

Effects of Certain Breadmaking Oxidants and Reducing Agents on Dough Rheological Properties¹

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ABSTRACT

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A dynamic rheometer was used to characterize the effect of glutathione, potassium bromate, and two ascorbic acid isomers on the rheological properties of wheat flour doughs. During resting after mixing (dough relaxation), G' decreased and the loss tangent increased. The major factor causing those changes was suggested to be a sulfhydryl-disulfide interchange. Free radicals appeared to be involved in the sulfhydryl-disulfide

interchange. Addition of potassium bromate to dough resulted an increase in G' and a decrease in loss tangent. L-threoascorbic acid was rheologically more effective than D-erythroascorbic acid. This was explained by the presence of an active glutathione dehydrogenase in wheat flour that is specific for both glutathione and L-threoascorbic acid.

Sperling (1986) cataloged the causes of stress relaxation into five categories: 1) a decrease in molecular weight caused by chain scission as a result of oxidative degradation or hydrolysis; 2) bond exchanges ongoing constantly in polymers, with or without stress (in the presence of a stress, however, the statistical rearrangements tend to reform the chains, so the stresses are reduced); 3) viscous flow caused by linear chains slipping past one another; 4) thirion relaxation as a reversible relaxation of the physical cross-links or trapped entanglements in elastomeric networks; 5) molecular relaxation, especially near the glass transition temperature (T_g), that tends to relieve any stress of chains during the experiment.

The role of the sulfhydryl groups in dough chemistry has

attracted the attention of many cereal chemists. The main premise has been that these sulfhydryl groups are potentially capable of undergoing a disulfide-sulfhydryl interchange that involves the cleavage or reformation of disulfide bonds mediated by sulfhydryl groups in flour or by relatively small amounts of added sulfhydryl compounds.

Reduced glutathione (GSH) and oxidized glutathione (GSSG) are both naturally occurring in wheat flour (Kuninori and Matsumoto 1964, Hird et al 1968, Tkachuk 1969). Graveland et al (1978) reported that flour contained 5-7 mmol of sulfhydryl groups and 11-18 mmol of disulfide per kilogram. Kuninori and Sullivan (1968) studied disulfide-sulfhydryl interchange in wheat flour by adding radioactive glutathione. They reported that significant interchange took place in a flour-water dough, but not in a flour suspension. They postulated that mixing promoted the reaction of disulfide groups and GSH. Another possibility is that a free radical ($GS\cdot$) is formed during mixing and may be involved in the disulfide-sulfhydryl interchange. Reaction with ($GS\cdot$) can cause session of protein disulfide forming a protein thiyl radical.

When interchain disulfide bonds are cleaved, the resulting

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depolymerization of the gluten proteins decreases the molecular weight and thereby reduces the elasticity and increases the extensibility of dough. Evidence for the occurrence of the interchain disulfide bonds in gluten, particularly in the glutenin fraction, have been presented by Beckwith and Wall (1966) and Yoshida et al (1980). Some of these interchain disulfide bonds may be important in maintaining the gluten structure: a reduction of 3-4% of the disulfide bond with mercaptoethanol caused a depolymerization of 80% of the high molecular weight gluten proteins (Jones et al 1974).

It is generally accepted that the rheological properties of dough and its three-dimensional network are dependent on the arrangement and number of disulfide bonds and sulfhydryl groups of the protein. The vital contribution of disulfide bonds to dough stability has been shown in rheological studies by the addition of either sulfhydryl compounds or sulfhydryl-blocking reagents. A small amount of cysteine or reduced glutathione dramatically increases the extensibility of dough. Bloksma (1972) showed that both the viscous and elastic component of dough deformation were increased by reduced glutathione.

Bloksma (1972) studied the relationship between the sulfhydryl and disulfide contents of dough and its rheological properties. He reported that only small fractions of the total sulfhydryl and disulfide groups were rheologically effective, and these fractions were much smaller than the chemically reactive ones. Jones et al (1974) estimated, on the basis of farinograph measurements, that ~25-35% of sulfhydryl groups and 4-13% of the disulfide bonds were rheologically effective. However, a small decrease in crosslinking was sufficient to cause a considerable rheological effect because of disappearance of rheologically effective groups (Bloksma 1972). In most breadmaking processes, after mechanical development of the gluten network, the structure must be stabilized by oxidants. Minute amounts of oxidizing reagents such as potassium bromate or dehydroascorbic acid (DHAA) improve the handling and baking characteristics of wheat flour. Loaf volume increased, and bread had a better crumb grain (Jorgensen 1939). Most current theories on the improver action of oxidants agree that sulphhydryl groups are involved in the reaction mechanism. The bromate is assumed to oxidize low molecular SH-peptides (glutathione) and consequently hamper sulfhydryl-disulfide interchange of gluten molecules (Bloksma 1972).

The sulfhydryl-disulfide interchange reaction also is used to explain the action of ascorbic acid, but the number of steps involved in its improver action are higher than with bromate.

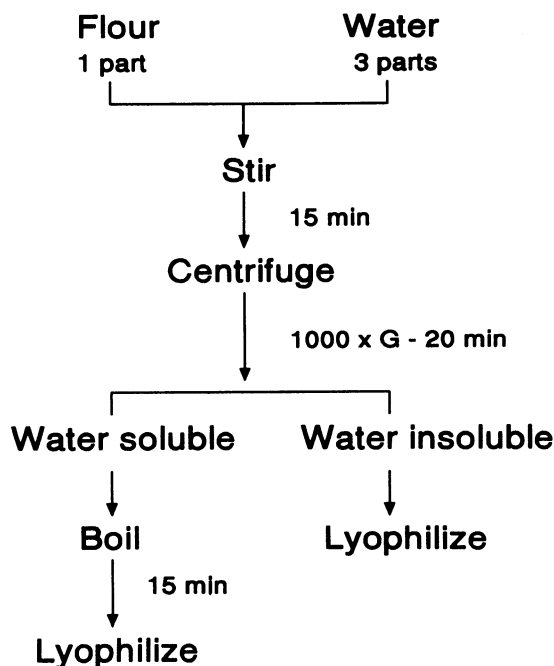


Fig. 1. Flour fractionation procedure.

The four stereo isomers of ascorbic acid and their dehydro-forms display quite different activities as improvers. Walther and Grosch (1987) reported that the specificity of the enzyme glutathione dehydrogenase (dehydroascorbate reductase, EC 1.8.5.1) was responsible for the difference in the improver action. L-threodehydroascorbic acid was the best and D-threodehydroascorbic acid was the worst substrate of the four stereoisomers. This enzyme, which was discovered in wheat by Kuninori and Matsumoto (1963, 1964), oxidizes glutathione to its corresponding disulfide with DHAA as the oxidant. The enzyme appears to be specific for both glutathione and L-threodehydroascorbic acid. Thus, the improver action of L-threodehydroascorbic acid is explained by the oxidation of glutathione to the oxidized form. The decrease in glutathione decreases the rate of sulfhydryl-disulfide interchange in the dough. The order of substrate specificity of glutathione dehydrogenase for the four stereoisomers corresponds well with their improver action in dough (Mair and Grosch 1979).

The purpose of this study was to characterize the effect of potassium bromate, glutathione, and ascorbic acid on the rheology of flour-water doughs using a dynamic rheometer.

MATERIALS AND METHODS

Commercial bread flour, 12.1% protein (N \times 5.7), 0.45 ash, (14% mc) from Ross Industries (Cargill, Wichita, KS) was used. The flour was fractionated into water-insoluble and soluble fractions according to the procedure shown in Figure 1. One part of flour was suspended in three parts of distilled water and stirred continuously for 15 min. Then the suspension was centrifuged for 20 min at 1,000 \times g. The insoluble residue, gluten plus starch, was frozen and lyophilized. The water-soluble fraction was boiled for 15 min and then frozen and lyophilized. Both the water-insoluble and water-soluble fractions were ground in

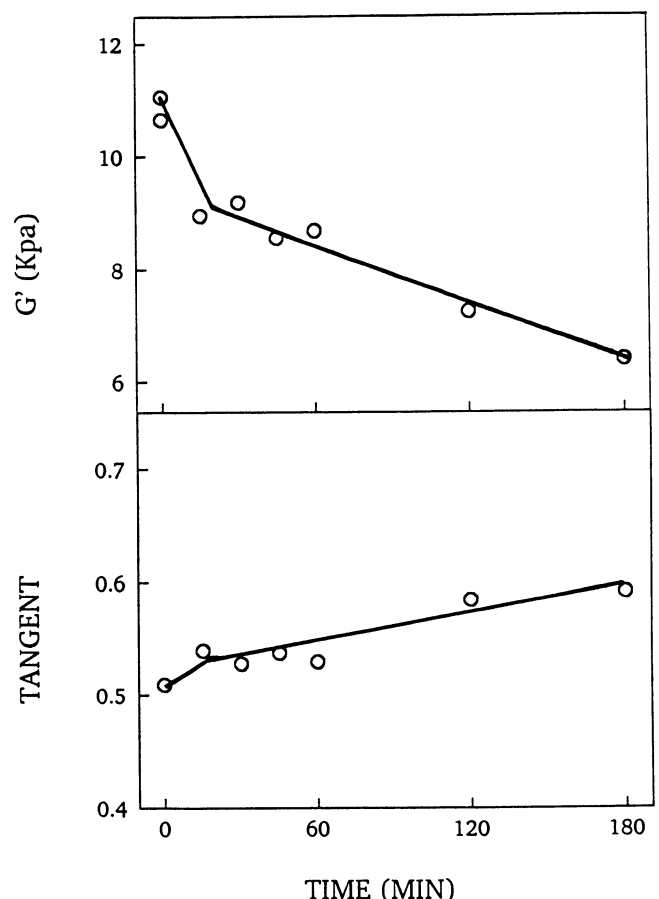


Fig. 2. Dough rheological changes during resting time. Flour-water dough (O) tested immediately after mixing or after resting in a bowl covered with a plastic plate at ambient temperature (23°C) for various times.

a Ross Mill until they passed a 10XX sieve. The water solubles and insolubles were blended and rehydrated by holding at 85% rh until the flour reached about 14% moisture. Mixing time and water absorption were determined with a mixograph.

All chemicals used in the study were reagent grade. Potassium bromate was from Baker & Adamson; L-threoascorbic acid from Fisher Scientific; and glutathione and D-erythroascorbic acid from Eastman Kodak. Also obtained from Eastman Chemical Products was Tenox-4, a food-grade antioxidant containing 20% butylated hydroxyanisole (BHA), 20% butylated hydroxytoluene (BHT), and 60% corn oil. Solutions of each chemical were prepared fresh daily. For the interaction studies, the two solutions to be studied were added to the water just before mixing.

The rheometer used was as described previously (Faubion et al 1985, Dreese 1988a). Dynamic measurements were taken at an oscillation frequency of 5 Hz and 1% strain. Dough was prepared with optimum mixing time and absorption except where noted. The doughs were tested immediately after mixing or after resting in a bowl covered with a plastic plate at ambient temperature (23°C) for 60 min, 120 min, and 180 min. The results are presented as plots of storage moduli G' and loss tangent versus storage time. Data were evaluated using the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Effect of Resting Time

When a flour-water dough was rested at room temperature for 180 min, it became softer. G' decreased, while the loss tangent increased (Fig. 2). During resting, a number of factors may affect dough rheology: relaxation of the stresses introduced during mixing, continued hydration of flour components, and redistribution of water. It is also possible that an alteration of gluten and starch may occur because of enzymatic action. Another possibility

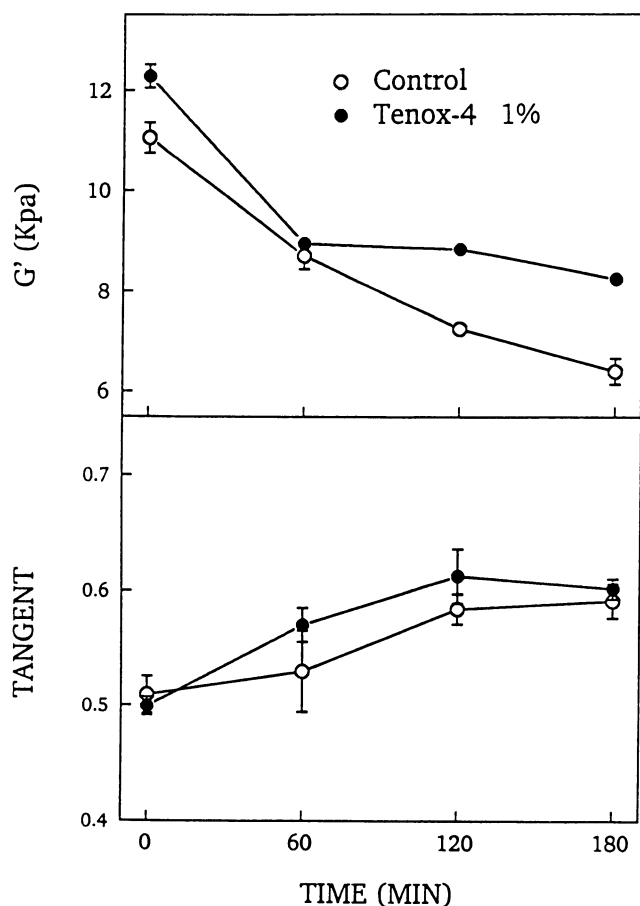


Fig. 3. Effect of antioxidant on dough rheology. Flour-water dough control (○) and flour-water dough with 1% Tenox-4 (●) based on flour weight.

is that the sulfhydryl-disulfide interchange continues during dough resting and, as a result, the average molecular weight of the gluten protein decreases.

Dough tested immediately after mixing had higher G' and smaller loss tangent than did doughs that were allowed to rest in a bowl for 15 min before testing (Fig. 2). Loading the dough in the rheometer immediately after mixing (~1 or 2 min required), did not allow sufficient time for the stresses to relax. Therefore, placing the dough between the parallel plates of the rheometer and allowing it to rest resulted in no change in the rheological properties (Dreese et al 1988b).

However, dough allowed to rest in a bowl undergoes rearrangements that release the stress. As a result, the dough becomes softer, G' becomes smaller, and the loss tangent becomes larger. When doughs were rested in a bowl at room temperature for 30 and 45 min before testing, the rate of decrease of G' was much slower than that for dough during the first 15 min of resting.

Glutathione, both reduced and oxidized, are naturally occurring in wheat flour (Kuninori and Matsumoto 1964, Hird et al 1968, Tkachuk 1969). During mixing, disulfide bonds are ruptured and create thiyl radicals (MacRitchie 1975). This free radical can then participate in and increase the rate of sulfhydryl-disulfide interchange reactions. Because the viscoelastic properties of dough are primarily related to its continuous protein phase, a decrease in the average molecular weight of gluten protein would be

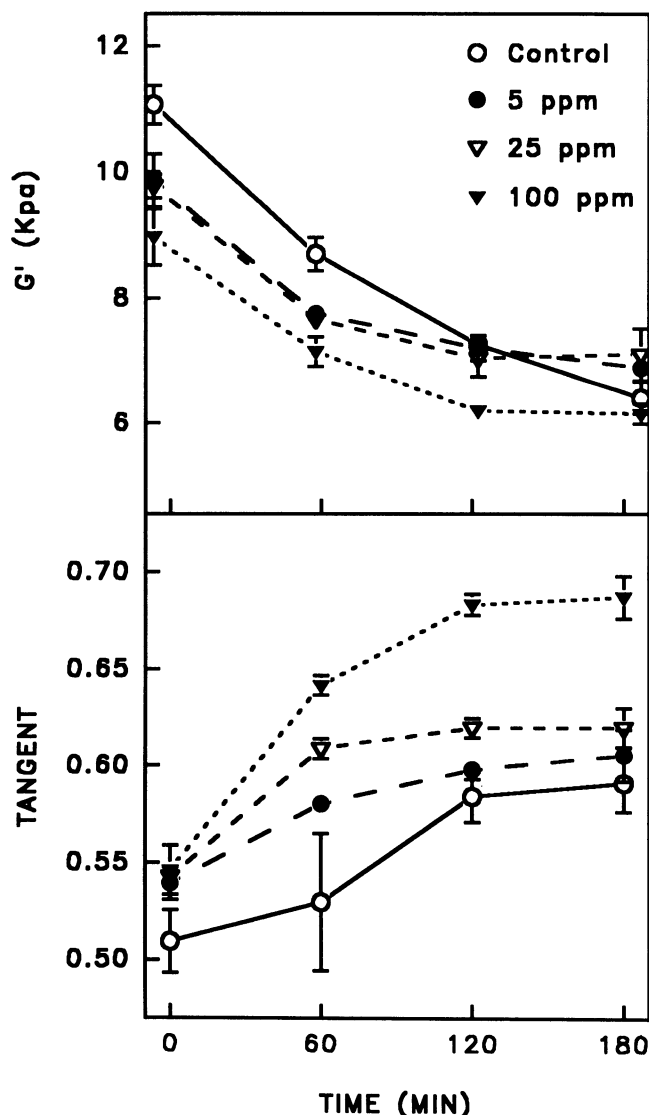


Fig. 4. Effect of glutathione on dough rheology. Flour-water dough control (○). Flour-water dough treated with 5 ppm (●), 25 ppm (△), or 100 ppm (▲) glutathione based on flour weight.

expected to cause a reduction in G' and an increase in the loss tangent.

To determine whether free radical-mediated sulfhydryl-disulfide interchange reactions were major factors causing the dough to become softer during rest, a free radical scavenger was added to the dough. By terminating the radicals, the scavenger would be expected to materially decrease the rate of interchange in the dough. The mixing time increased significantly with the addition of 1% of Tenox-4, as shown previously by Schroeder and Hosney (1978). Dough containing Tenox-4 did not decrease in G' after the first 60 min (Fig. 3). The rapid decrease in G' during the first 60 min was considered to be a stress relaxation. The effect of Tenox-4 can be explained by its action as a radical scavenger, thus stopping the sulfhydryl-disulfide interchange during resting. Increasing the Tenox-4 content to 2 and 3% only slightly increased the G' . This experiment provides evidence to support the assumption that free radicals are involved in the sulfhydryl-disulfide interchange during dough resting. These data indicate that strain relaxation occurs rapidly (<60 min) and is followed by a slow decay of G' brought about by free radical-mediated sulfhydryl-disulfide interchange reactions.

Effect of Glutathione

Glutathione is a tripeptide, with two negatively charged carboxylate groups and one positively charged amino group.

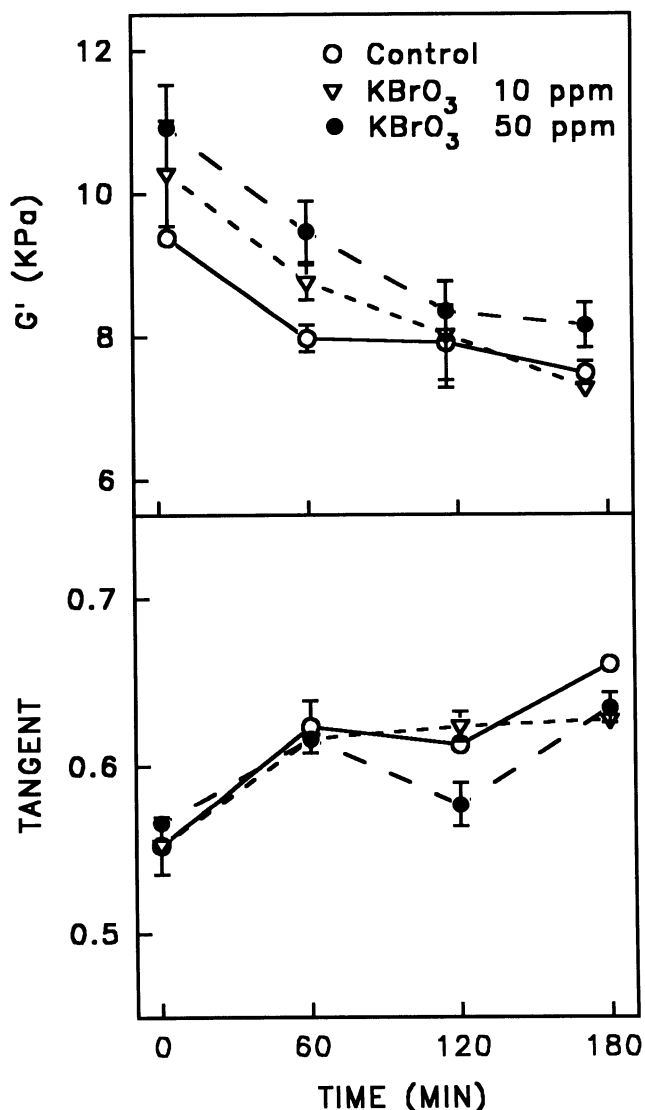


Fig. 5. Effect of potassium bromate on dough rheology. Flour-water dough control (○). Flour-water dough containing with 10 ppm (●) or 50 ppm (Δ) potassium bromate.

Obviously, these functional groups may interact with charged residues of proteins. However, the most reactive group in glutathione is the sulfhydryl of the cysteine side chain. This group can serve as a nucleophile, a reductant, and a scavenger of free radicals. Reactions involving glutathione as a reductant usually lead to formation of glutathione disulfide.

Addition of glutathione to a flour-water dough reduced G' and increased the loss tangent (Fig. 4). The doughs become relatively less elastic as the glutathione content was increased from 5 ppm to 100 ppm, as expected. According to the continuous network model of protein structure (Blokksma and Bushuk 1988), the elasticity of dough is at least partly due to disulfide crosslinks in the network of protein molecules. The storage modulus (G') would be expected to increase with increased disulfide bonds in gluten and the loss modulus (G'') to increase with increased low molecular sulfhydryl content. The presence of glutathione increases the rate of thiol-disulfide interchange reactions, which decreases the size of large proteins and results in lower molecular weight. The actual occurrence of such a reaction has been demonstrated by chemical experiments (Kuninori and Matsumoto 1963, 1964).

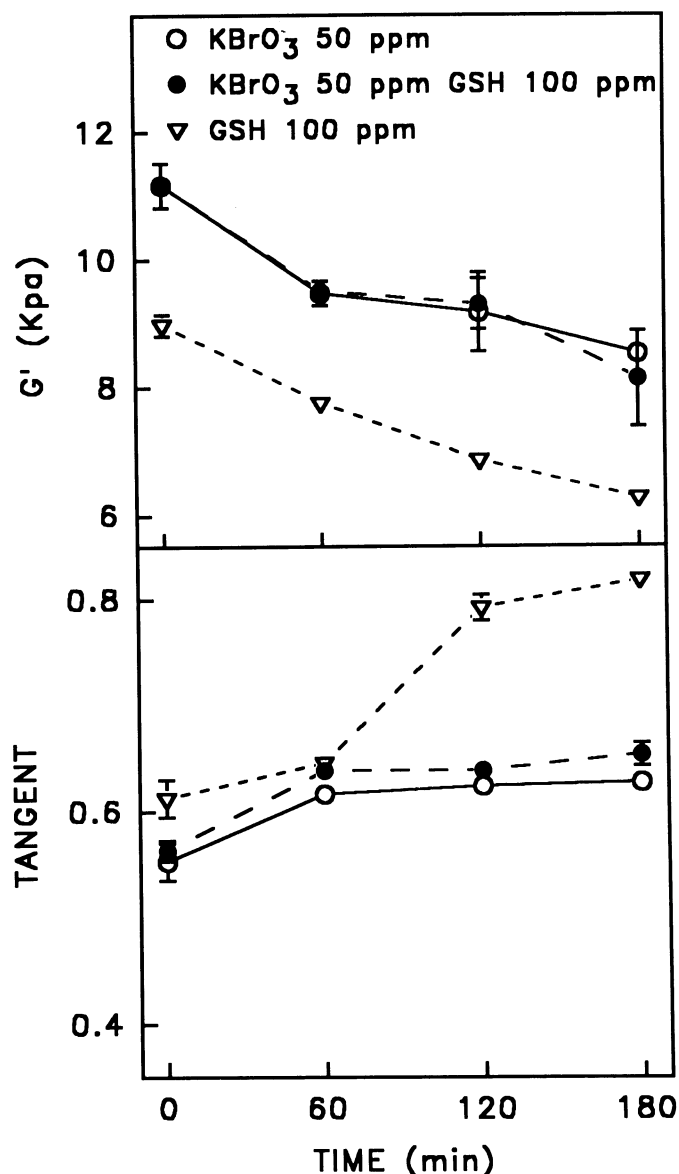


Fig. 6. Effect of potassium bromate and glutathione on dough rheology. Flour-water dough control (○) (58% abs, pH = 4.9) containing 50 ppm $KBrO_3$. Flour-water dough (58% abs, pH = 4.9) containing 50 ppm $KBrO_3$ (●). Flour-water dough (58% abs, pH = 4.9) containing 100 ppm glutathione (Δ).

During the first 60 min of resting, the decrease in G' of doughs with different glutathione contents paralleled the decrease in G' of the control dough (Fig. 4). However, the G' of doughs containing glutathione remained constant after 2 hr of resting, whereas the G' of the control flour-water dough continued to decrease. As shown above, sulfhydryl-disulfide interchange continues when the dough rests for 180 min. Low molecular weight sulfhydryl groups are more mobile than disulfide groups in protein. Therefore, added glutathione has more of a chance to react with low molecular weight sulfhydryl groups to form a stable disulfide bond (GS-SG). This can be viewed as free radical scavenging. As a result, this reduces the chance of intermolecular cross-links breaking and gives a constant G' .

Effect of Potassium Bromate

Potassium bromate has well-known effects on the rheological properties of dough. A widely accepted explanation for its action is that potassium bromate removes sulfhydryl groups by oxidizing them to disulfide bonds (Blokma and Bushuk 1988). Adding potassium bromate to flour-water dough increased G' slightly relative to the control during the first 60 min (Fig. 5). As stated previously, sulfhydryl groups naturally occur in flour. The increase of G' might be caused by a decrease of sulfhydryl groups in the dough. Increasing the concentration of potassium bromate from 10 ppm to 50 ppm did not change G' significantly. One

reason could be that 10 ppm potassium bromate was sufficient to give the maximum effect (Fig. 5).

When the dough was mixed with 50 ppm potassium bromate and 100 ppm of glutathione, and the pH adjusted to 4.9, the G' and loss tangent were about equal to those for dough mixed with 50 ppm potassium bromate alone at pH 4.9 (Fig. 6). This indicates that potassium bromate completely overcomes the effect of glutathione, presumably by oxidizing it to its disulfide.

Effect of Ascorbic Acid and its Isomers

Immediately after mixing, the G' of doughs containing L-threoascorbic acid or D-erythroascorbic acid was not changed from that of the control. With increased rest time, G' of the doughs containing the two isomers increased slowly. L-threoascorbic acid was more effective than D-erythroascorbic acid (Fig. 7). The oxidizing effect of ascorbic acid can be explained by two sets of reactions. First, the ascorbic acid was oxidized to its corresponding dehydroascorbic acid. This reaction was very fast, being complete during mixing.

In the second reaction, L-threodehydroascorbic acid oxidized the sulfhydryl groups to the corresponding disulfide. This reaction was catalyzed by the enzyme glutathione dehydrogenase (EC 1.8.5.1).

Because sulfhydryl groups were oxidized to a relatively stable disulfide, they were less active in thiol-disulfide interchange. Consequently, G' increased slowly relative to the control. This is in agreement with the improver action of ascorbic acid as shown by Mair and Grosch (1979).

Dough mixed with 30 ppm glutathione and 50 ppm L-threoascorbic acid had a slightly lower G' than the dough containing

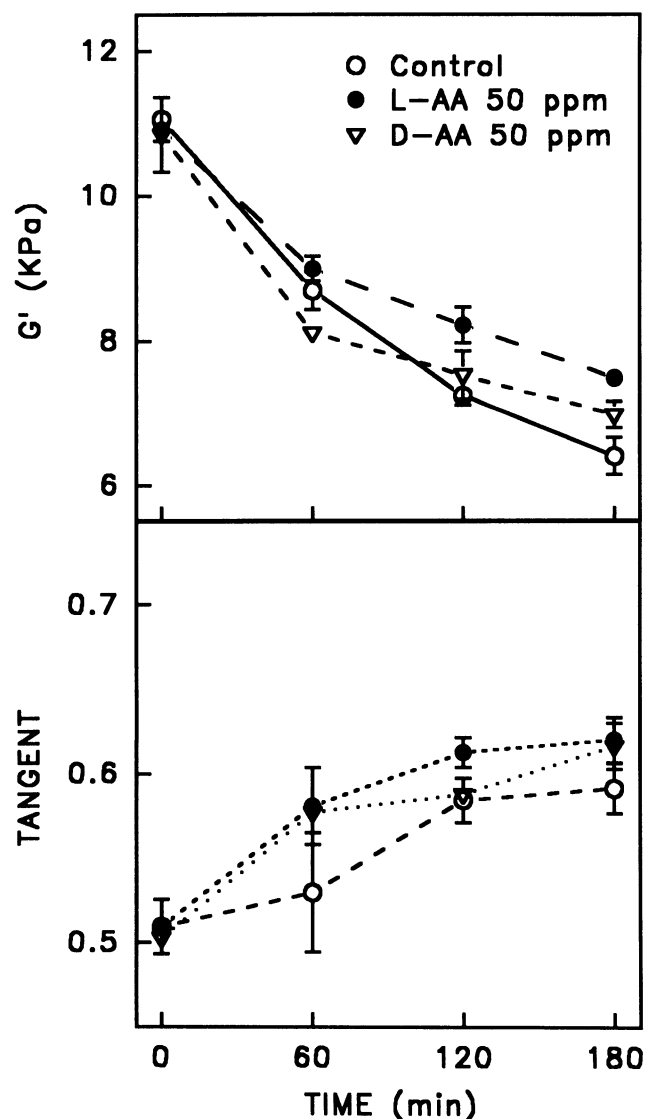


Fig. 7. Effects of ascorbic acid on dough rheology. Flour-water dough control (○). Flour-water dough treated with 50 ppm D-erythroascorbic acid (△) or 50 ppm L-threoascorbic acid (●).

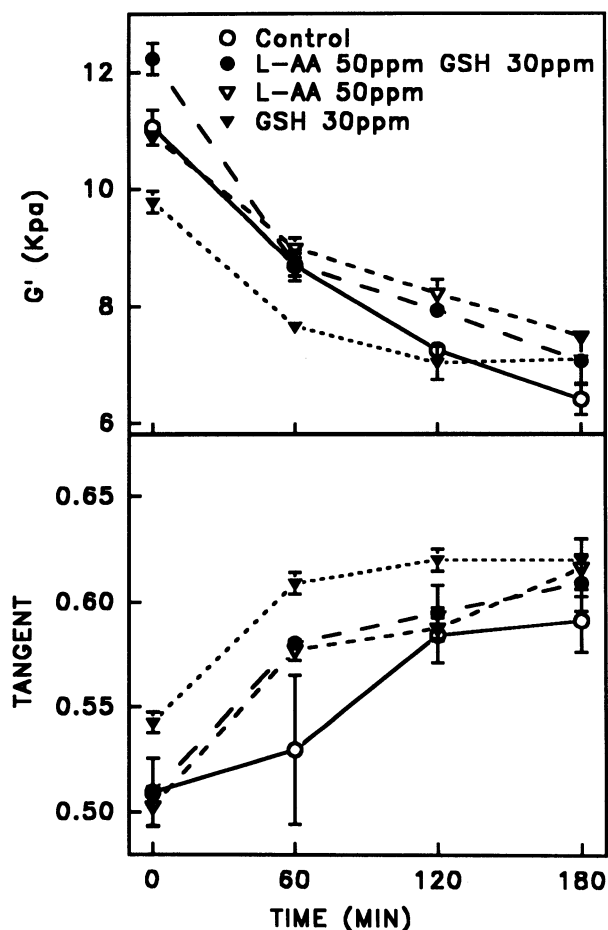


Fig. 8. Effects of L-threoascorbic acid and glutathione on dough rheology. Flour-water dough control (○). Flour-water dough treated with 50 ppm L-threoascorbic acid and 30 ppm glutathione based on the flour weight (●). Flour-water dough treated with 50 ppm L-threoascorbic acid (△) or 30 ppm glutathione (▲).

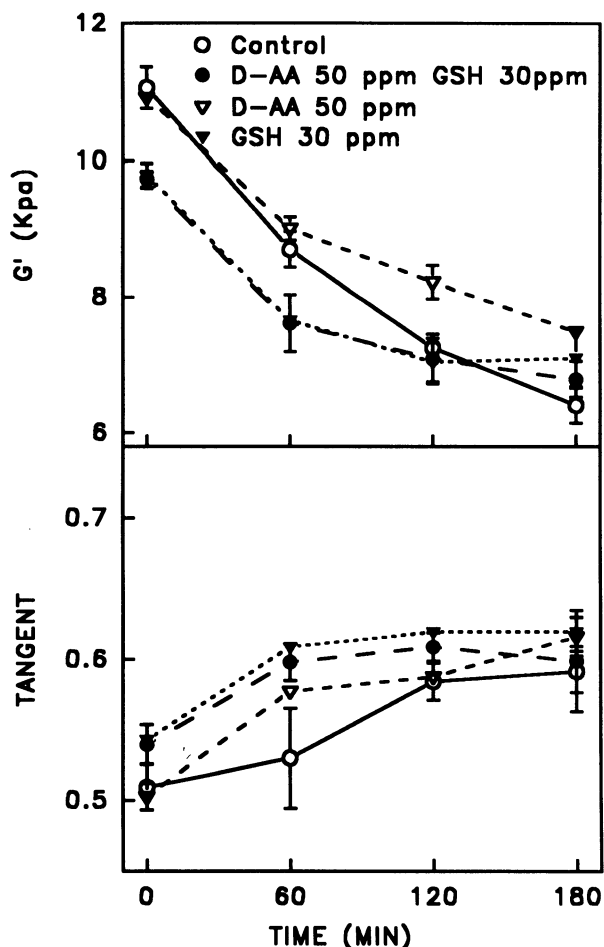


Fig. 9. Effects of D-erythroascorbic acid and glutathione on dough rheology. Flour-water dough control (○). Flour-water dough treated with 50 ppm D-erythroascorbic acid and 30 ppm glutathione (●). Flour-water dough treated with 50 ppm D-erythroascorbic acid (△) or 30 ppm glutathione (▲).

only 50 ppm L-threoascorbic acid (Fig. 8). This indicated that the L-threoascorbic acid eliminated the effect of glutathione.

Dough mixed with 30 ppm glutathione and 50 ppm D-erythroascorbic acid, had essentially the same G' as dough containing only glutathione (Fig. 9). This indicated that D-erythroascorbic acid was not used by glutathione dehydrogenase. In a similar experiment, D-threodehydroascorbic acid did not oxidize glutathione (data not shown).

These rheological differences showed that L-threoascorbic acid is a much stronger improver in a dough than D-threoascorbic acid. Of course this was already known from baking experiments (Walther and Grosch 1987). This specificity suggests that the enzyme glutathione dehydrogenase catalyzes the oxidation of glutathione to the corresponding disulfide with L-threodehydroascorbic acid as the oxidant, but not with D-threodehydroascorbic acid as an oxidant.

If the above is true, removal of the enzyme glutathione dehydrogenase should result in L-threodehydroascorbic acid being less active as an improver. Because the enzyme is assumed to be present in the water-soluble part of flour, it would be inactivated after that fraction was boiled for 15 min. Therefore, the reconstituted flour should be enzyme-free.

When reconstituted flour dough was mixed with 50 ppm glutathione and 100 ppm L-threoascorbic acid or mixed with 50 ppm glutathione and 100 ppm D-erythroascorbic acid, no significant differences occurred in G' or loss tangent between the two treatments. The G' of these two treatments were essentially the same as that for dough containing only glutathione (Fig. 10). This shows that the reaction between sulfhydryl groups and

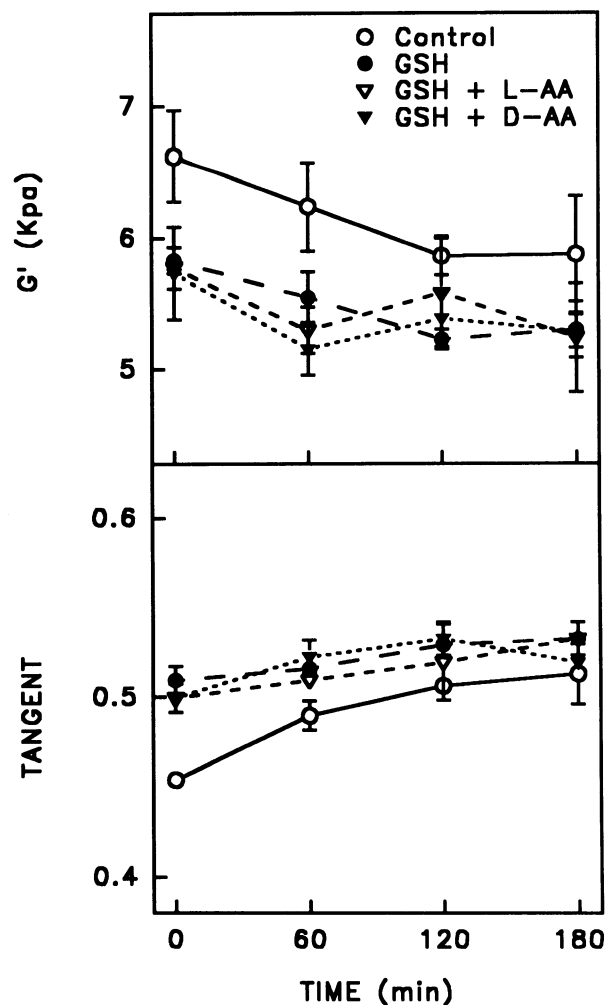


Fig. 10. Effects of D-erythroascorbic acid, L-threoascorbic acid, and glutathione on reconstituted flour-water dough (○). Reconstituted flour-water dough treated with 30 ppm glutathione (●). Reconstituted flour-water dough treated with 50 ppm L-threoascorbic acid and 30 ppm glutathione (△). Reconstituted flour-water dough treated with 50 ppm D-erythroascorbic acid and 30 ppm glutathione (▲).

dehydro-L-threoascorbic acid was catalyzed by an enzyme, presumably glutathione dehydrogenase.

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