SOY FLOUR AS A WHITE BREAD INGREDIENT
II. Fractionation of Raw Soy Flour and Effects of the Fractions in Bread[1]

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ABSTRACT

An unheated, defatted soy flour was fractionated by mild solvent separation, precipitation, and dialysis techniques. The baking performance of the flour and its fractions, employed at a level of 3% (wheat flour basis), was tested by a small scale procedure using several levels of potassium bromate. Chemical studies were made on the fractions in an attempt to relate their composition to baking performance. The raw soy flour caused reproducible adverse effects on loaf volume, but appropriate heat treatment improved loaf volume when baked with a satisfactory level of bromate. Heat treatment of several fractions adversely affected loaf volume; others were not significantly influenced, and one was improved.

The most injurious fractions were encountered in the dialysate from the supernatant after precipitation of most of the protein at pH 4.2 from the water-soluble fraction. Still another dialysate fraction contained most of the undesirable flavor, although it was otherwise of fair baking quality. A protein fraction, not precipitated at pH 4.2, was excellent in baking quality and showed high antitryptic activity.

Analyses of various fractions demonstrated that chlorides, sugars, and sulfhydryl groups were not responsible for the poor baking quality of the fractions in which they were found. Effects of fractions on dough pH were eliminated as a cause of poor baking quality. Quantitative analysis of the two most injurious fractions indicated significant levels of zinc, calcium, magnesium, and phosphate ions. In baking tests only zinc and phosphate ions corresponding to the levels in 3% of the most injurious fraction were found harmful. Zinc and phosphate ions in the amounts employed in the baking tests (0.0048 and 0.60% respectively, flour basis) retarded gas production; whereas the adverse effects of raw soy flour itself, and of the acid-precipitable protein, were primarily on gas retention. Thus, the inorganic constituents may be of relatively little importance in the performance of soy flour iself.

In a previous paper the authors reported that the addition of 1% experimentally prepared, defatted, raw soy flour to a Southwestern Bakers' Patent flour somewhat improved loaf volume as determined by a bromate baking formula containing 1 mg. of potassium bromate per 100 g. of flour, whereas higher levels had a deleterious effect when baked with the same bromate dosage (24). The raw soy flour markedly improved the stability of nonfermenting doughs as measured by normal and rest-period farinograms, the extent of the improvement increasing with the soy flour level employed over the range of 1 to 5%.

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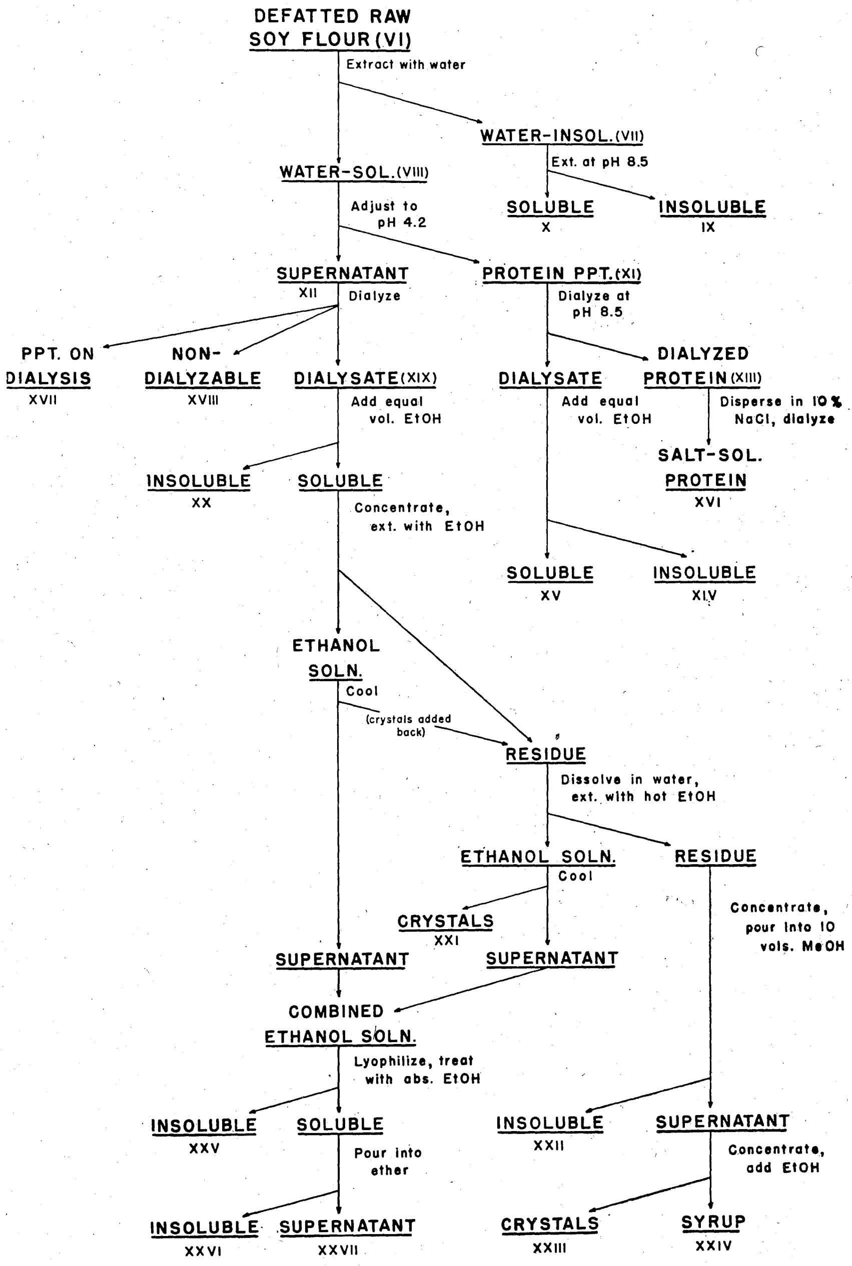

Fig. 1. Schematic outline of fractionation procedure applied to experimental unheated defatted soy flour.

Heat treatment of the soy flour at 100°C. or higher for 1 hour at 7.9% moisture decreased the water dispersibility of the proteins, decreased its baking quality as reflected by the bromate baking test, and decreased its beneficial effect on the stability of doughs to extended mixing in the farinograph.

The experiments reported in the present paper were undertaken to explore the properties of various fractions prepared from soy flour. Through a study of the composition and baking characteristics (as influenced by heat treatment and the presence of an oxidizing improver) of such fractions, it might be possible to eliminate the harmful effects of certain constituents by suitable modifications in processing soy flour or by changes in the bread formula or baking procedure.

Fractionation of Soy Flour

Initial Extraction Steps. The defatted raw soy flour (500 g.) described by Pollock and Geddes (24) was fractionated according to the scheme outlined in Fig. 1. The first step was a 2-hour water extraction at pH 6.5 and 30°C., using a solvent:solid ratio of 20:1 with continuous stirring at medium speed. The thin slurry resulting was centrifuged by three passes through a Sharples supercentrifuge, giving the water-insoluble and water-soluble fractions, designated nos. VII and VIII, respectively. Both fractions were frozen, crushed, and dried from the frozen state in a refrigerated chamber under 1 to 2 mm. pressure, using a small radiant heat input. The water-insoluble fraction (VII) obtained from one batch of soy flour without drying was re-extracted by continuous stirring with 20 parts of very dilute aqueous sodium hydroxide at pH 8.5. After centrifuging at 28,000 r.p.m. (one pass), the centrifugate was adjusted back to pH 6.5 with dilute hydrochloric acid; the insoluble (IX) and soluble (X) fractions were then dried in the frozen condition.

Fractionation of Water-Soluble Material: Precipitation of Protein. To precipitate most of the soluble protein, the water-soluble fraction (VIII) obtained from the supercentrifuge was adjusted to a pH of 4.2 with dilute hydrochloric acid solution. The acid-precipitated protein was recovered in a centrifuge, washed, twice redissolved in dilute sodium hydroxide solution at pH 8.5, and reprecipitated. The moist protein was frozen and dried by lyophilization to yield fraction XI.

The supernatant from No. XI was frozen, crushed, and dried giving fraction XII. This material consisted principally of low molecular weight substances and required care in lyophilization.

Additional Protein Purification Measures. A new preparation of moist protein (No. XI), after washing at pH 4.2 and redissolving at pH 8.5, was placed in sections of Visking casing and dialyzed for 4 days at 2°–4°C. against slightly alkaline solvent. Fresh external medium was provided daily, and the protein solution was protected against bacterial growth by small quantities of chloroform and toluene. The dialyzed protein was precipitated at pH 4.2, centrifuged, quick-frozen, and lyophilized (fraction XIII).

The combined dialysate from No. XIII was concentrated to a small volume under reduced pressure at 30°–35°C. To the concentrated dialysate, an equal volume of 95% ethanol was added, yielding a precipitate (fraction XIV). The supernatant, fraction XV, was concentrated under reduced pressure to remove the ethanol, diluted with water, frozen, and dried.

A second batch of dialyzed protein (No. XIII) was stirred 4 hours with 20 parts of 10% sodium chloride solution at 30°C. The clear supernatant upon centrifuging was dialyzed at 2°–4°C. against frequent changes of eight volumes of distilled water until a negative test for chloride ion was obtained. The protein precipitated during dialysis was centrifuged, frozen, and lyophilized (fraction XVI).

Further Treatment of Supernatant (XII) from Original Protein Precipitation. A fresh portion of XII was dialyzed at 2°–4°C. for 4 days against 1.5 volumes of distilled water, changed daily. The dialysate collected during the first 3 days was retained. During dialysis a trace of solid material precipitated, which was separated by centrifuging, frozen, and lyophilized (fraction XVII). The centrifugate, constituting the remainder of the nondialyzable fraction, was isolated in the same manner (fraction XVIII).

The dialysate portions above were adjusted as obtained to pH 6.5 and concentrated by perstillation in a 40°C. forced-draft oven to about one-sixth their original volumes. The combined concentrated dialysate was frozen and dried in the frozen state without application of heat. The dried material was extremely hygroscopic; since it was impractical to handle, sufficient water was added to give a heavy syrup (fraction XIX).

Fractionation of Dialysate (XIX) with Ethanol. The concentrated dialysate (XIX) from three batches of defatted soy flour was accumulated, and an equal volume of 95% ethanol was added, precipitating fraction XX. The supernatant alcoholic solution was concentrated under reduced pressure to a heavy syrup, which was extracted five times with 95% ethanol at 27°C. Crystalline products separating from the cold ethanol extracts on standing were returned to the residue. The residue was dissolved in water to give a syrup and extracted five times with hot 95% ethanol (70°C.). Cooling of the combined hot ethanol extracts gave crystalline fraction XXI.

The cold and hot ethanol extracts, when combined and concentrated to a small volume under reduced pressure, separated into immiscible layers. The mixture was dried in the frozen state and the solid treated with absolute ethanol. The ethanol-insoluble residue, being very hygroscopic, was converted to a syrup (fraction XXV) for ease of manipulation. The absolute ethanol solution was again concentrated to a small volume and poured into five volumes of ethyl ether, precipitating fraction XXVI, which was dried to a gummy solid. The ether solution, upon evaporation, yielded a trace of red-brown syrup (fraction XXVII).

Fractionation of Original Ethanol-Insoluble Residue with Methanol. The residue, after extracting the concentrated dialysate fraction with cold and with hot ethanol, was taken up in water to form a light syrup. When poured into ten volumes of methanol, the material gave a bulky precipitate, which was filtered rapidly, washed, and dried over phosphoric anhydride (fraction XXII).

From the filtrate, methanol was removed under reduced pressure. Upon cooling at 2°–4°C., crystals were obtained. The supernatant was concentrated further and ethanol added to incipient crystallization; upon cooling overnight a second crop of crystals was obtained. The procedure was repeated to give a third and fourth crop. All crystalline products were combined to form fraction XXIII. To remove all alcohol, the supernatant was evaporated under reduced pressure to a semisolid syrup and water added to give a medium heavy syrup, which was retained as fraction XXIV.

Heat Treatment of Fractions. The soy flour fractions in general were heat-treated for 1 hour at 100°C., using the apparatus described in a previous paper (24).

Because of the limited quantities and variable nature of the fractions, it was not feasible to bring them all to the same moisture content for heat treatment. The moisture contents at which the fractions were heated are shown in Table II. For certain fractions available in limited supply, the equipment was modified by using a smaller stainless steel cylinder (3.8 by 15.2 cm., inside dimensions) to prevent excessive moisture loss during heat treatment.

Syrupy fractions were heat-treated by exposure in open tubes in an oil bath at 80°C. for 30 minutes with frequent stirring.

Subfractionation Methods

Certain subfractionation techniques, not part of the original procedure, were adopted as results of baking tests and analytical data on the fractions became available. They were applied to those fractions which proved most active in the baking tests.

Water-Soluble and Insoluble Components of Fraction XX. Fraction XX, shown to be highly injurious to bread quality, was only partially water-soluble. To determine whether the activity lay in the soluble or insoluble portion, a 3.55 g. sample was shaken at 30°C. with four successive 15-ml. portions of distilled water for 30 minutes each. At each stage, the mixture was centrifuged and the supernatant solution removed. The combined supernatant and a slurry of the insoluble residue were frozen and lyophilized to obtain insoluble (XX_1) and soluble (XX_2) subfractions.

Ion Exchange Separation. Fraction XXII, suspected of containing considerable inorganic material, was separated into neutral and combined acidic and basic subfractions by an ion exchange procedure: a 3% solution containing 6 g. of fraction XXII was passed slowly through a column of Amberlite (cationic) resin IR-120 (acid form). The eluate was passed directly through a column of Duolite (anionic) resin A-4 (alkaline form). The resins were washed with six volumes of distilled water; the combined eluate and washings were concentrated under reduced pressure to a medium heavy syrup, which was retained as neutral subfraction $XXII_a$.

The portions exchanged by the respective resin columns were removed by regenerating the columns in the usual manner with hydrochloric acid and sodium hydroxide solutions. The eluates thus contained excess mineral acid and alkali; these were removed by passage again through the appropriate resins. The final eluates were combined, concentrated under reduced pressure, and evaporated to dryness in a vacuum oven at 50°C. and less than 10 mm. pressure. The dried material, the acidic and basic constituents of fraction XXII, was designated $XXII_b$.

Treatment of Fraction XXII with Ethanol-Water Mixtures. In an attempt to separate the components present in fraction XXII by solubility differences, a 40 g. sample was extracted with two portions each of 80, 70, and 60% aqueous ethanol. Each extraction was carried out by shaking 40 minutes, centrifuging, and removing the supernatant. Each pair of extracts was combined and concentrated under reduced pressure to a syrup. The residue, after the 60% ethanol extraction, was separated into a water-soluble portion (retained as a syrup) and an insoluble solid (frozen and lyophilized). Thus, the subfractions obtained were as follows:

Subfraction $XXII_1$	Extracted from XXII by 80% ethanol
Subfraction $XXII_2$	Extracted from XXII by 70% ethanol
Subfraction $XXII_3$	Extracted from XXII by 60% ethanol
Subfraction $XXII_4$	Water-insoluble portion of residue after 60% ethanol extraction
Subfraction $XXII_5$	Water-soluble portion of residue after 60% ethanol extraction.

Baking Tests and Analytical Procedures

Experimental Baking Tests. To test the soy flour fractions, many of which were available only in small quantities, a modification of the small-scale baking test described by Geddes *et al.* (10,11), employing 25 g. of wheat flour, was used. The formula and procedure are summarized in Table I. Each mix yielded one 40 g. dough; replicates were baked on successive days. Loaf volumes were determined by an adaptation of the conventional seed displacement method to a special small-scale apparatus which was calibrated with dummy loaves.

The soy flour fractions were included in dough formulas to the extent of 3% (dry basis) of the weight of wheat flour (14% moisture basis). Water absorption was adjusted as required to give uniform consistency, as judged by handling properties.

TABLE I

BASIC FORMULA AND PROCEDURE — SMALL-SCALE LABORATORY BAKING TEST

	% OF WHEAT FLOUR (14% Moisture Basis)	WEIGHT
		g/mix
Flour[a]	100	25.0
Yeast	3	0.75
Sugar	5	1.25
Salt	2	0.5
Potassium bromate[b]	variable	....
Water[c]	60	15.0

Mixing time[d]	2.5 min.
Dough weight	40 g.
Fermentation[e] at 30°C., 90% R.H.	
Punched[f] after	95 min.
Molded after	25 min.
Proofing time	55 min.
Oven time[g] at 232°C.	15 min.

[a] Commercial Southwestern Bakers' Patent Flour, ash 0.43%, protein 11.9%, both expressed on a 14% moisture basis.

[b] Potassium bromate was included to the extent of 0, 2, 4, or 6 mg/100 g wheat flour.

[c] Proper absorption was established at 60% according to dough handling properties.

[d] Mixing was done with a 25–35 gram Micro Mixer, supplied by the National Mfg. Co., Lincoln, Nebraska. The mixing head was operated at 120 r.p.m.

[e] Carried out in a "Humi-Temp" fermentation cabinet.

[f] Punching was done in a National dough sheeter, with rolls modified to an effective width of 1.5 inches and set 3/16 inch apart. For panning, doughs were sheeted twice, at 3/16 and 1/8 inch roll settings. Molding was done on a National dough molder. Pan size: top 7.1 by 4.6 cm; bottom 6.0 by 3.6 cm; depth 3.2 cm.

[g] Baking was carried out in a National Rotary hearth-type oven.

Moisture, Ash, and Nitrogen Determinations. Moisture in soy flour fractions was in most cases determined by the forced-draft oven procedure, described in a previous paper (24). However, dialyzable fractions high in sugar were heated in a vacuum oven at 80°C. under less than 10 mm. pressure for 5 hours and the loss in weight determined. Nitrogen and ash determinations were made as previously described (24). For fractions available only in small quantities, nitrogen was determined on a semimicro scale using selenium oxychloride as a catalyst.

Determination of Inorganic Constituents. Qualitative and quantitative tests for ions were applied to fractions containing appreciable inorganic material. Cations were determined qualitatively by semimicro methods after ashing (5).

Chloride was determined quantitatively in certain fractions as a measure of the quantity of sodium chloride derived from pH adjustments with acid and alkali during fractionation. The chloride was precipitated with excess standard silver nitrate, the organic matter destroyed with potassium permanganate, and the residual silver nitrate titrated with standard potassium thiocyanate solution using saturated ferric alum solution as an indicator.

A procedure was devised for the determination of zinc, calcium, magnesium, and phosphate on a single sample. Soy flour fractions were "wet-ashed" by digesting with concentrated sulfuric and nitric acids and hydrogen peroxide as prescribed by Sullivan and Near (27). Zinc was determined gravimetrically by precipitation as the sulfide, ignition and weighing as zinc oxide according to Furman (9). Phosphate was assayed in the filtrates by precipitation as the molybdate, ignition and weighing as $P_2O_5 \cdot 24MoO_3$ (15,23).

Prior to the analysis for calcium, excess molybdate reagent was removed by the procedure of Furman (9). Calcium was precipitated as the oxalate, which was ignited and weighed as the oxide (15). Analysis for magnesium was then accomplished by destroying excess ammonium oxalate, precipitation as magnesium ammonium phosphate, followed by ignition and weighing as magnesium pyrophosphate (23).

Sulfhydryl Groups. Sulfhydryl groups were determined qualitatively by the nitroprusside test and quantitatively by the amperometric method of Larson and Jenness (17).

Chromatographic Methods. Sugars were determined qualitatively and quantitatively in soy flour fractions derived from the dialysate fraction of soy flour using the chromatographic methods described by Koch *et al.* (14).

For quantitative sugar estimation, unknown solutions were applied to the paper in standard aliquots; guide strips along the sides of the chromatograms containing known sugars were removed, developed, and used to locate the individual sugars resolved from the test solution originally placed on the chromatogram. By reference to the guide strips, areas of the chromatogram containing the individual sugars were cut off, and the sugars were extracted by 15-minute treatment with a measured volume of water. The sugars were then determined spectrophotometrically by the phenol-sulfuric acid method of Dubois *et al.* (6).

Determination of Antitryptic Activity. Fraction XVIII, the nondialyzable fraction of the supernatant after protein precipitation, was obtained in a manner quite similar to the method employed by Klose *et al.* (13) in preparing a trypsin inhibitor fraction. The antitryptic activity of fraction XVIII was therefore of interest, and was determined by the method of Borchers *et al.* (2).

Determination of Dough pH. A Coleman Model 3 C pH electrometer was adapted to measure the pH of 1- to 2-g. dough portions. The measurement was accomplished by inserting the dough piece in the 5-ml. cup containing a few drops of potassium chloride solution; contact with the calomel electrode was established through a potassium chloride bridge. The glass electrode was inserted, the system allowed to equilibrate, and the pH determined in the conventional manner. Dough pH values thus obtained were in good agreement with values for larger dough samples and with those found in the literature (16).

Determination of Carbon Dioxide Production and Retention in Doughs. Production and retention of carbon dioxide in fermenting doughs which contained soy flour fractions and other adjuncts were measured with the Chopin Zymotachygraphe (4) at 30°C.

Doughs used for gas production and retention tests were made up of 125 g. Southwestern wheat flour (14% moisture basis) and other ingredients in the proportions given in Table I. Potassium bromate was included at a level of 2 mg. per 100 g. of flour; the various adjuncts were added at a level of 3% dry matter based upon flour at 14.0% moisture. Each dough was allowed to ferment for 6 hours.

Results

Results of Fractionation Procedure. The approximate percentage distribution of the fractions in defatted raw soy flour prepared as outlined in Fig. 1, together with their moisture and nitrogen contents are presented in Table II. Quantitative separation and recovery of all fractions was not practical; where feasible, losses were estimated, and the distribution figures are approximate only. The total weight of material recovered included 1.5% of sodium chloride, which was calculated as having been derived from pH adjustments and distributed among several fractions.

The isolated protein (fraction XIII) after purification contained 15.7% nitrogen and was nearly white and essentially tasteless. However, it was rendered low in water dispersibility by the isolation treatment involving acid. Re-extraction of the water-insoluble material

TABLE II

MOISTURE AND NITROGEN CONTENTS AND APPROXIMATE PERCENTAGE DISTRIBUTION OF SOY FLOUR FRACTIONS

FRACTION No.[a]	FRACTION	MOISTURE CONTENT "AS IS" BASIS	NITROGEN CONTENT (DRY BASIS)	CALCULATED % IN RAW DEFATTED SOY FLOUR (DRY BASIS)
		%	%	%
VI	Laboratory-defatted raw soy flour	8.7	9.12	100
IX	Fraction of water-insol. portion, insoluble at pH 8.5	7.6	3.14	21.1
X	Fraction of water-insol. portion, soluble at pH 8.5	6.2	12.82	13.9
XIII	Protein precipitated at pH 4.2 and dialyzed	6.0	15.70	30.4
XIV	Fraction of protein dialysate insoluble in 1:1 ethanol	11.0	11.78	0.3
XV	Fraction of protein dialysate soluble in 1:1 ethanol	8.4	3.82	0.9
XVII	Precipitate during dialysis of protein supernatant	6.0	13.62	0.7
XVIII	Nondialyzable fraction of protein supernatant	9.3	12.36	4.3
XX	Fraction of dialysate (of protein supernatant) insoluble in 1:1 ethanol	12.0	0.76	1.9
XXI	Crystals from hot ethanol extracts of dialysate	...	0.22	0.2
XXII	Precipitate upon addition of ethanol-insoluble portion of dialysate to methanol	10.2	1.27	9.6
XXIII	Crystals from methanolic supernatant	...	0.098	0.6
XXIV	Remainder of methanolic fraction[b]	25.7	1.28	6.0
XXV	Fraction of dialysate soluble in aqueous ethanol, insoluble in absolute ethanol[b]	28.2	2.36	7.2
XXVI	Precipitate upon addition of ethanol-soluble residue above to ethyl ether[b]	15.9	3.04	0.8
XXVII	Material soluble in ethyl ether	...	...	trace
	Total			97.9[c]

[a] See Fractionation Scheme, Fig. 1.

[b] Moisture content determined as loss of weight upon heating 6 hours at 80°C. and less than 10 mm. pressure.

[c] Includes 1.5% sodium chloride distributed among various fractions, calculated to have been derived from pH adjustments.

with very dilute alkali (pH 8.5) dispersed additional protein, as evidenced by the high nitrogen content of fraction X.

Baking Tests. The inclusion of 3% of raw soy flour in the baking formula significantly decreased loaf volume at lower bromate levels[4], as shown in Fig. 2. Color and flavor, as well as the grain and texture of the crumb, were also adversely affected. The experimental soy flour was inferior to a commercial unheated soy flour (II)[5] in general baking quality. Both showed a tendency to "buffer" dough against the injurious effect of excess potassium bromate.

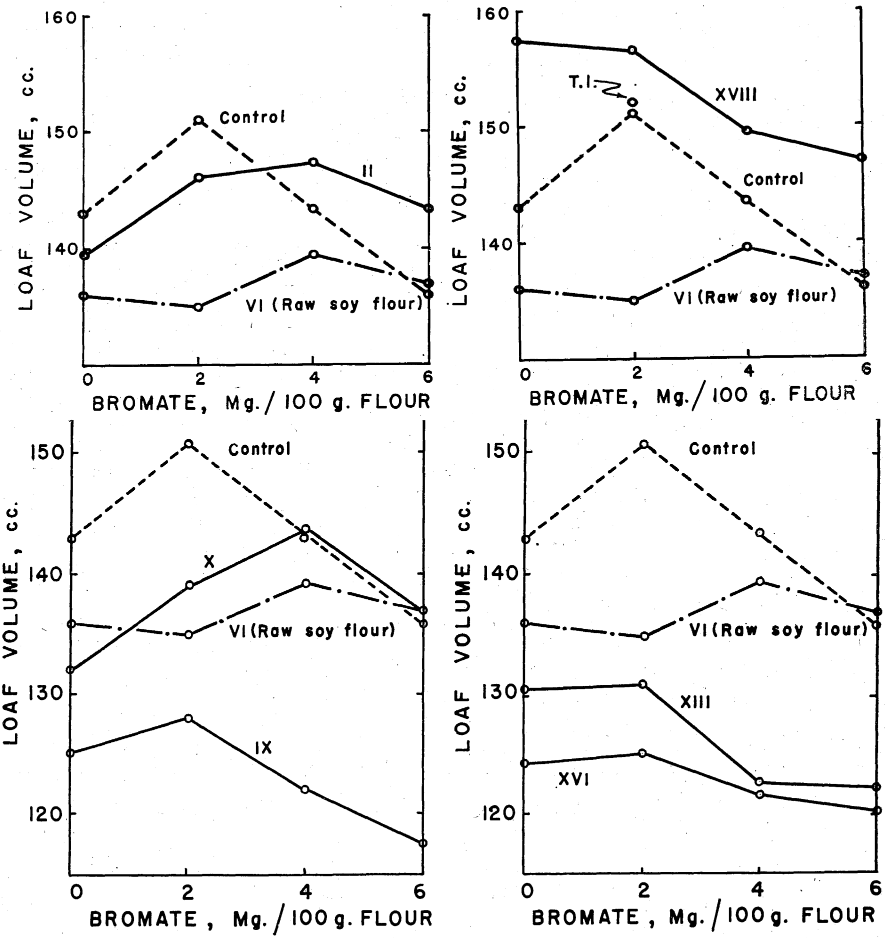

Fig. 2. Mean volumes of loaves containing 3% levels (flour basis) of raw defatted soy flour and several of its fractions in comparison with control (no soy flour).

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[4] The standard error for a single loaf volume determination by the 40 g. dough procedure, as calculated from the largest series of bakes was 2.7 cc.

[5] Courtesy Archer-Daniels-Midland Co., Minneapolis, Minnesota.

Fraction XVIII (Fig. 2) was of very good baking quality, far excelling raw soy flour and definitely improving the control loaf. Also shown is the performance at one bromate level of a trypsin inhibitor (TI) prepared by an independent procedure. These results will be discussed later.

In the course of fractionation, it became clear that undesirable color and flavor principles as well as adverse loaf volume effects were concentrated in the dialysate fraction (No. XIX, Fig. 1). As the further fractionation of the dialysate progressed, it was possible to separate, to a large extent, components responsible for these effects. In Fig. 2 are plotted mean loaf volumes obtained from formulas containing 3% of fractions XX, XXII, XXIV, and XXV baked at four bromate levels. Fractions XX and XXII greatly decreased loaf volume and affected grain and texture adversely; No. XX had the most undesirable flavor. Fraction XXIV was highly colored and caused poor grain and texture but was superior in flavor to raw soy flour and nearly equal in volume

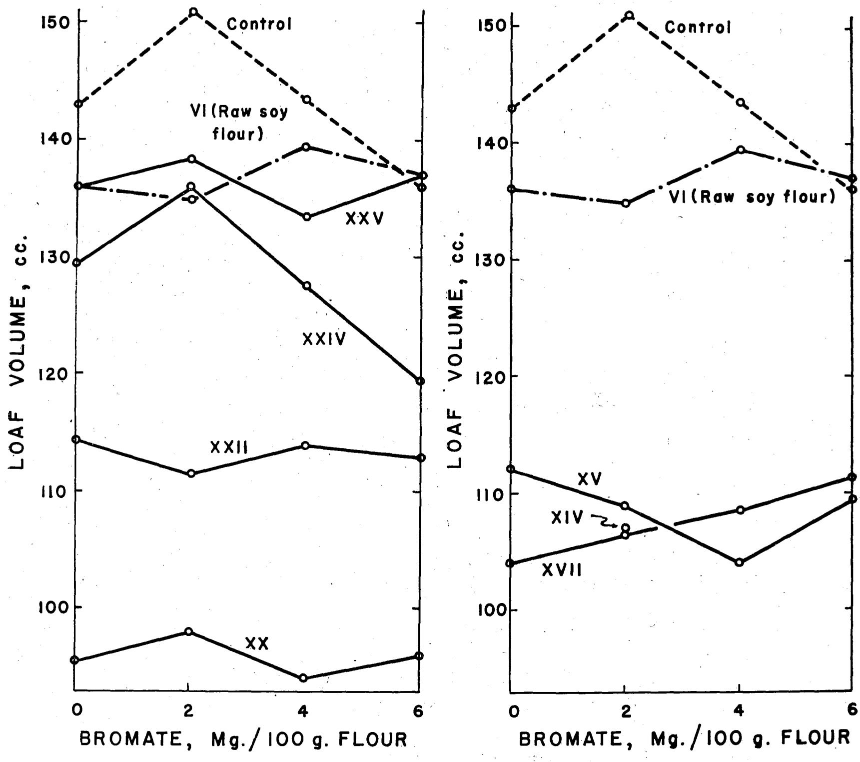

Fig. 3. Mean volumes of loaves containing 3% levels (flour basis) of raw defatted soy flour and several of its fractions in comparison with control (no soy flour).

potential. Fraction XXV affected chiefly flavor. Both XXIV and XXV tended to "buffer" against excess bromate.

The effects of three other injurious fractions, Nos. XIV, XV, and XVII, are shown in Fig. 2. Each was a minor fraction, constituting less than 1% of the raw soy flour. Fraction XVII is of interest as the only one which showed a positive response to potassium bromate.

Figure 3 depicts the performance of several fractions of medium baking quality. Fraction X (Fig. 3) was comparable with raw soy flour,

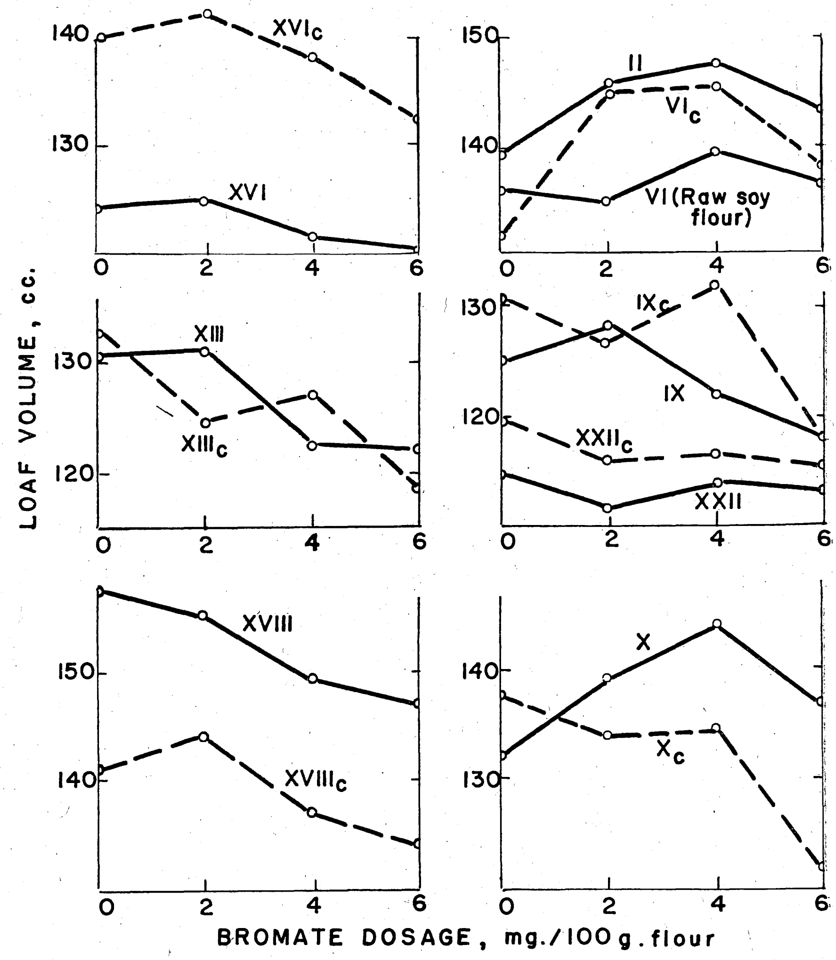

Fig. 4. Typical effects on loaf volume of heat-treating soy flour and soy flour fractions at 100°C. for 1 hour. Heat-treated fractions are designated by subscript "C."
Top row — fractions improved by heat treatment
Middle row — fractions not appreciably affected by heat treatment
Bottom row — fractions injured by heat treatment

while No. IX was lower in over-all quality. Both were superior to raw soy flour in flavor. Fractions XIII and XVI (Fig. 3), protein fractions prepared by different isolation techniques, were inferior in volume potential to raw soy flour but produced a better flavor.

The effects on the baking quality of raw soy flour and certain fractions, brought about by heat treatment for 1 hour at 100°C. and employing the moisture levels given in Table II, are shown in Fig. 4. Raw soy flour (VI) was improved by heat treatment (VI_c) so as to be similar to commercial soy flour II. A marked improvement in the baking quality of fraction XVI upon heat treatment (XVI_c) was noted; protein preparation XIII was virtually unaffected upon heating ($XIII_c$). Heat treatment proved injurious to fraction XVIII and to fraction X, both of which are high protein materials. None of the other fractions, including the most injurious in bread, was appreciably changed in baking quality by the conditions of heat treatment which were employed.

Chloride Content. The chloride contents of four fractions derived from the dialyzable material of soy flour are compared below with the general baking quality of these fractions:

Fraction No.	*Chloride Content as NaCl (dry basis)*	*Baking Quality*
	%	
XX	0.7	Poor
XXII	1.1	Poor
XXIV	14.4	Medium
XXV	16.5	Medium

It can be concluded that the presence of sodium chloride introduced through pH adjustments was not responsible for the poor baking quality of the most injurious fractions.

Antitryptic Activity. Antitryptic activities determined for fraction XVIII and for a trypsin inhibitor prepared as described by Klose *et al.* (13) were as follows:

	Nitrogen (dry basis)	*Inhibitor Units × 10³ per mg. (d.m. basis)*
Fraction XVIII	12.36	4.25
Trypsin inhibitor[6]	14.35	1.62

The high antitryptic activity of fraction XVIII suggests that the fractionation procedure up to this point was relatively mild. In the light of the researches of Learmonth (18,19) and of Melnick's patent (20), the marked improving effect of this fraction might perhaps be ascribed to its high antitryptic activity. However, there is no direct

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[6] Preparation of this inhibitor and assay of antitryptic activity were carried out by Dr. I. E. Liener.

TABLE III
CONCENTRATIONS OF SUGARS IN SOY FLOUR FRACTIONS AND RAW SOY FLOUR

SUGAR	SOY FLOUR FRACTION				RAW DEFATTED SOY FLOUR CALCULATED[a]
	XX	XXII	XXIV	XV	
	%	%	%	%	%
Stachyose[b]	1.9	31.8	10.7	0.9	3.8
Raffinose	...	2.8	4.0	1.5	0.6
Sucrose	2.6	10.8	37.8	35.0	6.2
Galactose	...	1.5[c]	3.0	2.7	0.5
Glucose	...	...	2.9	2.3	0.3
Fructose	...	...	1.9	2.9	0.3

[a] Calculated from the percentage distribution figures in Table II upon the assumption that the fractions for which analyses are given contained all of the sugars present in the soy flour.
[b] Calculated with reference to standard curve for raffinose.
[c] Single determination. All others are means of two or more determinations.

evidence available that the trypsin inhibitor decreases the activity of the wheat flour proteinases on the flour proteins.

Sugars. The determination of sugars in several soy flour fractions aided in their characterization, although the sugars were not considered responsible for poor baking quality. The concentrations of the sugars are shown in Table III. The sugars of the original soy flour are also shown; they are calculated with the aid of the percentage distribution figures in Table II upon the assumption that the fractions for which analyses are given contained all of the sugars present in the soy flour. Large quantities of sugars (45–60%) were detected in fractions XXII, XXIV, and XXV, all obtained from the dialyzable fraction of raw soy flour. Of particular interest are the high percentages of stachyose in fraction XXII and of sucrose in Nos. XXIV and XXV. Values for stachyose are approximate, since they were calculated from the standard curve for raffinose. However, the estimated stachyose and sucrose contents for soy flour are in good agreement with the literature values (3, 25, 26). The raffinose content is somewhat lower than is given in the literature. Values for reducing sugars (galactose, glucose, fructose) are somewhat high: this may be due to some hydrolysis of sucrose and stachyose during isolation (12).

Sulfhydryl Groups. The possible presence of sulfhydryl groups in the soy flour fractions was of interest in view of the well-known adverse effect of certain compounds of this type in bread dough. The following data are representative of the range of sulfhydryl titer and of baking quality encountered among the fractions:

Fraction	*Sulfhydryl (as Cysteine)*	*Baking Quality*
	%	
XX	Negligible	Poor
XXII	Negligible	Poor
XXIII	0.066	Medium
XVI	0.082	Medium
XVIII	0.050	Good
Trypsin inhibitor	0.050	Good

These results do not reveal any relationship between baking performance of the soy flour fractions and their sulfhydryl contents.

Hydrogen Ion Activity. The pH of control doughs varied from 5.70 to 5.85 upon mixing; doughs containing soy flour fractions (3%) ranged in general from 5.28 to 5.98. An exception was fraction XX, the most injurious fraction in bread, which yielded a more alkaline dough. The following pH and loaf volume data are of interest in this connection (means of duplicate values; bromate 2 mg. per 100 g. flour):

	pH	*Mean Loaf Volume*
		cc
Control	5.70	151
VI raw soy flour	5.78	135
XX	6.20	98
XXII	5.90	112
Sodium hydroxide (0.32 meq.)	6.50	142

To determine whether the adverse effect of fraction XX might be attributed solely to abnormal pH, dilute sodium hydroxide solution (0.32 meq.) was added to the baking formula with the above results. Artificial adjustment of dough pH to an alkalinity in excess of that caused by fraction XX or XXII produced a far superior loaf so that the deleterious influence of these fractions cannot be traced to their effects on dough pH.

Further Study of Fractions XX and XXII. Fraction XX was only partially water-soluble, and it was separated into its water-insoluble (XX_1) and water-soluble (XX_2) components. A baking test using 2 mg. bromate per 100 g. flour was applied with the subfractions in amounts corresponding to the 3% level of No. XX, which gave the following results:

Adjunct	*Percent Based on Whole Fraction*	*Dry Matter Added*	*Loaf Volume*
		g	*cc*
Control	. . .	. .	144
XX_1 (water-insoluble)	26	0.19	148
XX_2 (water-soluble)	74	0.56	110
XX	100	0.75	98

The results demonstrated clearly that the water-soluble constituents of fraction XX were responsible for its adverse effects in baking.

The results of ion exchange subfractionation of fraction XXII and the baking tests are summariezed in Table IV. The distribution of nitrogen in the subfractions revealed that constituent(s) high in nitrogen were unrecovered. A portion of the unrecovered material is con-

TABLE IV

RECOVERY AND BAKING TESTS OF ION EXCHANGE SUBFRACTIONS OF NO. XXII

FRACTION	DRY MATTER DISTRIBUTION	NITROGEN CONTENT DRY BASIS	BAKING TESTS[a]		
			Level[b]	Dough pH	Loaf Vol.
	%	%	%		cc
Control	...	...	0	5.86	138
XXII	100.0	1.27	3	5.90	112
$XXII_a$ neutral	44.2	.02	3	5.96	124
$XXII_b$ ionic	47.4	.64	1.5[c]	4.80	88
Unrecovered	8.4	11.4[d]	...	...	...
Sucrose	...	...	3	5.78	128

[a] Means of duplicate values; bromate 2 mg/100 g flour.
[b] Level based on wheat flour at 14% moisture.
[c] Corresponds approximately to 3% level of No. XXII.
[d] Calculated.

sidered to be substances of basic character retained by the resins; the calculated average nitrogen value of 11.4% is a minimum value, since the unrecovered material also included losses of fractions $XXII_a$ and $XXII_b$ which were much lower in nitrogen.

Qualitative chromatograms demonstrated the presence in $XXII_a$ of the same sugars as were previously found in fraction XXII itself; subfraction $XXII_b$ was essentially devoid of sugars. Amino acids or other ninhydrin-positive substances were revealed in $XXII_b$ but not in $XXII_a$ (detected by spraying chromatograms with a solution of 0.4 g. ninhydrin and 10 g. phenol in 90 ml. of 70% ethanol)[7].

The ionic subfraction $XXII_b$ had a pronounced injurious effect in bread, although this might be due in part to abnormally low dough pH. The neutral subfraction ($XXII_a$), known to contain sugars in large amounts, had little influence on loaf volume; its effect was similar to that of a 3% excess of sucrose. These results point to the ionic character of the most harmful substances in fraction XXII.

Subfractions Obtained by Extraction of No. XXII with Ethanol-Water Solutions. The distribution of subfractions and results of baking tests are shown in Table V. Although the differences observed in baking quality among the subfractions were not great, the smallest volume and lowest grain and texture score resulted from the use of No. $XXII_5$. Dough pH was not a primary factor. The history of this subfraction is similar to that of No. XX_2, in which a deleterious principle was also shown to reside. The solubility relationships offer evidence of the ionic and inorganic character of the injurious substances.

The Role of Inorganic Constituents in the Performance of the Deleterious Fractions. Inorganic constituents were present in appreciable quantities in several fractions, as indicated by ash contents of 20–45%.

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[7] These tests were made by Dr. J. E. DeVay, Department of Plant Pathology and Agricultural Botany.

On the basis of preliminary qualitative tests, quantitative determinations were carried out for zinc, magnesium, calcium, and phosphate in the most deleterious fractions. Results are shown in Table VI. Appreciable amounts of calcium, magnesium, and phosphate were found, especially in fraction XX. Although the quantities of zinc detected were quite small, zinc in small concentrations is known to have a marked effect on gas production and loaf volume (8).

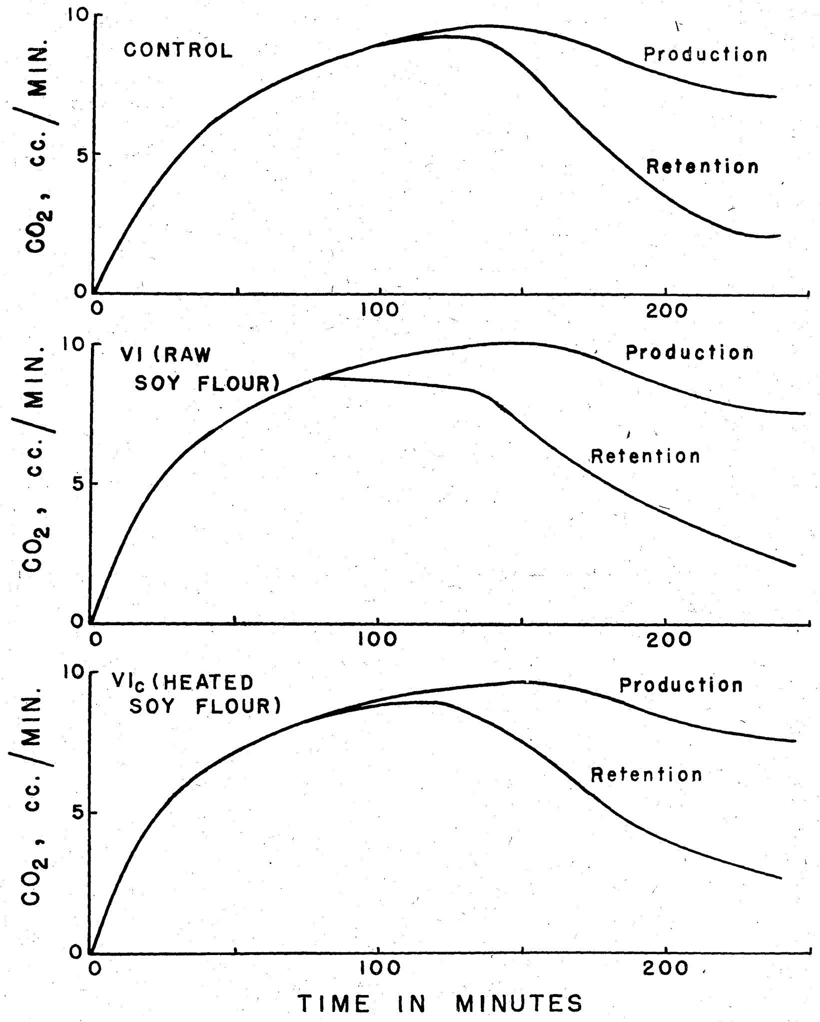

Fig. 5. Zymotachygraphe curves for doughs made without soy flour and with 3% of raw and heat-treated soy flour. Doughs were fermented at 30°C.

Since certain ions were detected in considerable quantity in the most injurious fractions, the effects of pure salts in bread were investigated. Cations were added as their chlorides, phosphate as a mixture of the monosodium and disodium salts; the level of each ion was selected to correspond to the amount found in the 3% level of fraction XX. The chlorides of sodium and potassium were included in these tests,

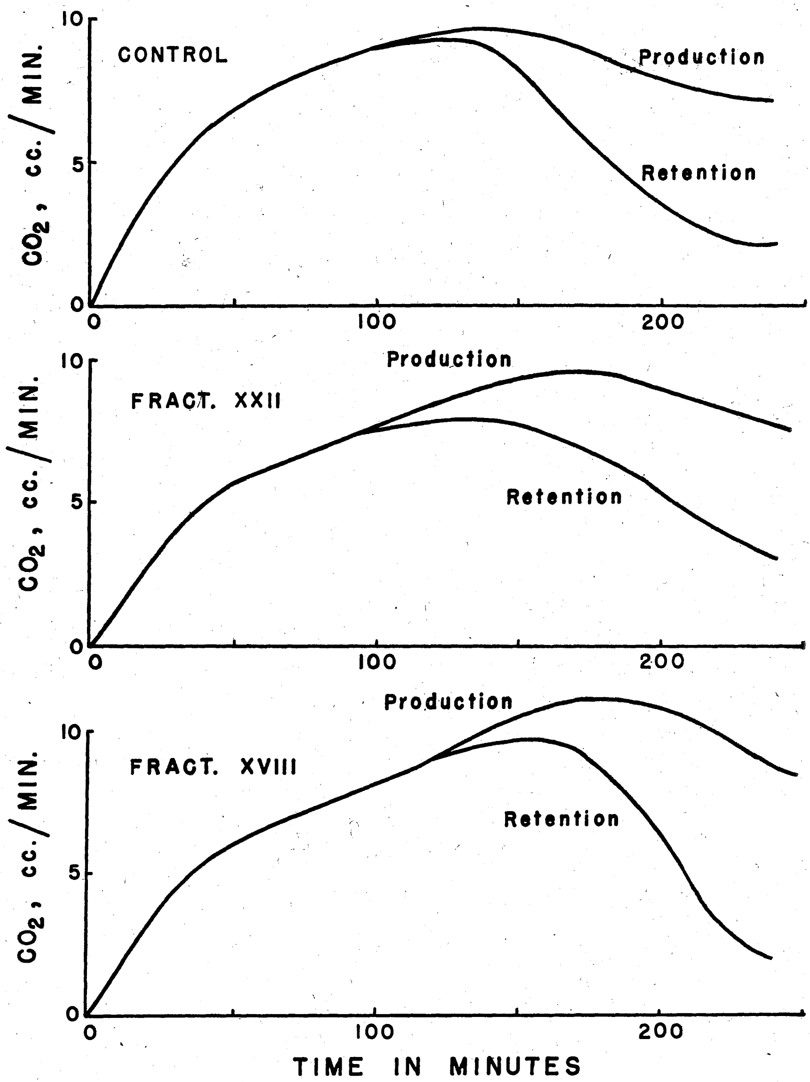

Fig. 6. Zymotachygraphe curves for doughs made without soy flour and with 3% additions of fractions XVIII and XXII. Doughs were fermented at 30°C.

although they were not determined quantitatively in the fractions. The levels of ions employed and the loaf volumes are presented in Table VII. Grain and texture scores were closely related to volumes, while flavor and color were not noticeably affected.

A 0.2% excess of sodium (as chloride) had no significant effect, whereas 1.0% excess was injurious. An equal weight of potassium (as chloride) was somewhat less injurious. Calcium and magnesium chlorides slightly increased loaf volume. A small quantity of zinc chloride profoundly affected bread quality, 0.0048% being sufficient to reduce loaf volume by nearly 20%. This result is in harmony with the observations of Finney *et al.* (8). Phosphate at the level used was also injurious to bread quality. Although the data do not indicate the individual effects of the ions, it seems certain that the zinc and the large phosphate content of the most injurious fractions were instrumental in causing poor volumes. However, in view of the adverse effects of other salts, the performance of these fractions must be attributed in part to a general

TABLE V

BAKING PERFORMANCE OF SUBFRACTIONS OF XXII OBTAINED BY EXTRACTION WITH ETHANOL-WATER MIXTURES[a]

	Adjunct	Distribution[b] (% of Fract. XXII, dry basis)	Nitrogen (dry basis)	Dough pH	Loaf Volume
			%		cc
Control			...	5.79	148
XXII			1.27	5.90	112
$XXII_1$	80% ethanol extract	5.0	1.09	5.67	117[b]
$XXII_2$	70% ethanol extract	25.0	1.18	5.72	114[b]
$XXII_3$	60% ethanol extract	35.2	1.32	5.75	115
$XXII_4$	Water-insoluble portion of residue after 60% ethanol extraction	2.4	0.96	6.08[b]	120[b]
$XXII_5$	Water-soluble portion of same	37.1	1.10	6.02	106
	Total	101.7[c]			

[a] Adjuncts included at 3% level, based on wheat flour at 14% moisture. Bromate 2 mg/100 g flour.
[b] Single determination. Others are means of duplicate values.
[c] Total greater than 100% due to additive errors in moisture determination.

TABLE VI

LEVELS OF INORGANIC CONSTITUENTS FOUND IN SEVERAL SOY FLOUR FRACTIONS

Ion	Fraction		
	XX	XXII	$XXII_5$
	%	%	%
Zinc	0.16	0.06	0.00
Calcium	5.76	0.34	0.53
Magnesium	4.15	1.71[a]	2.74[a]
Phosphate, as PO_4	20.00	3.64	6.47

[a] Single determination. All others are means of duplicate determinations.

TABLE VII

INFLUENCE OF ADDED SALTS ON BREAD QUALITY[a]

ION EMPLOYED	QUANTITY ADDED[b]	LOAF VOLUME[c]
	%	cc
Control, none	...	144
Sodium	0.20	142
Sodium	1.00	128
Potassium	1.00	137
Calcium	0.172	152
Magnesium	0.124	156
Zinc	0.0048	118
Phosphate (as monobasic sodium salt)	0.30	120
Phosphate (as dibasic sodium salt)	0.30	

[a] Bromate 2 mg/100 g flour.
[b] Based on weight of wheat flour. Included in addition to normal salt content of formula (2%).
[c] Means of duplicate values.

salt effect, in addition to specific effects of ions shown to be particularly harmful.

Effect of Soy Flour Fractions and Inorganic Ions on Gas Production and Retention during Fermentation. Figures 5, 6, and 7 show curves obtained by plotting against fermentation time the maximum gas production or retention recorded by the zymotachygraphe during each 10-minute cycle. In relating the curves to baking tests, it should be recalled that the fermentation period was 2.0 hours, followed by a 55-minute proofing period. The fact that the baking test involved manipulation of the dough at two stages (thus altering its gas-retaining properties) whereas the zymotachygraphe tests involved no such treatment, dictates caution in interpreting the curves.

Raw soy flour itself (Fig. 5) slightly increased gas production during the entire fermentation period but caused retention to fall off after about 1.5 hours. Heated soy flour (VI_c), although it hampered gas production, improved retention and was also superior in baking quality to the raw soy flour (Fig. 4, upper right). The curves indicate that gas retention is the limiting factor in these baking tests. Fraction XIII, a protein fraction for which no curve is shown, was very similar to raw soy flour in its effects on production and retention.

Fraction XXII, poor in baking quality, lowered both the production and retention of carbon dioxide (Fig. 6). The poor baking quality may be due to the decline in retention early (about 1.5 hours) in the fermentation period.

Fraction XVIII, which was of excellent baking quality, lowered gas production during the early part of the fermentation, but both production and retention were augmented during the critical proofing period (third hour).

As shown in Fig. 7, the inclusion of zinc ion, or phosphate, each in the amount corresponding to a 3% level of fraction XX, seriously retarded gas production. Under these conditions, the retention curve has little meaning. Although zinc and phosphate ions seemed to be important in the performance of the fractions of poorest baking quality, they influenced primarily gas production; soy flour and soy flour protein without oxidation or heat treatment affected chiefly gas retention. Thus, the inorganic constituents would seem to be of secondary importance in the over-all performance of soy flour.

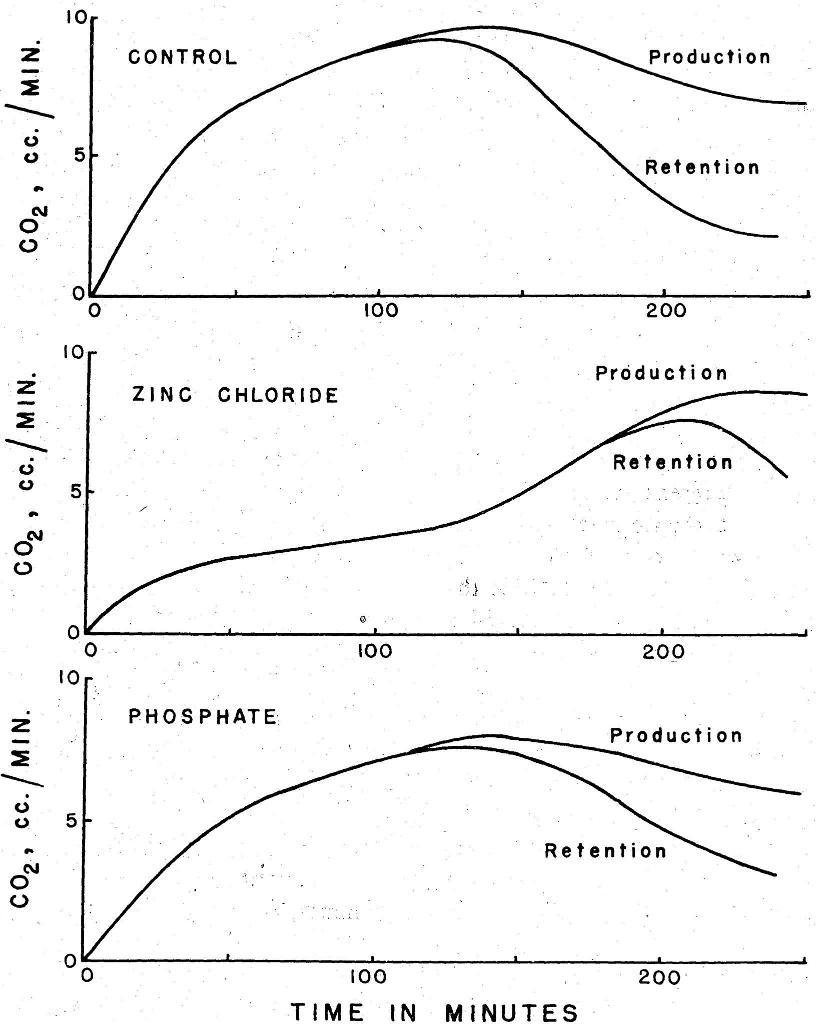

Fig. 7. Zymotachygraphe curves showing the influence of 0.0048% zinc ions and 0.60% phosphate ions (expressed on flour basis) on gas production and retention.

Discussion

The chief endeavor in this study was to obtain reproducible fractions from soy flour, to determine their influence on breadmaking, and to relate their chemical composition with baking performance. In the baking tests, the soy flour fractions were employed at a level of 3% (flour basis), without regard to the relative proportions present in the soy flour. In this way, the various fractions were employed at sufficiently high levels to secure definitive results which were influenced only by the nature of the fraction. It must be recognized, however, that the effects of individual fractions obtained in small yields may have little significance in the performance of soy flour itself. The possibility also exists that the properties of the fractions may have been modified by the techniques employed in obtaining them. An attempt was made to relate the baking qualities of recombined fractions to those of equivalent fractions. In many cases, the effects on loaf volume were equivalent, but, in several cases, the differences in loaf volume were statistically significant.

The beneficial effect of 1% raw soy flour, which was observed in baking tests at a low bromate level (24) and destroyed by heat treatment, may have been due to the presence of fraction XVIII, which was found to be very heat-labile. No beneficial effects of raw soy flour were observed when 3–5% levels were used (24) without increasing the oxidizing improver level. Baking tests on the fractions obtained in the present study revealed widely different effects of heat. At the higher bromate levels employed in these tests, raw, extracted soy flour itself was improved by heat. The baking quality of soy flour thus appears to be the resultant of the baking quality of individual fractions and the differential effects of heat and bromate level.

Although the restricted nature of the baking tests does not warrant a broad interpretation of the results, it would appear to be difficult to improve the baking quality of soy flour by the removal of any specific constituents, since major components were as injurious as soy flour itself and adverse effects on loaf volume and flavor were traced to different fractions. However, as pointed out by several other workers (1, 7,21,22), there is promise that soy flour performance in breadmaking can be enhanced by appropriate heat treatment, the use of oxidizing improvers, and the adjustment of fermentation schedules.

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