

Action of Oxidants on Water Sorption, ^2H Nuclear Magnetic Resonance Mobility, and Glass Transition Behavior of Gluten

GEORGE CHERIAN¹ and PAVINEE CHINACHOTI^{1,2}

ABSTRACT

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Oxidation of vital wheat gluten with potassium bromate and ascorbic acid significantly extends or broadens glassy-rubber transition to a higher final temperature range or moisture content. Thermomechanical and deuterium nuclear magnetic resonance (NMR) data show that the increased stiffness due to oxidation could be detected from the thermomechanical and deuterium NMR mobility level, indicating increased rigid fractions. The oxidation also resulted in increased water sorption, but no significant change in "freezable" water, and a much decreased mobile deuterium

NMR signal. Room temperature sorption of water resulted in a glassy-rubbery transition over a ≈ 10 – 20% mc range for the control. For the oxidized sample, it started at $\approx 10\%$ mc, but the transition was gradual and extended into much higher moisture ranges that corresponded to a more rigid fraction of deuterium (NMR) signal. This suggests that the oxidative interactions led to a more rigid gluten fraction, extending the transition to a higher temperature range, perhaps resulting in a more elastic dough.

Quality of baked products is greatly dependent on the rheological and mechanical properties of the flour components. Gluten contributes significantly to the dough and final product functionality (Schofield 1986), including loaf volume, uniformity, texture, water holding capacity, dough strength, and mixing tolerance of the dough (Magnuson 1985). The thermosetting character of the gluten also influences, to some extent, the structural and textural properties after baking.

Additives are added to a dough to improve its performance. They include oxidizing and reducing agents, dough conditioners, and enzymes, etc. Action of reducing and oxidizing agents on dough properties occurs during different stages of processing (mixing, proofing, heating, etc.) and, consequently, changes the development of the dough protein structure (Fitchett and Frazier 1986). Various chemical interactions are involved in dough structure formation, including hydrogen bonding, electrostatic bonding, van der Waals forces, hydrophobic interactions, and disulfide (SS) bonding (Bloksma 1975, Ewart 1977, Kaufman et al 1986).

Dough mixing in air or oxygen results in a decrease of thiol groups present in gluten due to oxidation (Tsen and Bushuk 1963). Tkachuk and Hlynka (1968) reported a two-step oxidative process by bromates: 1) a reduction of bromate to bromite and 2) a reduction of the bromite to bromide. The first stage was accompanied by oxidation of the sulfhydryl (SH) groups. However, rheological changes in a dough after adding bromates and iodates might not be entirely based on changes in SH groups (Bloksma 1972). The mode of action of L-ascorbic acid is different. Sandstedt and Hites (1945) suggested that L-ascorbic acid was converted enzymatically to its dehydro-form, which then acted on SH groups. Others reported that the oxidation could be either enzymatic or nonenzymatic (Elkassabany and Hoseney 1980). At present, the precise mechanism of ascorbic acid action on wheat dough is still not fully understood.

Storage modulus of a dough increases with addition of oxidizing agents. Dreese et al (1988) and Attenburrow et al (1990) reported that a dough with added potassium iodate had an increase in storage modulus that was disproportional to the degree of SS formation. Also, Muller (1969) found that only a small amount of potassium iodate additive was involved in SS crosslinking, thus some other mechanisms may be involved.

Rheological properties of dough and baking performance are related to hydration (Finney and Shogren 1972). Hydration of wheat flour and its components has been investigated by several researchers (Leung et al 1979, 1983; Richardson et al 1985). Leung et al (1983) studied water mobility in a flour-water system as determined by proton and deuterium relaxation. Although the data did not directly relate to different rheological properties of the dough, studying the nature of hydration by nuclear magnetic resonance (NMR) has proven to be a powerful tool in understanding dough properties. At present, not much is known about how oxidant additives affect the nature of hydration and related mechanical properties.

In a previous study (Cherian and Chinachoti 1996), vital wheat gluten was analyzed in great detail by water sorption study, ^2H NMR, and glassy-rubbery transition characterized by differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). Gluten underwent a glassy-rubbery transition upon sorption of water in the ≈ 10 – 20% mc range (covering the monolayer to multilayer mc range). ^2H NMR also showed a change in intensity that was parallel to that of the glassy-rubbery transition with matching onset, midpoint, and final moisture contents. Thus, ^2H NMR confirmed that short-range mobility changed with plasticization and sorption. Further study of the various roles of additives would provide more information about the rheological impact of oxidizing agents on dough properties.

This work characterizes the thermomechanical changes of wheat gluten during hydration as determined by an NMR mobility study and the specific role of oxidants in these changes.

MATERIALS AND METHODS

Materials

Hard red spring wheat gluten (Sigma Chemical Co., St. Louis, MO) with a protein content of 80% (db) was used in this study. Potassium bromate and ascorbic acid were obtained from Manildra Milling Co. (Atchinson, KS). Deuterium oxide (D_2O , 99.9% enrichment) was obtained from Cambridge Isotope Ltd. (Cambridge, MA). Salts for preparation of saturated salt solutions were obtained from Sigma Chemical Co. (Fairlawn, NJ).

Methods

Samples were prepared by mixing 100 g of gluten with 150 mL of water. The oxidizing agents (50 ppm of potassium bromate or 200 ppm of ascorbic acid; concentration based on supplier's recommended level for dough conditioning) were dissolved in an ali-

¹Department of Food Science, University of Massachusetts, Amherst, MA 01003.

²Corresponding author. Phone: 413/545-2276. Fax: 413/545-1262.

quot of the added water. The gluten was mixed in a farinograph (Brabender, South Hackensack, NJ) at intermediate speed for 3 min. The material was pressed between plexiglass plates to form a bar of uniform thickness (≈ 2.5 mm thick). Samples were kept in a freezer for 2 hr and then cut into rectangular strips and further freeze-dried (model 50 SRC, Virtis Sublimator, Gardiner, NY). The freeze-dried samples were conditioned at 25°C to various moisture contents using saturated salt solutions of different relative humidities or water activities (0–0.97 a_w) (Greenspan 1977). Samples with >25% mc were sprayed directly with D₂O and allowed to equilibrate in a closed minidesiccator before analysis. All samples were compared with a control (no oxidants added).

²H NMR Mobility Measurement

Samples were hydrated at different water activities with ²H-enriched water (added to salt to obtain saturated solutions). All samples were equilibrated for three to seven days at 25°C. The samples (300 mg) were packed in an NMR tube with a vortex plug to minimize moisture loss. An NMR spectrometer (XL-300, Varian, Inc., Palo Alto, CA) was used for the study. Acquisition parameters included: spectral width 40,000 Hz; acquisition time 0.05 msec; pulse width 16.5 μ sec; equilibrium recycle time between transients 0.05 μ sec, and deadtime 200 μ sec. Up to 50,000 transients were accumulated, depending on moisture content of the sample. Pure D₂O of varying amounts was used for constructing a standard curve (intensity vs. mg of D₂O) (Chinachoti and Stengle 1990). All samples were measured in duplicate. Linewidth and signal intensity (from the peak area) were measured from each spectrum. The percent of detected intensity for each sample was compared to an external reference (pure D₂O) to measure the percent of signal detected:

$${}^2\text{H signal of sample} \times \text{D}_2\text{O} \times 100 / {}^2\text{H signal of sample} \times \text{g of pure D}_2\text{O}$$

Differential Scanning Calorimetry

A ≈ 10 -mg sample was placed in a hermetically sealed, stainless steel pan and heated (model DSC 110, Seiko Instruments, Inc., Torrance, CA) from –60°C to 200°C at 5°C/min. The calorimeter was calibrated with indium. The sample pan was run with an empty pan for reference. Change in specific heat was used to detect a glass transition by observing a baseline shift with the onset,

midpoint, and final temperature recorded (Ferry 1980). All samples were measured in duplicate; experimental error was within 5%. Freezable water was measured from endothermic enthalpy for ice melting, assuming that the enthalpy of melting in the sample was the same as that of pure water.

Dynamic Mechanical Analyzer

Rectangular bars of sample ($\approx 40 \times 10 \times 2.5$ mm) were tested with DMA (model DMA 100, Seiko Instruments). Samples were mounted in a flexure mode, cooled to –80°C, and then immediately heated to 200°C at 5°C/min. Sinusoidal stress of various frequencies (1, 5, 10, 20, and 50 Hz) was applied, but only 1 Hz results are presented here. Responding strain was recorded and data reported as storage modulus (E'), loss modulus (E'') and $\tan \delta$ (ratio of E''/E'). Transition temperature was observed from $\tan \delta$ peak. All samples were measured in duplicate; experimental error was within 3%. Detailed discussion on theory and method of analysis may be found elsewhere (Murayama 1978, Hallberg and Chinachoti 1992).

Sorption Isotherm

Samples (0% mc, ≈ 2 g) were equilibrated at 25°C in proximate equilibration cells (Lang et al 1981) against saturated salt solutions (0–0.97 a_w) (Greenspan 1977). During the course of equilibration, samples were weighed daily after an initial four-day period until no consecutive change in weight was observed. The final moisture content was measured from weight and vacuum oven moisture determination (60°C, vacuum 29 in. Hg, 24 hr).

RESULTS

Deuterium NMR

²H NMR spectra showed Lorentzian shape with linewidth at half height decreasing with D₂O content similarly for all samples in a curvilinear fashion (Fig. 1). Most of the line broadening effect occurred <10% D₂O content; linewidth increased with decreasing D₂O content from 200 Hz at 30% D₂O to 6,000 Hz at 2% D₂O. Essentially, there was no significant line broadening observed at >20% D₂O. No significant difference was found in linewidth between the control and the oxidant-treated samples at a given D₂O content.

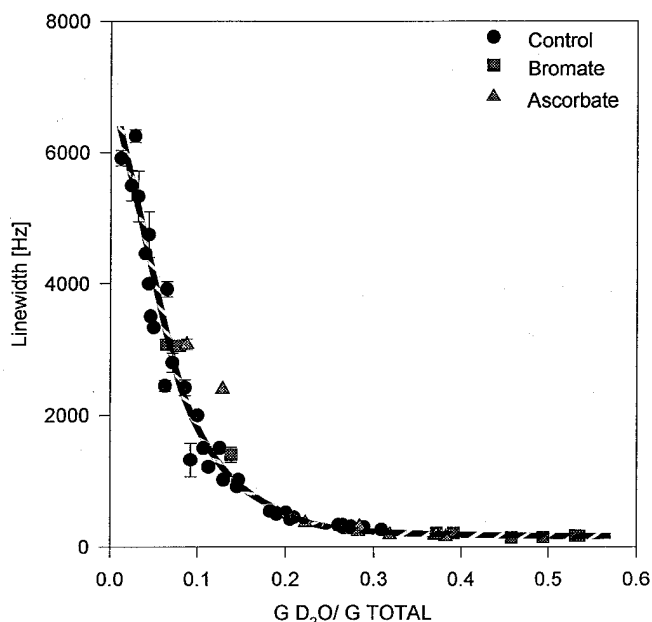


Fig. 1. Change of ²H nuclear magnetic resonance linewidth with D₂O content for gluten treated with potassium bromate and ascorbic acid and a control (no treatment).

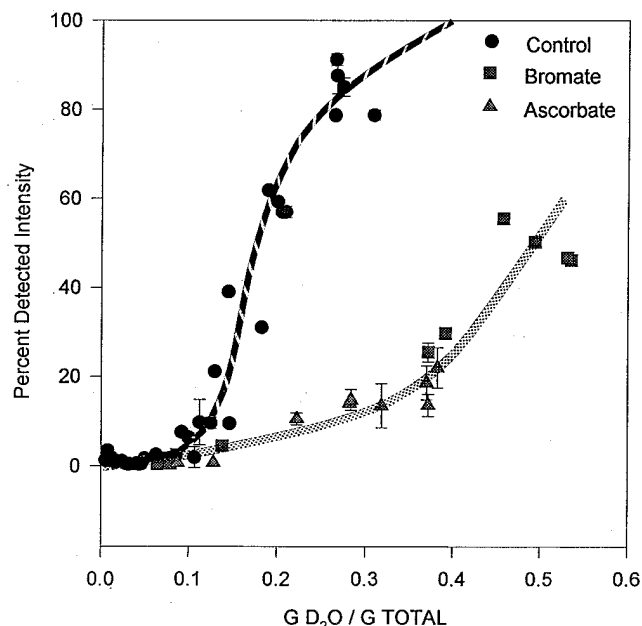


Fig. 2. Detected intensity (%) from ²H nuclear magnetic resonance at various D₂O contents for gluten treated with potassium bromate and ascorbic acid and a control (no treatment).

Figure 2 shows ^2H NMR intensity of observable deuterium expressed as percent detected intensity. Samples with <100% detected intensity indicated that, some of the deuterons were not detected because relaxation times were shorter than the NMR timeframe (200 μsec deadtime before the first acquisition). At D_2O content <10%, the data indicated that most of the deuterons were undetected (Fig. 2). But >10% D_2O , the ^2H NMR intensity of the control sample increased dramatically from <1% to $\approx 85\%$ at 26% D_2O (Fig. 2). This was previously thought to be due to an increased mobility of D_2O and gluten side chains upon hydration (Cherian and Chinachoti 1996). Changes were more gradual in oxidant-treated samples (Fig. 2). At D_2O contents >10%, the control showed a significantly greater ^2H NMR signal when compared with that of the oxidant-treated samples. No significant difference was found between the two treated samples, even though the levels of oxidants added were not identical.

Some of the ^2H nuclei might be exchangeable with the protons on the protein side chains. As the protein side chain became more rigid at lower D_2O levels, the more solid ^2H signal would be increasingly lost in the baseline as the T_2 relaxation time shortened sufficiently. Additional disappearance of the ^2H intensity in the oxidized gluten samples could be a result of a higher immobilized fraction of D_2O or higher solidity of the protein side chains, possibly due to SS bond formation (Kaufman et al 1986). It is possible that excess oxidant might have other effects not related to the SS bond formation.

Almost all deuterium nuclei should be detected at some level where most of deuterium nuclei are associated with free or mobile D_2O . All curves in Figure 2 should eventually reach 100% at high D_2O levels. Earlier work showed that the onset, midpoint, and final moisture content for the control corresponding to the change in ^2H NMR intensity agreed very well with the corresponding values for the glassy-rubbery transition (DMA) (Cherian and Chinachoti 1996).

Sorption Isotherm

The gluten samples treated with either potassium bromate or ascorbic acid showed a significantly greater water sorption ability (>0.30–0.85 a_w) than did the control (Fig. 3). The discrepancies at 0.97 a_w (where the opposite was observed) is not explainable, but it is possible that at a high a_w , the slow equilibration time might lead to a lack of true equilibrium in some samples (the difficulty

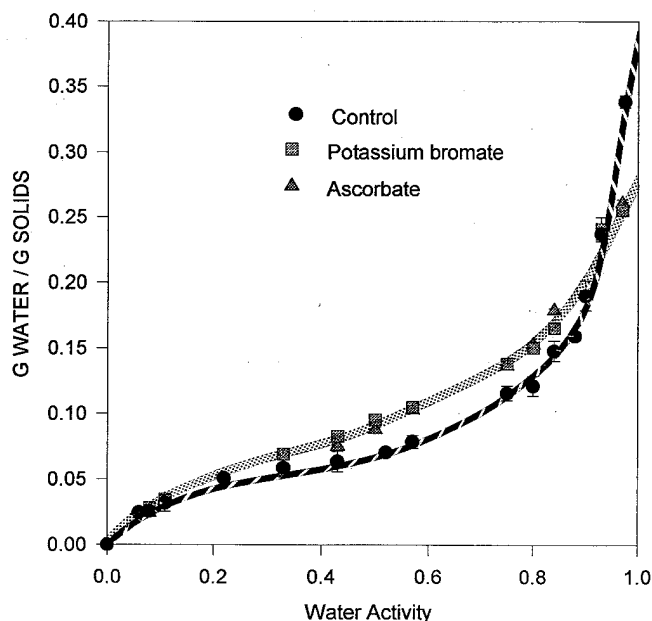


Fig. 3. Sorption isotherms at 25°C for gluten treated with potassium bromate and ascorbic acid and a control (no treatment).

in measuring water sorption at a very high a_w) and possibly to invisible microbial spoilage. However, the lower water sorption in the oxidized sample (0.97 a_w) could be an indicator of lower swelling property or solubility caused by the higher degree of crosslinking in the treated samples. The BET monolayer moisture content (Brunnauer et al 1938) was ≈ 0.05 g of water/g total for all three samples.

The increased amount of sorbed moisture in oxidized gluten samples could indicate a greater sorption capability because of changes in the conformation from oxidation. It is possible that the oxidants may expose hydrophilic moiety in gluten, resulting in higher water sorption ability. It is also possible that added oxidants themselves sorbed some moisture, but their contribution in the amount of moisture sorbed was probably small (<0.001 g/g of solids), assuming the most hygroscopic (sugar-like) sorption ability. Thus, it was concluded that the most of the discrepancies were

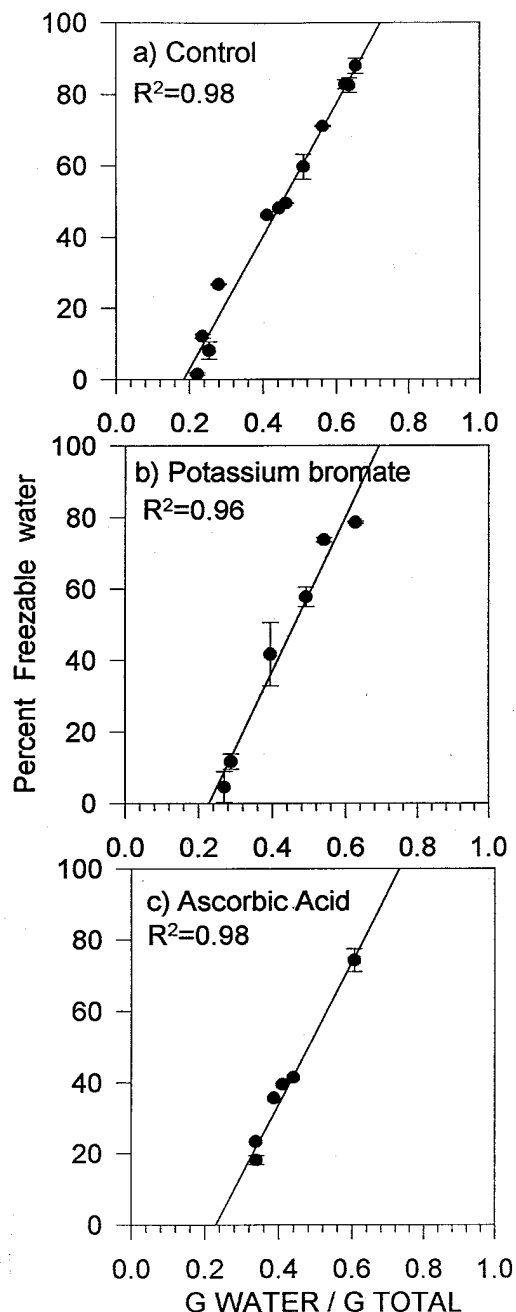


Fig. 4. Freezable water (%) observed for gluten treated with potassium bromate and ascorbic acid and a control (no treatment).

related to the expected increase of SS bonding. At the same level with 200 ppm of bromate, more than 50% of the free SH groups was lost to SS bond formation (Matsumoto et al 1960).

Hydrophobic interaction might also be involved. A review indicates that glutenin is hydrophobic and thus contributes significantly to the dough functional properties (Bushuk 1985). A solid-state NMR study suggested that glutenin subunits that are not tightly folded (gliadins), are associated through hydrophobic interactions involving aromatic and aliphatic side chains (Schofield and Baianu 1982). These interactions are important to intermolecular subunits and their functionality. Thus, function changes in a dough caused by oxidizing agents involve many complex combinations of hydrophobic interactions, hydrogen bonding and inter- and intrachain SS bonding (Bushuk 1985). Much of this must have a significant impact in water sorption because conditioning dough with an oxidant increases water sorption (Tieckelmann and Steele 1991).

Freezable Water

Figure 4 shows there was no significant difference in freezable water and unfreezable water at moisture contents up to 70% for

the control gluten and gluten treated with potassium bromate or ascorbic acid (Fig. 4a-c). The extrapolation of the endothermic DSC data points on to the x-axis (moisture content) resulted in unfreezable water values of 0.18, 0.23, and 0.23 g of water/g total for the control and the two oxidized gluten samples, respectively. This could be the real difference between untreated and oxidized samples. The higher unfreezable water in the oxidized gluten could reflect the higher degree of association (hydration) of oxidized gluten (Fig. 3), which may have a significant impact in the dough rheology and functionality (Tieckelmann and Steele 1991).

Thermomechanical Measurement

The DMA data showed that, within the unfreezable moisture range ($\approx <20\%$) the glassy-rubber transition was observed as a decrease in E' and E'' , and a peak in $\tan \delta$ (E''/E'). All samples showed an extremely broad transition (80–200°C from onset to final temperature) suggesting a T_g distribution of different domains or local regions. Although many prefer to compare a single T_g value, we will also discuss the range of T_g . The midpoint $\tan \delta$ peak temperature is used as T_g for comparison but this is uncertain because it does not reflect the range of transition.

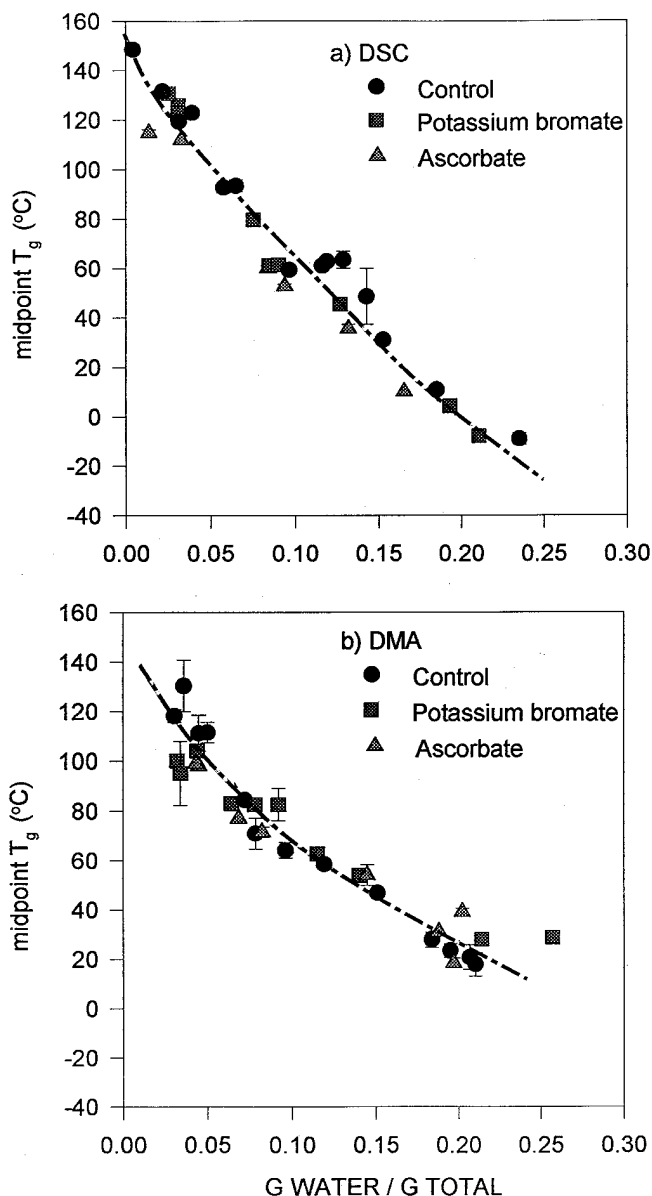


Fig. 5. Midpoint T_g as a function of moisture for gluten treated with potassium bromate and ascorbic acid and a control (no treatment). DSC = differential scanning calorimetry; DMA = dynamic mechanical analysis.

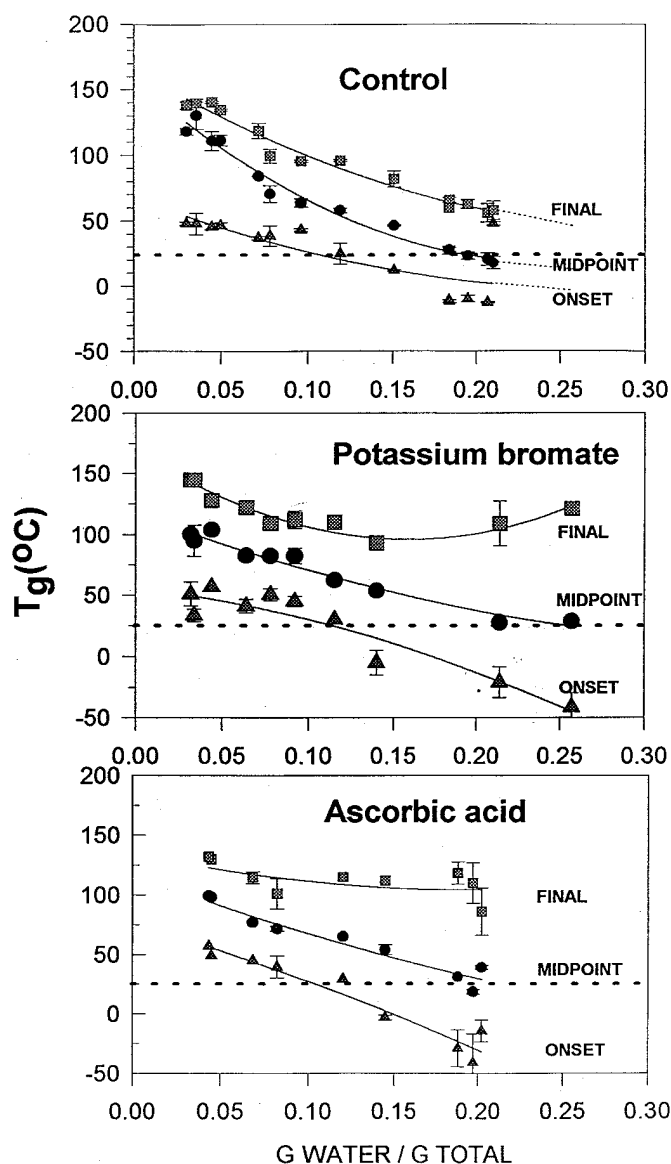


Fig. 6. Onset, midpoint, and final transition temperatures measured by dynamic mechanical analysis for gluten treated with potassium bromate and ascorbic acid and a control (no treatment). Horizontal lines indicate room temperature (25°C).

The midpoint T_g measured by DSC and DMA for the control, and treated gluten, are shown in Figure 5. The temperature decreased from $\approx 140^\circ\text{C}$ at almost 0 g of water/g total to 20°C (DSC) or 50°C (DMA) at 15% moisture (Fig. 5a and b). Gluten treated with ascorbic acid and potassium bromate had similar their temperature dependencies (Fig. 5a and b). There were some discrepancies observed in T_g midpoints at relatively higher moisture content between the two methods. This was probably due to a moisture loss during the DMA experiment. It may be misleading to say that oxidation had no impact on T_g .

Comparison of the onset and final temperatures of transition (by DMA, 1 Hz) indicated some effect of oxidation on the thermomechanical transition of gluten (Fig. 6). For the control, the transition range (breadth of transition) remained relatively unchanged ($80\text{--}100^\circ\text{C}$) with changing moisture content. However, the transition range increased significantly as the moisture content increased for the ascorbic acid and potassium bromate treatments. This indicates that the transition started at a lower temperature and terminated at a higher temperature.

The oxidized gluten samples could have shown a spread in thermomechanical transition into a higher temperature range for several reasons. First, there may have been a drying out effect that might have caused some transition to go to a higher temperature. However, this was ruled out because the control sample would have shown a more or less similar trend. Thermal expansion could also have been a factor. The data also suggested that the SS bond formation in the oxidized samples resulted in inter- and intramolecular interaction that could have caused an increase in the transition temperature range.

Comparison of E' among samples of a given moisture content reveals that E' values changed similarly among these three samples. The only difference was the higher temperature spread in oxidized samples with $>15\%$ moisture, which could help explain the effect of oxidants on dough rheology observed by using an extensigraph (Dreese et al 1988) and during oven spring where the spread in thermomechanical transition may change the elastic properties of the dough.

DISCUSSION

Adding oxidants caused a distinctive increase on the water sorption ability, freezable water content, and the thermomechanical behavior of gluten. Potassium bromate and ascorbic acid caused crosslinking of gluten structure and changed the flow properties, resulting in an increase in relaxation time and rigidity modulus (Mita and Bohlin 1983). For potassium bromate, crosslinking may form in the lamellar structure. But for ascorbic acid, its conversion into dehydroascorbic acid may have been responsible for the oxidation of gluten, although the precise mechanism is not yet understood (Elkassabany and Hosney 1980).

Stronger interaction between the oxidant-treated gluten and water becomes possible because of the conformational changes in the gluten, induced by the inter- or intrachain SS bonding (Kasarda et al 1976). Thus, the SS linkages and other resulting inter- or intramolecular interaction between the peptides result in changes in the gluten-water interaction and thermomechanical properties, increasing glass transition temperatures of some fractions.

On the other hand, ^2H NMR showed a significant reduction in detected signal intensity. Even though the linewidths of all samples were identical, the difference in intensity could be taken to mean that more fractions of the deuterons become incorporated in immobile side chains of the oxidized proteins. Thus, the formation of SS bonds that interconnect subunits also increased the rigidity and decreased the exchange rate of deuterons. With increasing D_2O levels, the signal detected is expected to increase to 100% at very high moisture (50% mc). Since ^2H NMR spectra were affected by the unknown contribution of exchangeable protons on

the proteins, it becomes difficult to quantify the change based on D_2O mobility. It would be interesting to evaluate further how SS bond formation (caused by oxidation) affects D_2O and gluten association on a molecular level in such a concentrated system far from extreme narrowing conditions (Belton 1990). Further investigation using a solid-state NMR to allow quantitative measurement of the more solid signals is being conducted in our laboratory.

The detected signal intensity change in the control sample occurred in the same moisture range where the glass transition occurred (0.10–0.30 g D_2O /g total) (Fig. 2 and Fig. 6a). For the oxidized gluten samples however, the room temperature glass-rubbery transition (Fig. 6 b and c) occurred over a much wider moisture range (same onset moisture but extremely high final moisture) (Fig. 6 a–c). At room temperature (25°C), all gluten were very much still in the glassy region at $<12\%$ mc (Fig. 6 a–c). However at $>12\%$ mc, the oxidized samples had a broader transition moisture range, showing the transition or extending of thermomechanical property (rigidity) into the higher moisture range (Fig. 6 b and c). This is one of the major effects of oxidation on gluten functionality, which may be related to the lower ^2H NMR signal. With increased crosslinking and rigidity, many of the fast relaxing components show less ^2H NMR signal. Thus, the deuterium signal loss could be explained thermomechanically. The increased water sorption might reflect the increased interaction with water which might also lead to a decreased signal intensity.

CONCLUSION

Water interactions (^2H NMR intensity, water sorption, and thermomechanical properties) of chemically treated gluten (potassium bromate and ascorbic acid) differed significantly from that of the control. Signal intensity detected by ^2H NMR corresponded with the thermomechanical transition change. The oxidant-treated sample had a lower ^2H NMR signal than the control, due to the increase in the glassy, rigid domains as well as possible increased interaction with water. The small increase in unfreezable water, water sorption capability, and a decrease in ^2H NMR signal in oxidized gluten could reflect the effect of SS bond formation on other interactions (e.g., hydrophobic interaction, hydrogen bonding, etc.).

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