The Effects of Bromate (0–30 ppm) on the Proteins and Lipids of Dough

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ABSTRACT

The bromate response in dough, as measured by an improvement in loaf volume, has been shown to be largely variety dependent, with some environmental effect too. For the samples and baking procedure used in this study, potassium bromate (5–10 ppm) was required to achieve the optimum loaf volume (at 2.5 min of constant mixing time). Subsequent additions of potassium bromate (up to 30 ppm) resulted in a toughening of the dough and a decline in loaf volume. Bromate changed the apparent molecular weight distribution of proteins and lipid extractability. Fractionation of glutenin proteins by size-exclusion high-performance liquid chromatography identified an extra large glutenin aggregate, P_1, associated with bromate addition. However the proportion of this aggregate continued to increase with bromate levels, beyond those required for maximum loaf volume. Thus, we concluded that this aggregate is not a major contributor to the loaf-volume effect. The composition of the protein extractable with 70% aqueous ethanol was also examined by size-exclusion high-performance liquid chromatography. The high molecular weight fraction from this material (a lipid-mediated aggregate) followed a trend similar to that of the loaf-volume response to bromate. Lipid extractability changed with bromate addition, although lipid composition was unaltered. The bromate-response factor appears to be the result of noncovalent aggregation of low molecular weight proteins together with a decrease in lipid extractability due to lipid binding.

Potassium bromate has been a commonly used bread improver since early this century (Kohman et al 1915). The improver action of potassium bromate is presumed to occur by the oxidation of the disulfide groups of gluten proteins in dough. This change improves gas-cell structure, retention of carbon dioxide, and loaf volume. Bakers have also noted that bromated doughs have improved tolerance to mixing and show improved dough-handling properties. In addition to the implication of proteins in the bromate response, evidence suggests that bromate also affects nonprotein components such as nonstarch carbohydrates (Patil et al 1976, Ali and D'Appolonia 1979) and free lipids (Cunningham and Hlynka 1958).

Recently, there has been renewed interest in bromate and the mechanism of the bromate response because of reports that associated bromate with possible health risks. Kurokawa et al (1983) reported that rats given 250–500 ppm of potassium bromate in their drinking water showed a significant increase in the incidence of renal carcinomas. These findings, together with the difficulty in developing sensitive detection methods for bromate in bread, caused the bread industry to adopt a ban on bromate use rather than risk adverse publicity.

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In avoiding bromate, the baking industry has adopted alternative dough improvers. However, different quality requirements need to be introduced so that wheat breeders selecting new varieties can assess the potential loaf volume responses to new, nonbromate improvers. The aim of this study was to investigate the changes in protein and lipid composition resulting from the action of potassium bromate. This would allow the assessment of the efficacy of new dough improvers and the selection of new wheat varieties to produce good quality bread in the absence of potassium bromate.

MATERIAL AND METHODS

Commercial wheat samples of Australian cultivars (Hartog, Banks, Sunco, Sunfield, and Rosella) were milled on the pilot mill of the Bread Research Institute of Australia. The baker's flour was a commercial breadmaking flour (Bunge (Australia) Pty. Ltd). Table I shows the protein contents, lipid compositions, and dough properties of these flour samples.

Dough rheological properties were determined according to official testing methods (RACI 1985).

Each sample of flour was baked in duplicate. The baking test was the standard baking procedure adopted by the Victorian Institute for Dryland Agriculture (Panozzo et al 1990) to test breeding lines for breadmaking quality. The mixing time was standardized at 2.5 min, which may not necessarily give optimum dough development. The dough was mixed in a CM10 Hobart mixer. Final development was achieved during the 180-min fermentation period.

A series of baking tests used 300 g of flour and 0, 5, 10, 15, 20, and 30 ppm of potassium bromate. In addition, 0.5% malt, 2% yeast, and water amounts based on Farinograph water absorption were added. Upon completion of the fermentation stage, the doughs were scaled to 450 g, pan-proofed for 55 min, and baked. The remaining dough (30–50 g) was frozen and later freeze-dried. Loaf volumes were determined by rapeseed displacement.

Freeze-dried doughs were gently ground and sieved. Free lipid and bound lipid were extracted using *n*-hexane and water-saturated *n*-butanol, respectively, according to Bekes et al (1983). Each lipid determination was done in duplicate. Lipid composition was determined using the high-performance liquid-chromatography (HPLC) procedure described by Bekes and Batey (1991).

Total unreduced proteins were extracted from flour and freeze-dried dough samples by sonication in a buffered detergent solution. This procedure was reported to provide complete extraction without chemical rupture of disulfide (SS) bonds (Singh et al 1990). Fractionation by size exclusion (SE)-HPLC was done according to Batey et al (1991); the only modification was a lower (0.05%) concentration of trifluoroacetic acid in the eluting solvent. Ethanol-extractable proteins from flour and freeze-dried dough samples were prepared for SE-HPLC according to Bekes et al (1983). The SE-HPLC procedure was the same for both types of extracts.

RESULTS AND DISCUSSION

Bromate and Loaf Volume

The bromate response has its most obvious effect in baking: at an optimal bromate level, the loaf volume is maximized. The extent of this improvement, and the level of bromate required, vary with baking procedure and flour sample (cultivar and growing conditions). The optimum response for the range of cultivars and samples used in this study is illustrated in Figure 1A. The optimum potassium bromate level was between 5 and 10 ppm (flour mass basis). Further increases in the amount of bromate reduced loaf volume, probably because of increased dough strength and decreased elasticity. Actual loaf volumes, protein content, dough development time, dough strength, and elasticity varied between samples (Table I), but the optimum bromate was nearly constant (5–10 ppm).

Variation in Total Unreduced Protein

The effect of bromate addition on protein composition was initially examined using the HPLC procedure of Batey et al (1991), which permits extraction of virtually all the protein from flour and dough without significant rupture of SS bonds. Fractionation by SE-HPLC normally resolves four major peaks or intervals (Fig. 2A): glutenin (P_{II}), gliadin (P_{III}), and soluble proteins (P_{IV} and P_V). Upon addition of bromate, an extra peak (P_I) was observed with the shortest elution time (Fig. 2B). The relationship between the percentage of P_I and the bromate used is shown in Figure 1B. Quantitative comparison of the relative percentages of peaks (Table II) showed that amounts of P_I and P_{II} were constant across different bromate levels. P_I thus appears to consist of very large aggregates of glutenin subunits formed from glutenin (P_{II}) by bromate.

Relationships between bromate level, loaf volume (LV), and P_I were characterized by calculating linear or rank correlation coefficients separately for each cultivar or sample. Highly significant ($P<0.001$) linear correlations were found for each sample in the bromate-P_I relationship. The *r* values of the cultivars were: Sunfield 0.97, Sunco 0.96, Banks 0.99, Hartog 0.95, Rosella 0.98, and bakers flour 0.98. Pooled *r* values were 0.98. Using rank correlation for the characterization of the P_I-LV relationships, no significant correlation was found, which suggests that the apparent aggregation of glutenin to form P_I is not a major mechanism in the improver action of bromate with respect to loaf volume.

Variation in Ethanol-Extractable Protein

In a parallel study, the dough protein extractable with 70% ethanol was analyzed by SE-HPLC to produce three major peaks (Fig. 3). The peak with the highest apparent molecular weight (ethanol-extractable glutenin, P_A) has previously been described as lipid-mediated aggregate (LMA) (Bekes et al 1983). These protein-lipid complexes contained glutenins, gliadins, and globulins. The amount and composition of these aggregates showed inter-

TABLE I
Protein, Free Lipid (FL), and Bound Lipid (BL) Content and Dough Properties of Samples

				Extensigraph		Farinograph	
Sample	Protein[a]	FL[b,c]	BL[b,d]	Ext[e]	Res[f]	DDT[g]	WA[h]
Baker's flour	10.4	1,070	600	21.7	310	4.0	61.8
Rosella	10.4	990	680	24.2	320	4.0	57.4
Hartog	12.0	910	765	22.5	390	8.5	66.1
Banks	10.4	890	600	20.7	230	4.0	63.1
Sunco	13.0	1,220	780	28.6	360	10.2	62.3
Sunfield	12.3	1,120	680	26.5	240	4.9	66.1

[a] g/100 g of sample on dry basis.
[b] mg/100 g of sample on dry basis.
[c] *n*-hexane-extractable free lipids.
[d] Water-saturated *n*-butanol extractable bound lipids.
[e] Extensibility (cm).
[f] Maximum resistance (BU).
[g] Dough development time (min).
[h] Water absorbance (%).

varietal effects in Canadian (Zawistowska et al 1984), Hungarian (Karpati et al 1987, Lasztity et al 1988), and Australian (Bekes et al 1992) wheat samples. In earlier reports, the glutenin-like component of LMA was called different names, such as *high molecular weight gliadin* (Bietz and Wall 1980), *low molecular weight glutenin* (Kanazawa and Yonezawa 1973), and *aggregating gliadin* (Shewry et al 1983). These proteins were key factors in the formation of LMA during dough making (Lasztity et al 1988).

The amount of P_A reached a maximum in doughs with 5 ppm of bromate and decreased again in doughs with higher levels of bromate (Fig. 1D). This was similar to the trend observed in loaf volume with bromate addition (Fig. 1A). Each flour sample had a highly significant positive relationship between loaf volume and the proportion of P_A (r values from 0.91 to 0.98). Although linear for each sample, these relationships differed greatly in slope and intercept (Fig. 4). Thus, it appears that P_A may be a useful marker of bromate response, indicating the extent of the response (slopes in Fig. 4).

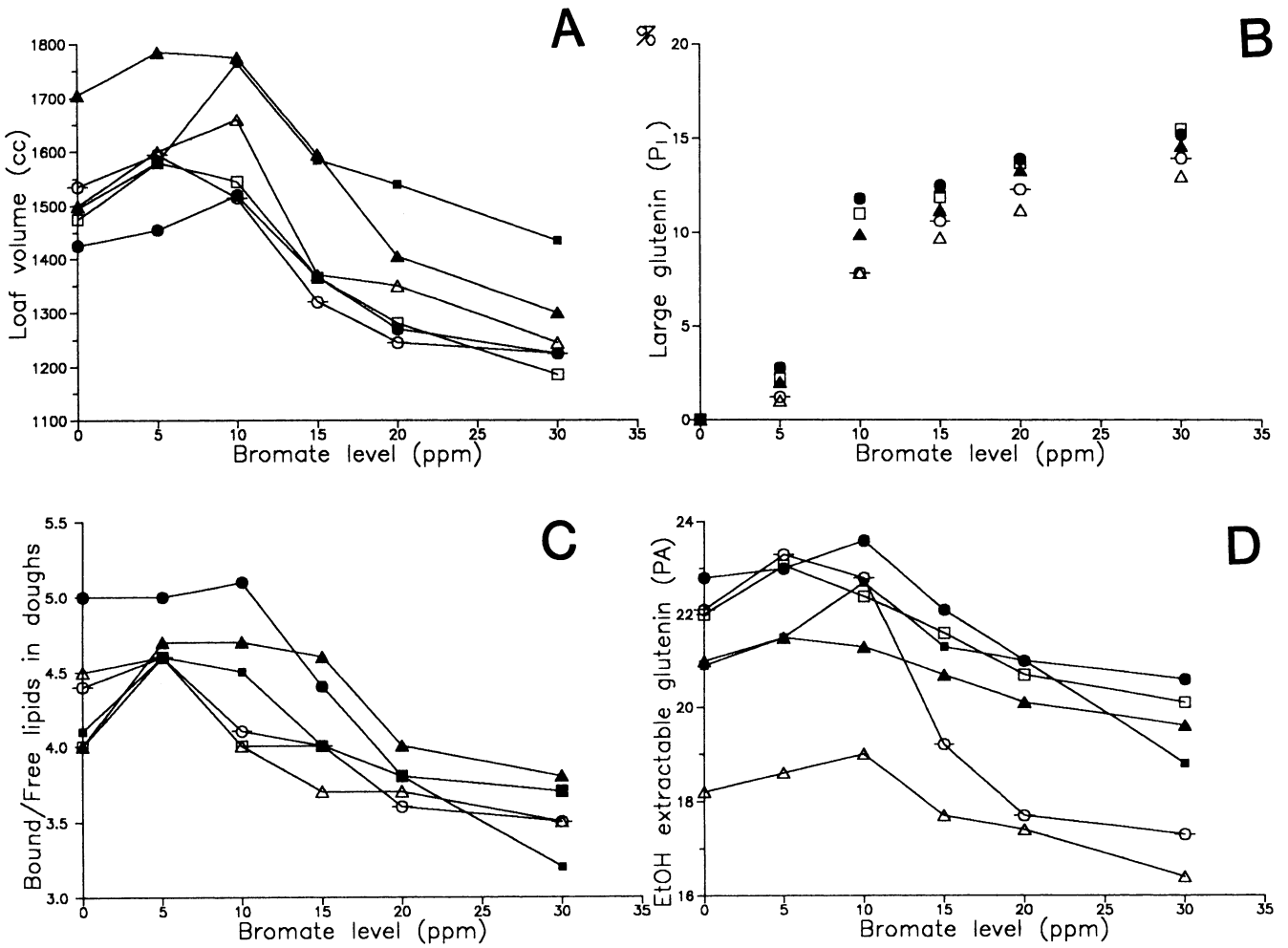

Fig. 1. Effects of bromate addition. **A,** Loaf volume. **B,** Amount of large glutenin fraction (P_I) observed on the size-exclusion, high-performance liquid chromatography profiles of the total protein extracts from the dough (see Fig. 2). **C,** Lipid extractability from dough. **D,** Amount of ethanol-extractable glutenin (P_A) observed on the size-exclusion, high-performance liquid chromatography profiles of the 70% aqueous ethanol proteins of dough. Cultivars used in the study included: Banks (●), Rosella (-○-), Hartog (□), Sunco (△), Sunfield (■) and baker's flour (▲).

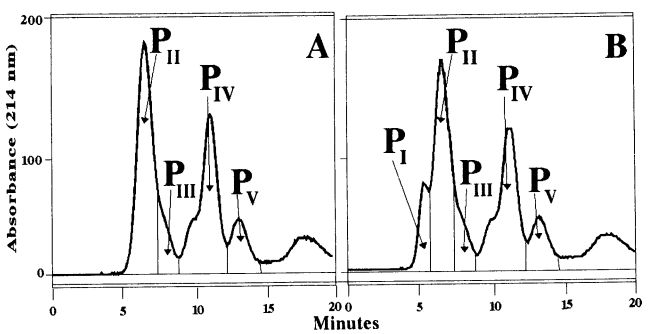

Fig. 2. Size-exclusion, high-performance liquid chromatography profiles of total protein extracts from dough mixed with 10 ppm of bromate (**B**) and without bromate, 10 ppm (**A**). P_I and P_{II} = glutenin, P_{III} = gliadin, P_{IV} and P_V = soluble proteins.

TABLE II
Effects of Bromate Level on the Distribution of Fractions (%)[a]

Bromate level (ppm)	Peak[b]				
	P_I	P_{II}	P_{III}	P_{IV}	P_V
0	0.0	41.7	7.7	37.7	12.9
5	1.1	40.6	7.9	37.5	12.9
10	9.5	32.6	7.3	38.3	10.2
15	11.6	30.8	7.6	38.1	11.9
20	12.0	29.7	7.6	38.0	12.7
30	18.7	23.0	7.6	38.3	12.4
LSD[c]	0.9	0.9	0.5	0.9	1.0
F value	1,465[d]	315[d]	1	11	0.5

[a] Extracted with sodium dodecyl sulfate sonication method.
[b] P_I and P_{II} = glutenin, P_{III} = gliadin; P_{IV} and P_V = soluble proteins.
[c] Least significant difference.
[d] Significant at $P < 0.001$.

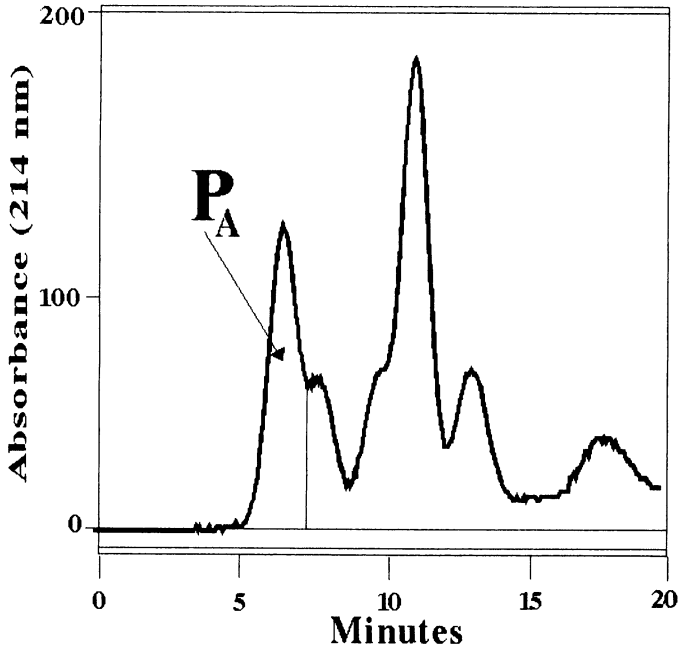

Fig. 3. Size-exclusion, high-performance liquid chromatography profile of the 70% ethanol-extractable proteins from dough. P_A = lipid-containing peak of ethanol-extractable glutenin.

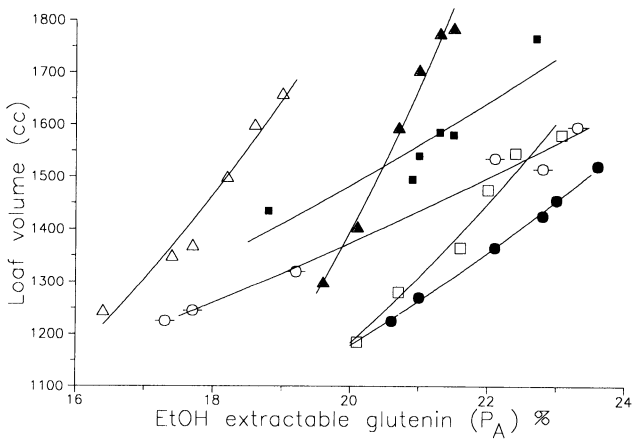

Fig. 4. Relationship between the amount of ethanol-extractable glutenin (P_A) and loaf volume. Cultivars used in the study included: Banks (●), Hartog (□), Rosella (-○-), Sunco (△), Sunfield (■), and baker's flour (▲).

Changes in Lipid Extractability

The addition of bromate also had an effect on the extractability of lipids, but not on the overall composition of the lipids, as analyzed by HPLC. The trend in the ratio of bound lipid to free lipid with bromate addition (Fig. 1C) was identical to that in the profile for ethanol-extractable glutenin (P_A), and similar to the profile for the loaf volume, peaking in doughs with 5 ppm of bromate, and then decreasing in doughs with the higher levels of bromate (Fig. 1D).

Although lipid extractability changed significantly with bromate addition, no such changes were observed in lipid composition as determined by HPLC analysis of combined free and bound lipid preparations. Apparently, bromate does not alter lipids that might be expected to be sensitive to oxidation, such as unsaturated fatty acids, their esters, or their phosphatide derivative, as claimed by Sullivan (1940). It is more likely that there is a connective link between the changes in P_A and in bound lipid with bromate addition, suggesting that the bromate response occurs by increasing the binding of lipids to proteins, possibly by changing their conformation by oxidation of protein sulfhydryl groups. These changes occur primarily during dough mixing because when freshly mixed dough samples were analyzed, identical results (not shown) were obtained.

CONCLUSIONS

We present new evidence that bromate addition has two effects, both causing higher level aggregation of dough components. Bromate addition increases apparent polymerization of glutenin subunits in the dough and increases the amount of large ethanol-extractable protein aggregates presumably formed by the interaction of lipids with low molecular weight subunits. The increased amount of a lipid-containing high molecular weight fraction in the ethanol-extractable protein seems to be related to decreased lipid extractability.

Our results show that the formation of large glutenin aggregates (P_1) caused by bromate addition are not consistently related to changes in loaf volume; the formation of these aggregates continued beyond the optimal level of bromate. Therefore, we conclude that noncovalent aggregation of low molecular weight protein components of dough, in parallel with decreased lipid extractability, is the effect making the more important contribution to the bromate response in baking.

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