Sulfhydryl Analysis. II. Free Sulfhydryl Content of Heated Doughs from Two Wheat Cultivars and Effect of Potassium Bromate

D. C. ANDREWS,[1] R. A. CALDWELL,[1] and K. J. QUAIL[2]

ABSTRACT

Cereal Chem. 72(3):330-333

The combined effects of heat and potassium bromate ($KBrO_3$) on the free sulfhydryl content of doughs prepared from two Australian commercial wheat flours were investigated. Flour derived from the cultivar Janz was not responsive to $KBrO_3$ in baking, whereas flour from the cultivar Oxley was responsive. Heating of dough samples with or without $KBrO_3$ caused a large decline in free sulfhydryls for both samples. Doughs prepared from each sample showed a significant loss of $KBrO_3$ during the heating protocol. Mixing alone caused a nearly 50% decline in the level of $KBrO_3$ from the quantity added. Results of this investigation indicate that although $KBrO_3$ had reacted during the period of heating, it was not directly linked to the decrease in free sulfhydryls.

One of the first patented bread improvers was potassium bromate ($KBrO_3$) (Kohman et al 1916). Despite being used for almost 80 years, there are still questions about its mode of action and influence in doughs. Lately, concerns about a potential health risk from $KBrO_3$ have led to legislation banning its use from breadmaking in some countries and voluntary industry withdrawal in others. An understanding of its complex reactions with individual flour constituents may assist in the development and selection of replacement chemical or biochemical agents.

The effect of adding small quantities of $KBrO_3$ to dough are well known (Kohman et al 1916, Sullivan et al 1940, Tkachuk and Hlynka 1961, Fitchett and Frazier 1986, Brown 1993). Apart from producing a more stable and robust dough, in most cases it also improves loaf volume and enhances texture and crumb structure. Many attempts have been made to better understand how the addition of $KBrO_3$ produces these results. One of the first theories proposed (Jorgensen 1945) was that bromate acted by inhibiting protease action in flour. That theory was quickly disputed and discarded. Earlier, Ziegler (1940) had shown that bromate could oxidize reduced glutathione, and this led to the theory that $KBrO_3$ oxidized the sulfhydryls in flour proteins. Work by Hird and Yates (1961), Lee and Samuels (1962), and Sullivan et al (1963) attempted to demonstrate that there was a relationship between the oxidation of sulfhydryls to disulfides and the addition of potassium bromate. Although there were no conclusive results, they did indicate that a decrease in sulfhydryls caused by the addition of $KBrO_3$ corresponded to an increase in disulfide levels. Tsen (1968) continued this work and also studied the effect of temperature on the process. He concluded that $KBrO_3$ oxidized sulfhydryls to intermolecular disulfides. The lack of precise methods for the measurement and quantification of sulfhydryls at the levels present in dough meant questions still remained. Results from work by these and other authors have led to the still currently accepted equation for the $KBrO_3$ reaction, as proposed by Tkachuk and Hlynka (1961):

$$\underset{\text{Bromate}}{BrO_3^-} + \underset{\substack{\text{Protein}\\\text{Thiols}}}{6\ RSH} \rightarrow Br^- + \underset{\substack{\text{Protein}\\\text{Disulfides}}}{3\ RSSR} + 3\ H_2O$$

The action of dough mixing causes the gluten matrix to stretch and at some points the disulfide bonds break, resulting in the formation of sulfhydryls (Tsen and Bushuk 1963, MacRitchie 1992). Most oxidizing agents, such as azodicarbonamide and potassium iodate, begin reforming these disulfide linkages soon after mixing ceases. $KBrO_3$ acts as a slow oxidizing agent in dough because of the relatively high activation energy of the $KBrO_3$ reaction. Although some oxidation occurs soon after cessation of mixing (Dempster et al 1952), the bulk of the $KBrO_3$ reaction occurs during the baking stage (Tsen 1968). There have been other mechanisms proposed for sulfhydryl-disulfide interchange reactions (Sanger 1953, Lee and Lai 1968, Jones and Carnegie 1969, Grosch 1986). All reactions proposed show the conversion of sulfhydryls to disulfides by oxidants, or the interchange of disulfides using a sulfhydryl intermediate.

The aim of the present investigation was to determine what effect the addition of $KBrO_3$ and temperature had on the free sulfhydryl content of flours and doughs derived from two wheat samples that respond differently to the addition of $KBrO_3$.

MATERIALS AND METHODS

Wheat Cultivars

Two commercial Australian wheat cultivars, Janz and Oxley, both with a protein content of 10.9% (N × 5.7, 14% mb), were used in this investigation. Samples were straight-run flours obtained from the 1991 Australian Interstate Wheat Variety Trials and milled on a Buhler test mill fitted with an entoleter (Butcher and Stenvert 1972) and stored at 15°C.

The two samples were test baked using 120 g of fermented doughs according to the Bread Research Institute method (Moss 1980) with some modifications. These included the replacement of the 1% malt extract with 0.5% medium activity malt flour (65 SKB units/g), a proof temperature of 35°C, and 85% rh. The two samples were mixed for 2.5 min with no $KBrO_3$ or with 20 ppm $KBrO_3$ added before fermentation. The samples were then baked at 255°C.

Temperature and $KBrO_3$ Treatment of Flour Doughs

Doughs were prepared with and without $KBrO_3$ and then heated. For the nonbromated samples, 80 g of flour and 48 ml of water (distilled, deionized) were mixed until the dough cleared, using a four-prong National pin mixer (Finney modified). Ten-gram pieces were scaled and heated at either 18, 35, 45, 55, 65, 75, or 85°C for 1 hr in covered but unsealed containers. The samples were immediately frozen, freeze-dried, powdered, and stored at −20°C. The bromated samples were prepared by mixing 80 g of flour with a 30 ppm $KBrO_3$ solution (48 ml) and processing as described above.

Temperature Treatment for Flour

Flour from both samples was heated in covered but unsealed containers for 1 hr at either 18, 35, 45, 55, 65, 75, or 85°C. The samples were then stored in air-tight containers at −20°C.

[1] Department of Agricultural Chemistry and Soil Science, University of Sydney, NSW, Australia.
[2] Bread Research Institute of Australia, North Ryde, NSW, Australia.

Sulfhydryl Determination

Free sulfhydryl content of all samples was determined using the method described previously (Andrews et al 1995), using the proteolysis extraction procedure. Results shown in Figures 1 (A,B) 2, and 3 (A–C) are the average of three determinations at each temperature; errors are the average deviation of the mean.

Gas Chromatographic Determination of $KBrO_3$ Residue

The $KBrO_3$ remaining in dough samples was determined by the gas chromatographic method of Oyamada et al (1984) and Sparkes et al (1992). Results in Figure 4 are the average of four determinations at each temperature; errors are the average deviation of the mean.

RESULTS AND DISCUSSION

The test-baking results for the Oxley sample showed an increase in loaf volume from 710 to 880 cm^3 when $KBrO_3$ was added, the Janz sample showed a decrease in loaf volume from 660 to 645 cm^3. On this basis, Janz was considered nonresponsive and Oxley was considered responsive to $KBrO_3$. The free sulfhydryl content of untreated Janz flour was 0.45 μmol SH/g of flour and untreated Oxley flour was 0.37 μmol SH/g of flour.

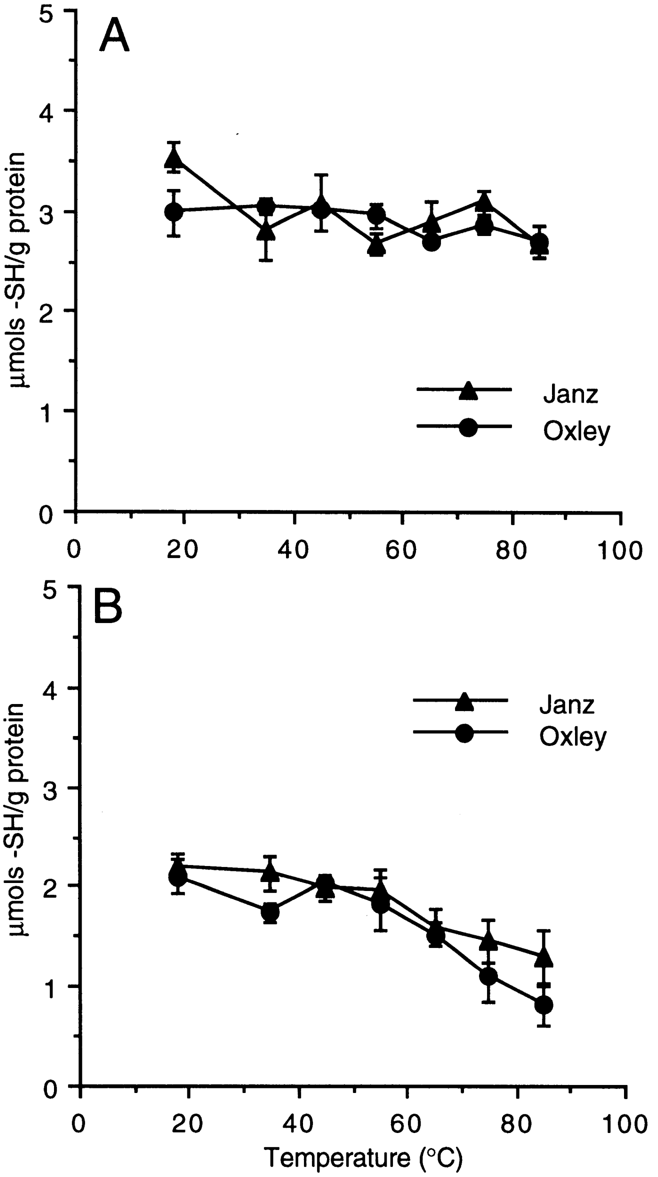

Fig. 1. A, Free sulfhydryl content of untreated Janz and Oxley flours after heating at the indicated temperature for 60 min. **B,** Free sulfhydryl content of nonbromated Janz and Oxley doughs after heating at the indicated temperature for 60 min.

The effect of mixing flour into doughs and subjecting those doughs to different temperatures is shown in Figure 1 (A,B). These results indicated that the heating of raw flours with a 14% moisture content caused little or no decrease in free sulfhydryl content, whereas the addition of water to 51% moisture content caused a rapid decline (~34%) in free sulfhydryl content at 18°C. Further processing of those doughs at higher temperatures produced a slight decrease (~12%) between 18 and 55°C, followed by a more pronounced loss between 55 and 85°C. These results are consistent with observations of Weegels et al (1994), who found that the free sulfhydryl content of hydrated gluten decreased on heating only when the moisture content was >20%. It has been postulated (Schofield et al 1983) that the transition between 55 and 85°C is a consequence of protein denaturation, although there are some who argue that starch gelatinization is responsible (Eliasson and Hegg 1980).

However, work by Schofield et al (1983) on the effect of heat on gluten functionality showed that the free sulfhydryl content of gluten was not changed by temperature but rather free sulfhydryls were transferred from an SDS-extractable form to an SDS-insoluble form. This transfer occurred between 55 and 75°C. At 60°C, there was a marked decline in gluten protein extracted into 0.1% SDS/0.1*M* Tris-HCl, pH 8.0. They concluded that the oxidation of sulfhydryl groups to disulfide bonds was not the mechanism by which polymerization of glutenin molecules occurred. Jeanjean et al (1980) also found that gluten protein extractability was markedly affected by the time that gluten was heated at 100°C. They concluded that the ethanol-soluble proteins are insolubilized by the formation of new intermolecular disulfide bonds.

Extraction of protein in the present investigation involved a combination of proteolysis and SDS, and was expected to be more efficient. Evidence for the efficiency of extraction, based on the absorbance at 280 nm of solutions resulting from the extraction of 40 mg of freeze-dried dough, is shown in Figure 2.

The effect of adding $KBrO_3$ on the sulfhydryl content of heated doughs is shown in Figure 3 (A–C). The initial level of $KBrO_3$ in these doughs was 67.4 nmol/g of dough (equivalent to 0.108 μmol/g of flour). This was approximately one-third the sulfhydryl content of the raw flours and, therefore, twice the amount required to oxidize sulfhydryls, according to Equation 1. However, for both bromated samples, the loss of sulfhydryl as a function of temperature was almost the same in quantity and pattern as that of the nonbromated samples.

The average net decline in sulfhydryl content of each sample due to the presence of bromate may be computed by averaging

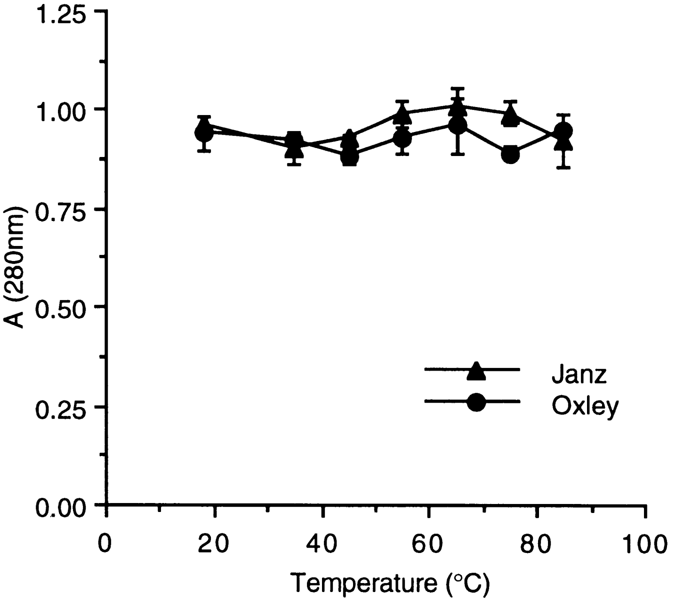

Fig. 2. Protein extraction efficiencies (as indicated by absorbance at 280 nm) for Janz and Oxley doughs at the indicated temperatures.

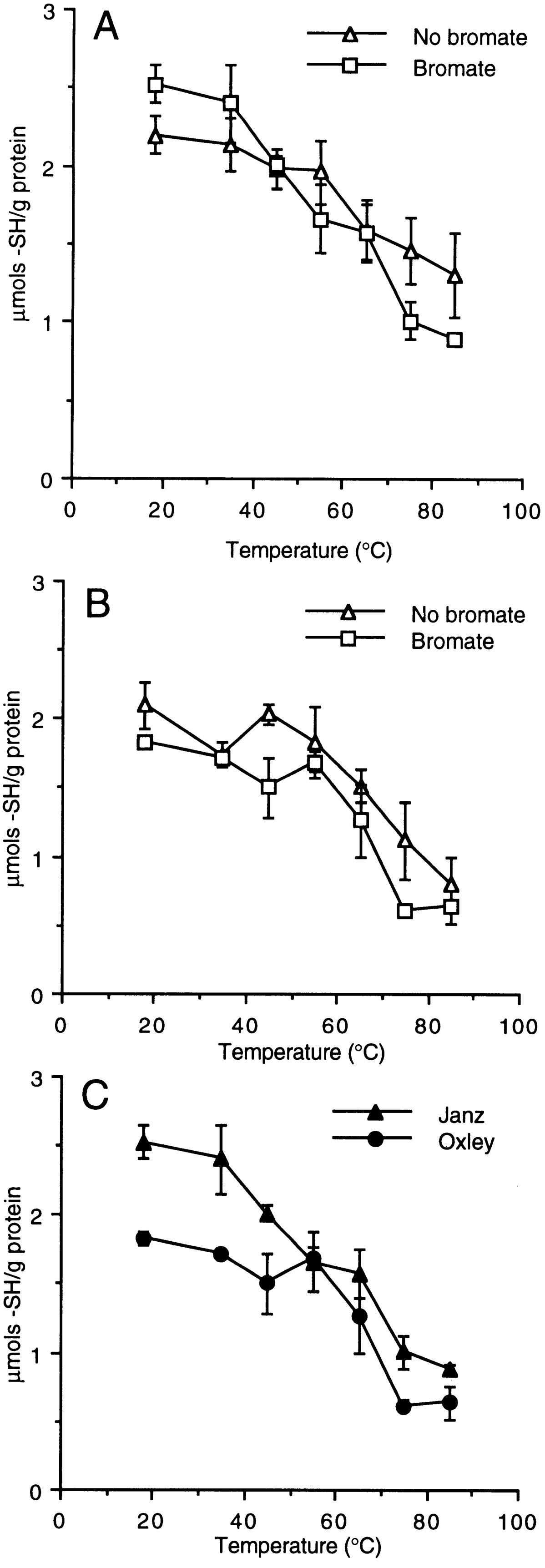

Fig. 3. A, Free sulfhydryl content of nonbromated and bromated dough samples derived from Janz and heated at indicated temperatures. **B,** Free sulfhydryl content of nonbromated and bromated dough samples derived from Oxley and heated at indicated temperatures. **C,** Free sulfhydryl content of Janz and Oxley bromated dough samples heated at indicated temperatures.

the difference in values at each temperature. For Janz, this figure was −0.08 μmol SH/g of protein, whereas for Oxley, it was −0.27 μmol SH/g of protein. It is tempting to speculate that the larger average decline observed for Oxley as compared to Janz was related to the improver action of $KBrO_3$ on flour from Oxley. However, the average decline in sulfhydryl content for Oxley represents <8% of the oxidative capacity of the $KBrO_3$ in the dough.

Sulfhydryl loss due to increasing temperature has been demonstrated by Tsen (1968), who concluded that increased temperature, specifically 75–95°C, played a key role in the oxidation of sulfhydryls by $KBrO_3$. This work, and observations by others (Sullivan et al 1940, Tkachuk and Hlynka 1961), have shown that $KBrO_3$ is a slow-acting oxidant requiring the higher temperatures found during baking for oxidation to occur. The results of this current study suggest that the role of $KBrO_3$ as a bread improver is not as an oxidizing agent for free sulfhydryls in flour proteins.

The breakdown of $KBrO_3$ during the baking process was investigated using gas chromatography, as described by Sparkes et al (1992). The method depends on $KBrO_3$ reacting with bromide ions to produce bromine. Bromine reacts with styrene in the presence of chloride ions producing a chloro-bromo-styrene derivative that is readily resolved and measured by gas chromatography with electron capture detection. This method has a detection limit of 25 ng of $KBrO_3$. Figure 4 illustrates the decrease in $KBrO_3$ level of the doughs as the temperature increases. The $KBrO_3$ level present initially in the dough was 67.4 nmol/g of dough. Mixing alone caused ~46% decline in the level of $KBrO_3$. The action of $KBrO_3$ during mixing was also investigated by Panozzo et al (1994). They suggested that the $KBrO_3$ reaction increased binding of lipids to proteins, thereby oxidizing the free sulfhydryls. Although we found no evidence of $KBrO_3$ causing any significant oxidation of free sulfhydryls, the data at 18°C in Figure 1 (A,B) suggest that mixing also produces a significant loss in sulfhydryl content. The two samples show similar patterns of $KBrO_3$ loss in Figure 4, however the decline in the Janz sample dough is significantly greater than that for Oxley. Both samples show a distinctive decline in the 55–75°C temperature range, a range that also corresponds to denaturation of wheat proteins (Pence et al 1953, Schofield et al 1983, Dreese et al 1988).

The loss of sulfhydryls due to change in temperature has been observed by Sullivan et al (1961) and Yoneyama et al (1970), although in these studies the difference in temperature was for flours stored below freezing and then brought to room temperature. The results of this study indicate that although $KBrO_3$ is consumed during heating, the loss of free sulfhydryls

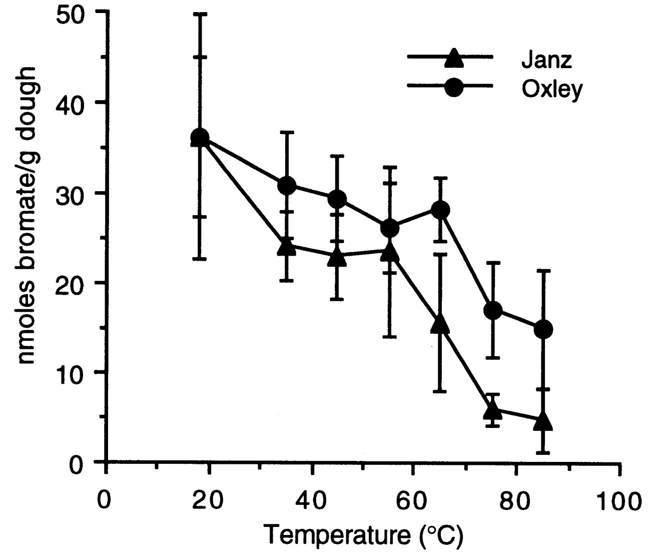

Fig. 4. $KBrO_3$ remaining in Janz and Oxley dough samples after heating at indicated temperatures.

(which also occurs during heating) is not dependent on the presence of $KBrO_3$.

CONCLUSION

Adding $KBrO_3$ to doughs appeared to have little or no effect on free sulfhydryl content. Despite the current theory that $KBrO_3$ converts sulfhydryls to disulfides, results of this study showed that only a small fraction of sulfhydryl loss can be attributed to the action of $KBrO_3$, even for a derived flour sample known to be highly responsive to $KBrO_3$ during baking. For both non-responsive and responsive $KBrO_3$ samples, the principal cause of oxidation of free sulfhydryls was heating, with some oxidation due to mixing. Further work will be required to fully understand the $KBrO_3$ reaction in doughs.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support of an Australian Postgraduate Research Award (Industry) and technical support from the Bread Research Institute of Australia Inc.

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[Received August 29, 1994. Accepted February 23, 1995.]