33 percent of the soyflour and contained 61 percent of the total soyflour protein. The insoluble residue represented 34 percent of the soyflour and contained 32 percent of the total soyflour protein.

The pH 4.5 isoelectric protein fraction when added to wheat-flour in the presence of sucrose monotallowate produced a loaf volume comparable to the controls of soyflour, sucrose monotallowate and wheat flour. The pH 4.5 water soluble fraction when similarily baked was deleterious to loaf volume.

The pH 4.5 water soluble fraction apparently had an effect on gas retention of the dough. Attempts, to isolate the substance deleterious to loaf volume by dialysis and ion-exchange columns, were unsuccessful since the fractions were altered during fractionation.

An interaction between wheat flour, sucrose monotallowate and soyflour protein was demonstrated by starch gel electrophoresis. The pH 4.5 isoelectric protein fraction, sucrose monotallowate, and the gluten fraction of wheat flour interact during dough mixing. Further evidence of the interaction of sucrose monotallowate with gluten was suggested by its unusual gluten washing characteristics.

The minimum amount of pH 4.5 isoelectric protein fraction that was needed to replace 0.4 gm. milk in the baking formula was 0.0375 gm.