Functional (Breadmaking) and Biochemical Properties of Wheat Flour Components. X. Fractions Involved in the Bromate Reaction[1]

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ABSTRACT

Defatted flour (extracted with petroleum ether) gave essentially no bromate response. Since supplementation of defatted flour with 1% sucrose monopalmitate gave a positive response, the native lipid fraction is not directly involved in the bromate reaction. Fractionating and reconstituting techniques have shown that potassium bromate improves gas retention by blocking a normally occurring, deleterious reaction, and that a nondialyzable entity from the water-soluble fraction is required for the bromate reaction. In addition, both the dialyzable and nondialyzable fractions from the most soluble gluten fraction (that remaining in solution after neutralizing the lactic acid suspension to pH 6.1 by sodium carbonate) are required for the bromate reaction. The entity in the dialyzable fraction has been tentatively identified as phosphoric acid. The nature of the overall reaction remains obscure.

Why adding potassium bromate ($KBrO_3$) to flour produces a dough with better machining properties and an improved loaf of bread is one of the most challenging problems of cereal chemistry. Despite much work on the action of maturing agents, the entire mechanism remains obscure (1).

Regardless of the mode of action, it is clear that optimum levels of $KBrO_3$ improve dough handling properties, loaf volume, and crumb grain. Optimum levels of $KBrO_3$ are necessary to obtain a high correlation between loaf volume and protein quality and quantity (2). Finney and Barmore (3) showed that mixing time and $KBrO_3$ compensate for each other, to some extent, but are not completely interchangeable. With inadequate bromate, overmixing improved bread. With optimum bromate, either undermixing or overmixing reduced loaf volumes and damaged crumb grains.

In general, optimum $KBrO_3$ increases with increased protein content and decreases with increased mixing time. Presumably the level is lower with long-mixing flours because of the oxidizing effects of molecular oxygen during mixing. Using fractionating and reconstituting techniques, Finney (4) showed that the water-soluble fraction as well as the gluten fraction was involved in the bromate reaction. Hoseney et al. (5) reached a similar conclusion.

Studies reported here were to further identify the wheat-flour fraction or fractions involved in the bromate reaction.

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MATERIALS AND METHODS

Flour

RBS-69A flour (Regional Bake Standard) was milled from a composite of numerous varieties of wheat harvested at many locations in the Southern and Central Great Plains. The flour had a protein content of 12.9% (14% m.b.), good loaf-volume potential, a medium mixing requirement of 3-7/8 min. and an optimum $KBrO_3$ requirement of 30 p.p.m.

Analytical Procedures

Protein and moisture were determined as described in AACC Approved Methods (6). The straight-dough baking procedure described by Finney and Barmore (3,7,8) and Finney (9) was adapted by Shogren et al. (10) for 10 g. of flour. The method employed optimum mixing time, water absorption, and $KBrO_3$, and a formula that included: flour, 10 g.; sugar, 0.6 g.; salt, 0.15 g.; shortening, 0.3 g.; yeast, 0.2 g.; nonfat dry milk, 0.4 g.; and 60°L. malt syrup, 0.05 g. Doughs were fermented 3 hr. and proofed 55 min. at 30°C. The standard deviation for the average of duplicate loaf volumes was 1.75 cc.

Fractionation Procedures

Flour was fractionated into gluten, starch, and water-solubles as described previously (5,10). The water-solubles were either lyophilized or further fractionated by dialysis. The starch was lyophilized, ground, and hydrated to about 12% moisture before use in reconstitution. The gluten was either lyophilized or further fractionated by the scheme shown in Fig. 1.

The fractions were reconstituted on the basis of their yield percentages and protein contents to give a reconstituted flour containing the protein content of the original flour (12.9%). When fractions were omitted or doubled, the amount of gluten (pH 4.7 fraction) in the reconstitute was held constant and the amount of starch was varied to maintain 10 g. (14% m.b.).

Cationic material was separated from the dialysate fraction by passing it through a column (1.2 X 24-cm.) of Amberlite IR-120 (H^+). The column was flushed with water (100 ml.) to remove anionic and neutral materials, and the cationic material eluted with 2N ammonium hydroxide (100 ml.). Both fractions were recovered by evaporation to dryness in a rotary evaporator at less than 50°C. The column was rejuvenated by flushing with water (100 ml.), followed by 100 ml. 1.0N hydrochloric acid (100 ml.), and water again until eluant was neutral.

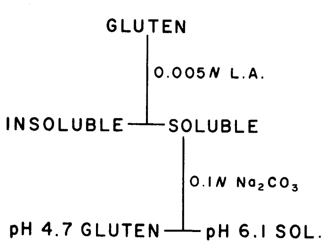

Fig. 1. Scheme for fractionating gluten.

Anionic material was separated from the neutral fraction by passing it through a column of Bio-Rad AG-1 (Cl^-). The column was flushed with water (100 ml.) to remove the neutral material, and the anionic fraction was eluted with 1N hydrochloric acid (100 ml.). The neutral fraction was recovered by evaporation in a rotary evaporator and the anionic fraction by evaporation under reduced pressure over solid sodium hydroxide. The column was rejuvenated by flushing with water until neutral.

RESULTS AND DISCUSSION

An 11-cc. loaf-volume response to optimum bromate (30 p.p.m.) was accompanied by a definite improvement in grain and texture (Table I).

In an effort to identify the flour fractions involved in the bromate response, we extracted the free lipids with petroleum ether (b.p., 35° to 60°C.). The resulting defatted flour gave little or no loaf-volume response with $KBrO_3$. However, it was not clear whether something in the lipid fraction was necessary for the bromate reaction or if the lack of lipid was not allowing the dough to express the beneficial effects of $KBrO_3$. Since it has been shown (11) that certain synthetic glycolipids can replace flour lipids, loaves were baked, with and without $KBrO_3$, from the defatted flour supplemented with 1% sucrose monopalmitate (Di-Nippon Sugar Mfg. Ltd., Tokyo, Japan). A 6-cc. bromate response was obtained. The saturated synthetic glycolipid would not be expected to enter into oxidation-reduction reactions; therefore, it appears that the lipid fraction is not directly involved in the bromate reaction.

The nondefatted flour was fractionated into gluten, starch, and water-solubles. Reconstitution of those fractions gave a flour with a normal bromate response (Table II), indicating that the bromate reaction was not altered by manipulations involved in fractionation. The gluten was further fractionated by solubilization in 0.005N lactic acid, as shown in Fig. 1. When the pH 4.7-gluten, starch, and water-solubles were reconstituted, thus leaving out the insoluble and pH 6.1 fractions, loaves showed no bromate response. Of particular interest was the loaf containing no bromate; its loaf volume, crumb grain, and texture were all comparable to those of the control flour that contained optimum (30 p.p.m.) $KBrO_3$. Including the insoluble fraction in the reconstituted flour did not alter the results. However, when the pH 6.1 fraction was included in the reconstitute, a

TABLE I. LOAF-VOLUME RESPONSE TO OPTIMUM $KBrO_3$ OF BREAD BAKED FROM RBS-69A, RBS-69A DEFATTED WITH PETROLEUM ETHER, AND DEFATTED RBS-69A SUPPLEMENTED WITH 1% SMP (SUCROSE MONOPALMITATE)

Flour and Treatment	$KBrO_3$ p.p.m.	Loaf Volume cc.
RBS-69A	0	71
RBS-69A	30	82
RBS-69A, defatted	0	67
RBS-69A, defatted	30	69
RBS-69A, defatted, + 1% SMP	0	81
RBS-69A, defatted, + 1% SMP	30	87

TABLE II. LOAF-VOLUME RESPONSE TO 30 p.p.m. $KBrO_3$ OF BREAD BAKED FROM RECONSTITUTED FLOURS WITH CERTAIN FRACTIONS OMITTED, REPLACED, AND/OR DOUBLED

Reconstituted Flour[a]	$KBrO_3$ p.p.m.	Loaf Volume cc.
Crude gluten + starch + WS	0	72
Crude gluten + starch + WS	30	81
pH 4.7-Gluten + starch + WS	0	80
pH 4.7-Gluten + starch + WS	30	82
pH 4.7-Gluten + starch + WS + 6.1	0	72
pH 4.7-Gluten + starch + WS + 6.1	30	82
pH 4.7-Gluten + starch + 6.1 + NH_4Cl	0	72
pH 4.7-Gluten + starch + 6.1 + NH_4Cl	30	67
pH 4.7-Gluten + starch + 2(6.1) + NH_4Cl	0	73
pH 4.7-Gluten + starch + 2(6.1) + NH_4Cl	30	66
pH 4.7-Gluten + starch + 2(WS)	0	70
pH 4.7-Gluten + starch + 2(WS)	30	80

[a]WS = water-soluble fraction; 6.1 = pH 6.1-soluble fraction; NH_4Cl = 5 mg. ammonium chloride.

normal bromate response was obtained. Thus, two points are clear: a) Something in the pH 6.1 fraction is involved in the bromate reaction, and b) $KBrO_3$ improves gas-retaining ability of gluten protein by blocking a deleterious reaction that usually occurs in unfractionated flour.

The pH 4.7-gluten, starch, and the pH 6.1 fraction were reconstituted, omitting the water-soluble fraction. As shown previously (12), when the water-soluble fraction was omitted from the reconstituted flour, gas production was low unless a source of ammonia was provided; therefore, 5 mg. of ammonium chloride (NH_4Cl) was added to each reconstituted dough. When the water-soluble fraction was omitted, 30 p.p.m. $KBrO_3$ was deleterious to loaf volume. Doubling the amount of the pH 6.1 fraction in the reconstitute did not significantly alter baking results. However, doubling the normal water-soluble fraction in the reconstituted dough, while omitting the pH 6.1 fraction, gave a normal bromate response. Therefore, it appears that the active agent in the pH 6.1 fraction may also be in the water-soluble fraction and that the water-soluble fraction contains a necessary component not found in the pH 6.1 fraction.

The pH 6.1 fraction was further fractionated by the scheme shown in Fig. 2.

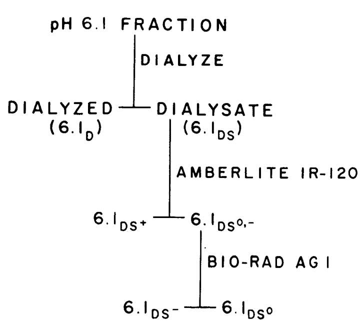

Fig. 2. Scheme for fractionating the pH 6.1 fraction.

Reconstitution of the pH 4.7-gluten, starch, water-solubles, 6.1_D (material retained in the dialysis bag), and the 6.1_{DS} (material passing through the dialysis bag) gave a dough with a normal response to $KBrO_3$ (Table III). Thus, the dialysis procedure did not adversely alter the pH 6.1 fraction. However, when either the 6.1_D or the 6.1_{DS} was omitted from the reconstituted doughs, no bromate response was obtained. Thus, both fractions are required. Reconstitution of the pH 4.7-gluten, starch, pH 6.1 fraction, and the WS_D (material retained in the dialysis bag) gave a dough with a normal bromate response, indicating that the WS_{DS} (material passing through the bag) was not necessary, providing NH_4Cl (yeast food) was added. However, reconstituting the pH 4.7-gluten, starch, and a double amount of WS_D gave a dough with essentially no bromate response, indicating that the WS_{DS} fraction apparently contains a necessary bromate-response component also found in the pH 6.1 fraction. Thus, at levels normally found in flour, WS_D, 6.1_D, and 6.1_{DS} fractions all appear to be essential for a normal bromate response.

The 6.1_{DS} fraction was fractionated further by passing the fraction through a cation exchanger, Amberlite IR-120 (H^+). The resultant two fractions $6.1_{DS}{}^{+}$ (cationic material) and $6.1_{DS}{}^{o,-}$(neutral and anionic materials) were individually reconstituted with the pH 4.7-gluten, starch, water-solubles, and the 6.1_D fractions. When baked into bread, the reconstituted loaf containing the cationic $6.1_{DS}{}^{+}$ fraction (characterized by paper chromatography as amino acids and peptides) gave no bromate response (Table IV). When the fraction containing the neutral and anionic materials ($6.1_{DS}{}^{o,-}$) was reconstituted with the pH 4.7-gluten, starch, water-solubles, and the 6.1_D fraction, a normal bromate response was obtained.

The $6.1_{DS}{}^{o,-}$ fraction was fractionated further into a neutral fraction $6.1_{DS}{}^{o}$ and anionic fraction $6.1_{DS}{}^{-}$ by passing it through an anion exchanger (Bio-Rad AG 1(Cl^-). The $6.1_{DS}{}^{o}$ fraction (characterized by paper chromatography as mainly sugars), when reconstituted with the pH 4.7-gluten, starch, water-solubles, and the

TABLE III. LOAF-VOLUME RESPONSE TO 30 p.p.m. $KBrO_3$ OF BREAD BAKED FROM RECONSTITUTED FLOURS WITH CERTAIN FRACTIONS OMITTED, REPLACED, AND/OR DOUBLED

Reconstituted Flour[a]	$KBrO_3$ p.p.m.	Loaf Volume cc.
pH 4.7-Gluten + starch + WS + 6.1	0	72
pH 4.7-Gluten + starch + WS + 6.1	30	82
pH 4.7-Gluten + starch + WS + 6.1_D	0	80
pH 4.7-Gluten + starch + WS + 6.1_D	30	82
pH 4.7-Gluten + starch + WS + 6.1_{DS}	0	80
pH 4.7-Gluten + starch + WS + 6.1_{DS}	30	80
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS}	0	72
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS}	30	80
pH 4.7-Gluten + starch + WS_D + 6.1 + NH_4Cl	0	73
pH 4.7-Gluten + starch + WS_D + 6.1 + NH_4Cl	30	82
pH 4.7-Gluten + starch + 2(WS_D) + NH_4Cl	0	78
pH 4.7-Gluten + starch + 2(WS_D) + NH_4Cl	30	80

[a]WS = water-soluble fraction; 6.1 = pH 6.1-soluble fraction; WS_D = dialyzed water-soluble fraction; 6.1_D = dialyzed pH 6.1-soluble fraction; 6.1_{DS} = dialysate from the pH 6.1-soluble fraction; and NH_4 Cl = 5 mg. ammonium chloride.

TABLE IV. LOAF-VOLUME RESPONSE TO 30 p.p.m. $KBrO_3$ OF BREAD BAKED FROM RECONSTITUTED FLOURS WITH CERTAIN FRACTIONS OMITTED, REPLACED, OR BOTH

Reconstituted Flours[a]	$KBrO_3$ p.p.m.	Loaf Volume cc.
pH 4.7-Gluten + starch + WS + 6.1	0	72
pH 4.7-Gluten + starch + WS + 6.1	30	82
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS^+}	0	80
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS^+}	30	82
pH 4.7-Gluten + starch + WS + 6.1_D + $6.1_{DS^{o,-}}$	0	73
pH 4.7-Gluten + starch + WS + 6.1_D + $6.1_{DS^{o,-}}$	30	83
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS^o}	0	76
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS^o}	30	78
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS^-}	0	73
pH 4.7-Gluten + starch + WS + 6.1_D + 6.1_{DS^-}	30	79
pH 4.7-Gluten + starch + WS + 6.1_D + H_3PO_4	0	73
pH 4.7-Gluten + starch + WS + 6.1_D + H_3PO_4	30	80

[a]WS = water-soluble fraction; 6.1_D = dialyzed pH 6.1-soluble fraction; WS_D = dialyzed water-soluble fraction; 6.1_{DS^-} = anionic fraction of pH 6.1 dialysate; 6.1_{DS^+} = cationic fraction of pH 6.1 dialysate; 6.1_{DS^o} = neutral fraction of pH 6.1 dialysate; and H_3PO_4 = 30 p.p.m. phosphoric acid.

6.1_D fraction, gave no bromate response. However, 6.1_{DS^-} fraction, similarly reconstituted, gave almost a normal bromate response. One of the major components in that fraction was tentatively identified by paper chromatography as phosphoric acid (H_3PO_4). Reconstituted loaves containing pH 4.7-gluten, starch, water-solubles, 6.1_D, and 30 p.p.m. H_3PO_4, gave almost a normal bromate response. The level of 30 p.p.m. H_3PO_4 agrees with the wheat-flour inorganic phosphorus reported by Watson et al. (13). Also Hlynka (14) reported that adding phytic acid to dough reduced the $KBrO_3$ requirement.

In summary, bromate improves gas retention by blocking a deleterious reaction. Nondialyzable entities in the water-solubles and pH 6.1 fractions, along with a dialyzable fraction of the pH 6.1 fraction identified as H_3PO_4, are required for the bromate reaction. The nature of the reaction remains obscure.

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