

# Glucose Oxidase in Breadmaking Systems<sup>1</sup>

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## ABSTRACT

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The mechanism of glucose oxidase action in breadmaking was investigated by studying the baking performance of glucose oxidase, the active ingredient that it produced, and its effect on the rheological properties of dough. Glucose oxidase improved the loaf volume of bread made by 45-, 70-, and 90-min fermentation processes. Although the increase in loaf volume was significant, it was less than that obtained with an optimum level of  $\text{KBrO}_3$ . With the 90-min fermentation process, the crumb grain of bread was

similar for loaves oxidized with optimum levels of glucose oxidase or  $\text{KBrO}_3$ . The rheological properties of doughs containing glucose oxidase and doughs containing no oxidant were compared. Doughs made with glucose oxidase had higher  $G'$  and  $G''$  and lower  $\tan \delta$  values than doughs made without an oxidant. Hydrogen peroxide was responsible for a drying effect in doughs. This drying effect of glucose oxidase was reduced significantly by incorporation of free radical scavengers into the dough.

Calcium peroxide has been used for many years as an effective oxidant for production of breads in the United States. The  $\text{H}_2\text{O}_2$  produced by  $\text{CaO}_2$  is presumed to be the active ingredient. A disadvantage of  $\text{CaO}_2$  is its limited solubility in water. Therefore, in commercial use  $\text{CaO}_2$  is always dry blended with flour. Glucose oxidase, an enzyme found in a number of fungal sources, also produces  $\text{H}_2\text{O}_2$ . The enzyme acts on  $\beta$ -D-glucose and, in the presence of  $\text{O}_2$ , produces D-gluconic acid and  $\text{H}_2\text{O}_2$ . Glucose oxidase complies with FAO/WHO and GRAS requirements for food-grade enzymes. In addition, it is readily soluble in water and has been reported to have stability for at least one year when stored at 2–4°C. Therefore, glucose oxidase could be used as an alternative for  $\text{CaO}_2$  by the baking industry.

Potassium bromate, another chemical oxidant, has been one of the most common bread improvers in North America. Now the use of  $\text{KBrO}_3$  faces an uncertain future because of questions concerning safety (Kurokawa et al 1983). The baking industry is faced with a major challenge of finding a replacement for  $\text{KBrO}_3$  and glucose oxidase could be a candidate.

The use of glucose oxidase in combination with other enzymes and surfactants for the production of bread has been reported (Haarasilta and Vaeisaenen 1989, Haarasilta et al 1989, Nakai et al 1995). The mechanism by which glucose oxidase improves bread is not fully understood. Therefore, the objectives of this research were to determine: 1) baking performance of glucose oxidase in breads, 2) its active ingredient, and 3) its effect on the rheological properties of dough.

## MATERIALS AND METHODS

Commercial flours (A and B) were obtained from Cargill (Wichita, KS). A sample a hard red winter wheat cultivar, Larned, was obtained from a seed producer. The wheat was tempered for 18 hr to 15.5% moisture and milled into a straight-grade flour using a Buhler experimental mill. The composition and properties of the flours are given in Table I. Glucose oxidase (17,165 glucose oxidase units [GU]/g) was supplied by Novo Nordisk A/S (Bagsvaerd, Denmark). One GU oxidizes 1.0  $\mu\text{mole}$  of  $\beta$ -D-glucose to gluconic acid and  $\text{H}_2\text{O}_2$  per minute at pH 5.1 at 35°C. The  $\text{CaO}_2$  (marketed under the trade name CM paniplus 240) was obtained

from ADM Company (Olathe, KS). The  $\text{KBrO}_3$  was obtained from Fisher Scientific (Fair Lawn, NJ). Tenox 4, a food-grade antioxidant containing 20% butylated hydroxytoluene (BHT) and 20% butylated hydroxyanisole (BHA) dispersed in 60% corn oil, was obtained from Eastman Chemical Products Inc. (Kingsport, TN). Fleischmann's instant yeast (Fenton, MO) that contained no ascorbic acid was used.

## Baking

The oxidants or enzymes were added to the baking formula during the mixing stage. Breads were made with commercial bread flour A and Larned flour by the pup loaf formula using a 90-min fermentation, straight-dough process (AACC 1995). Bread was also produced by short-time (70- and 45-min) fermentation, straight-dough processes (Finney et al 1976) using Larned flour. For the short-time baking systems, the only change made in the formula was the level of yeast. Amounts used were 2.0 g of yeast/100 g of flour for 90 min, 2.4 g of yeast for 70 min, and 4.0 g of yeast for 45 min. In the 45-min fermentation system, doughs were sheeted, molded, and panned after fermentation. The doughs were proofed for a constant time (27 min) to attain a proof height of 7.5 cm and then baked. In the 70-min fermentation system, doughs were sheeted (punched) after 40 and 60 min, then molded and panned after 70 min. The doughs were proofed for a constant time (45 min) to attain a proof height of 7.5 cm and then baked. The loaf volume of bread was measured using rapeseed displacement.

Loaf volumes of all the breads were determined, and the internal and external characteristics evaluated subjectively. The crumb grain of optimally oxidized bread has a large number of elongated cells with a distinct slip plane (an area where the cells slide past each other as the dough expands). In bread that is underoxidized, the crumb grain has many small round cells with thin cell walls, no distinct slip plane, and the loaf has sharp corners and a pitted bottom. An overoxidized crumb grain of bread has large round cells with thick cell walls, a very prominent slip plane, often containing large open cells, and the loaf has round corners and a deep crease on the bottom.

## Dynamic Frequency Sweep

The optimum water absorption and mixing time for commercial flour B was estimated with a 10-g mixogram (AACC 1995). Flour

TABLE I  
Composition and Properties of Flours

Flour	Moisture (%)	Protein (%)	Absorption <sup>a</sup> (%)	Mixing Time <sup>a</sup> (min)
Commercial A	13.0	10.8	69	4.5
Commercial B	11.7	11.1	69	4.0
Larned	11.1	10.0	73	3.0

<sup>a</sup> Bread dough made by a 90-min fermentation bake formula.

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and water were mixed to optimum development using a pin mixer (National Mfg. Co., Lincoln, NE). Glucose oxidase (20 GU), CaO<sub>2</sub> (15, 60, and 120 ppm), and Tenox (4.0%) were added to some doughs. The control dough contained no additives. A sub-sample of dough was placed in a lightly greased container. The dough was covered and allowed to rest for 30 min at room temperature before testing. Dynamic rheological analysis was performed with a Rheometrics RDS 7700 using a cone and plate fixture at 1% strain. The cone has an angle of 0.1 radians. After placing the dough between the cone and the plate, the minimum gap was adjusted to 0.05 mm. The excess dough was trimmed with a knife, and the edges were coated with automotive lubricant (Blue Guard 500+, Farmland Industries, Kansas City, MO) to reduce water loss from the dough. After loading, the dough was allowed to rest for 5 min before testing. All measurements were made at room temperature.

### Spread Test

The straight-dough formula with 100 g of commercial flour B (14% moisture basis), 1.5 g of salt, 6.0 g of sugar, 4.0 g of nonfat dry milk, 3.0 g of shortening, 2.0 g of yeast, and optimum water (69%) was used for the spread test. Glucose oxidase (20 GU), corn oil (2.4%), or Tenox (4.0%) were added to some doughs. The control dough had no additives. Doughs were mixed to optimum development using a National pin mixer and allowed 90 min of fermentation with sheeting after 52 and 25 min and molding after 13 min. The dough after molding was allowed to rest on a smooth plate in a fermentation cabinet (30°C, 90% rh) for 33 min. The width and height of the doughs were measured with Mitutoyo dial calipers after 0 and 90 min of fermentation. The spread ratio was calculated as width over height, with higher values indicating more spread (Hoseney et al 1979).

### Statistical Analysis

All experiments were at least duplicated. Analysis of variance (ANOVA) was performed using SAS procedures (SAS Institute, Cary, NC). Duncan grouping was applied to compare the means.

## RESULTS AND DISCUSSION

To compare the performance of various additives, the bread-baking systems must be optimized in terms of absorption, mixing time, and concentration of additives. Breads containing glucose oxidase were compared with those containing KBrO<sub>3</sub> under optimized conditions.

TABLE II  
Optimization of the Level of Glucose Oxidase in Breads

Treatment <sup>a</sup>	Loaf Volume (cm <sup>3</sup> )	Crumb Grain
90-min fermentation (Flour A)		
No oxidant	887a <sup>b</sup>	Underoxidized
2.5 GU	880a	Optimum oxidation
10 ppm of KBrO <sub>3</sub>	912a	Optimum oxidation
90-min fermentation (Larned)		
No oxidant	723c	Underoxidized
5.5 GU <sup>b</sup>	762b	Optimum oxidation
30 ppm of KBrO <sub>3</sub>	792a	Optimum oxidation
70-min fermentation (Larned)		
No oxidant	727c	Underoxidized
20 GU <sup>b</sup>	780b	Underoxidized
60 ppm of KBrO <sub>3</sub>	905a	Optimum oxidation
45-min fermentation (Larned)		
No oxidant	710c	Underoxidized
45 GU	777b	Underoxidized
80 ppm of KBrO <sub>3</sub>	910a	Optimum oxidation

<sup>a</sup> GU = glucose oxidase units.

<sup>b</sup> Values followed by the same letter in the same column are significantly different ( $P < 0.05$ ).

### Bread Produced by a 90-min Fermentation Process

The optimum levels of glucose oxidase and KBrO<sub>3</sub> for bread made with commercial flour A were 2.5 GU and 10 ppm, respectively (Table II). Breads made with <2.5 GU had an underoxidized crumb grain, and those with >2.5 GU had overoxidized crumb grain. The loaf volumes of breads without an oxidant and with oxidants (2.5 GU or 10 ppm of KBrO<sub>3</sub>) were not significantly different. However, significant differences were observed in the crumb grain. The crumb grain of breads made without an oxidant had fewer elongated cells and many round cells with thin cell walls, whereas the crumb grain of breads made with glucose oxidase or KBrO<sub>3</sub> had many elongated cells and fewer round cells with thin cell walls. Higher levels of glucose oxidase (5.5 GU) and KBrO<sub>3</sub> (30 ppm) were required to produce optimum bread using Larned flour (Table II). Presumably this was because Larned is a weak flour and, therefore, required higher oxidant levels than the relatively strong commercial bread flour. In comparison to bread made without an oxidant, addition of optimum levels of glucose oxidase or KBrO<sub>3</sub> increased the loaf volumes by ≈40 cm<sup>3</sup> and 70 cm<sup>3</sup>, respectively. Bread made with glucose oxidase had relatively lower loaf volumes than breads made with KBrO<sub>3</sub>. However, the crumb grains of the breads were similar and were improved significantly by addition of either oxidant. In general, all breads made with Larned flour and glucose oxidase had higher volumes and improved crumb grain characteristics when compared to the no additive control. Thus, glucose oxidase was found to be an effective oxidant in breads made by a 90-min fermentation process.

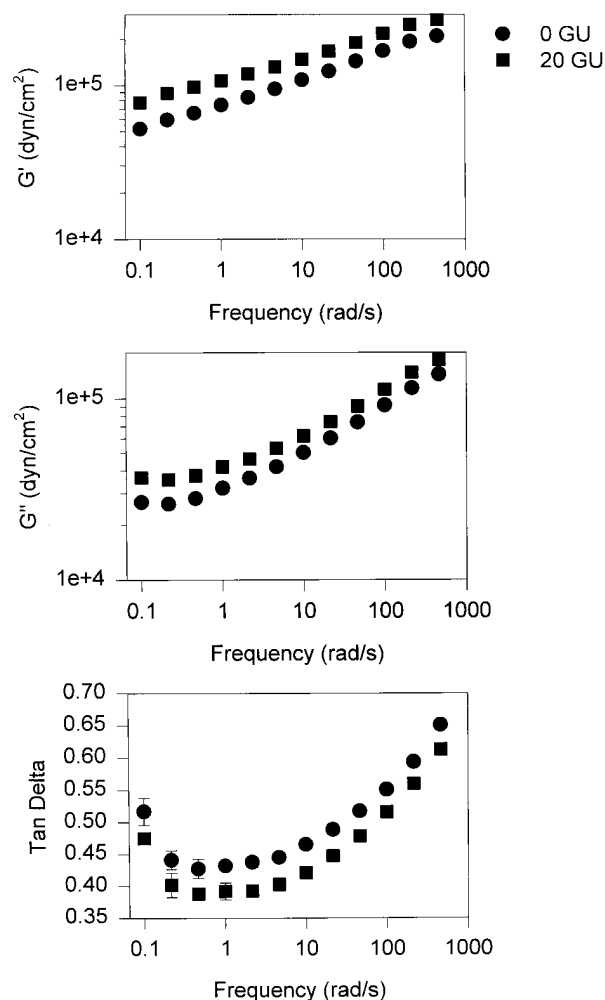


Fig. 1. Effect of glucose oxidase (20 GU) on viscoelastic properties of doughs made with commercial flour B at 1% strain as measured with a dynamic rheometer using a cone and plate. If error bars are not visible, they are smaller than the symbol.

## Bread Produced by Short-Time (70- and 45-Min) Fermentation Processes

The optimum level of  $\text{KBrO}_3$  for bread made by a 70-min fermentation process and Larned flour was 60 ppm. In comparison to bread made without an oxidant, breads made with  $\text{KBrO}_3$  had significantly higher volume and improved crumb grain. However, glucose oxidase was not as effective as  $\text{KBrO}_3$  in improving either the loaf volume or crumb grain of the breads (Table II). At its optimal level of 20 GU, glucose oxidase significantly increased the loaf volume when compared to the no-oxidant control. Doughs made with glucose oxidase felt very dry and strong. Because of their dryness, they were easy to handle during sheeting and molding operations. However, these doughs did not give sufficient oven spring. Therefore, the resultant bread had lower volume and a poor break and shred. Also, the bread crumb grain had many round cells with thin cell walls and essentially no elongated cells, characteristic of an underoxidized loaf.

The  $\text{H}_2\text{O}_2$  produced by glucose oxidase may have been responsible for the strong and dry doughs. The  $\text{H}_2\text{O}_2$  together with peroxidase (native to flour) is presumed to cause the water-soluble pentosans in the dough to gel. Hydrogen peroxide and peroxidase have been known to cause oxidative gelation of water-soluble pentosans (Hoseney and Faubion 1981, Crowe and Rasper 1988, Izydorczyk et al 1990). The gelation limits water mobility, giving the drier dough. This also may change dough rheology. Doughs containing glucose oxidase were presumably too strong and, therefore, did not give oven spring.

Bread made with a short-time fermentation (45-min) system and containing optimum  $\text{KBrO}_3$  (80 ppm) had many elongated cells in the crumb grain, good break and shred, and higher volume than breads made with the same system but no oxidant (Table II). On the other hand, bread made with the same procedure but with glucose oxidase had many round cells with thin cell walls and essentially no elongated cells in the crumb grain. At its optimum level (45 GU), glucose oxidase significantly increased the loaf volume when compared to the no-oxidant control. However, the volume was much lower than that produced with the optimum level of  $\text{KBrO}_3$ . The doughs made by the 45-min fermentation system and those containing glucose oxidase felt dry and had strong handling properties.

Glucose oxidase appears to work by a different mechanism than  $\text{KBrO}_3$ . Glucose oxidase caused both drying and strengthening effects when it was added to doughs. Doughs containing  $\text{KBrO}_3$  showed a strengthening effect but no drying effect. With short-time fermentation systems, the level of glucose oxidase necessary to obtain the optimum strengthening apparently resulted in the dough becoming much too dry. This resulted in doughs that appeared to be overoxidized.

## Drying Effect of Glucose Oxidase

The rheological properties of doughs containing glucose oxidase (20 GU) and doughs containing no oxidant (control) were compared at constant absorption. Both an elastic modulus ( $G'$ ) and viscous modulus ( $G''$ ) values for doughs made with glucose oxidase were significantly higher than similar values for control doughs (Fig. 1). More force was needed to deform doughs containing glucose oxidase. In other words, glucose oxidase significantly increased the complex modulus ( $G^*$ ) of doughs. Also, the  $\tan \delta$  values ( $G''/G'$ ) for doughs made with glucose oxidase were significantly lower than those for control doughs. Thus, doughs containing glucose oxidase had relatively more elastic properties relative to its viscous properties than doughs made without an oxidant.

The rheological properties of doughs made with various levels of  $\text{CaO}_2$  (15, 60, and 120 ppm) also were determined. All doughs made with  $\text{CaO}_2$  had higher  $G'$  and  $G''$  values and lower  $\tan \delta$  values than control doughs (without  $\text{CaO}_2$ ) (Fig. 2). The rheological properties of doughs made with  $\text{CaO}_2$  were similar to those of doughs made with glucose oxidase. This experiment further confirmed that  $\text{H}_2\text{O}_2$  produced by glucose oxidase or  $\text{CaO}_2$  was responsible for

the drying effect on doughs. The drying effect of  $\text{CaO}_2$  in bread doughs has been reported by several researchers (Potavina and Kasatkina 1972, Dubois and Ash 1974, Tieckelmann and Steele 1991). These results further agree with our earlier conclusions from baking studies that  $\text{H}_2\text{O}_2$  is the active ingredient responsible for the oxidative and drying effects of glucose oxidase.

## Formation of Gel by Glucose Oxidase

We presumed that the  $\text{H}_2\text{O}_2$  produced by glucose oxidase together with peroxidase causes the flour water-soluble pentosans to gel. The water-soluble pentosans have the ability to form hydrated networks or gels after treatment with oxidants capable of producing free radicals. The  $\text{H}_2\text{O}_2$  by itself does not generate a radical, but in the presence of the enzyme peroxidase it does (Yamazaki 1977). Gel formation is presumably the cause for the drying effect of glucose oxidase. The spread test compared doughs containing glucose oxidase with doughs containing both glucose oxidase and free radical scavengers (BHA and BHT). We assumed that the free radical scavengers would prevent formation of gel.

Doughs without any fermentation had a larger spread ratio than fermented doughs (Table III); the spread was reduced significantly with fermentation. Yeast influences the rheological properties of dough during fermentation (Hoseney et al 1979). Doughs made with glucose oxidase (20 GU) had significantly more elastic properties (lower spread ratio) than doughs made without any oxidant. Glucose oxidase is a fast-acting oxidant. It reduced the spread ratio immediately after mixing. Tenox contains 20% BHA and 20% BHT (active ingredients) and 60% corn oil (inert ingredients). Doughs made with corn oil (2.4%) and glucose oxidase (20 GU) had spread ratios

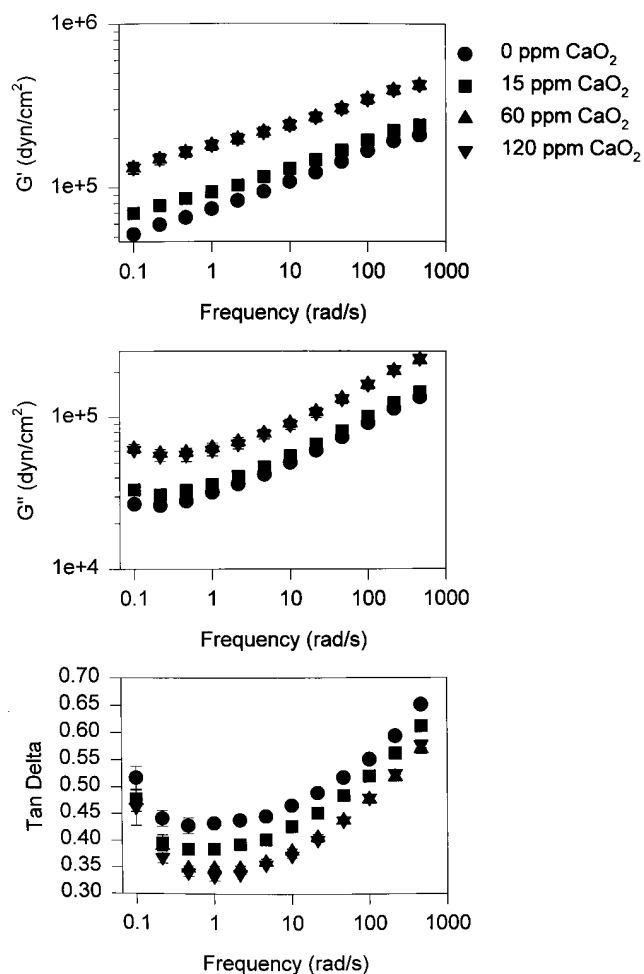


Fig. 2. Effect of  $\text{CaO}_2$  on viscoelastic properties of doughs made with commercial flour B at 1% strain as measured with a dynamic rheometer using a cone and plate. If error bars are not visible, they are smaller than the symbol.

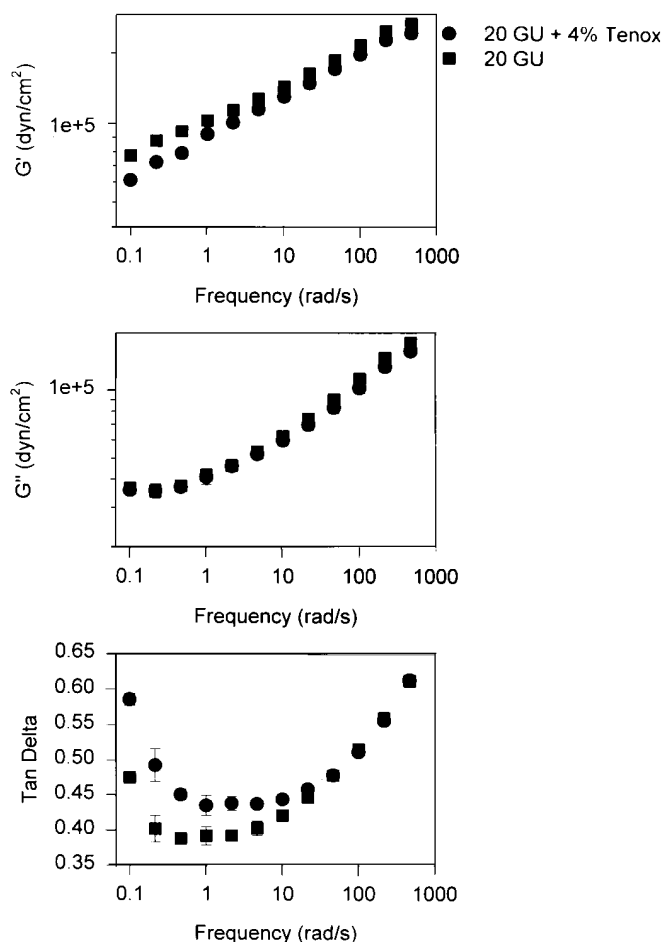
**TABLE III**  
Effect of Glucose Oxidase and Tenox on Spread Ratio<sup>a</sup>  
of Doughs Made with Commercial Flour B

Treatment <sup>b</sup>	Spread Ratio	
	0 min	90 min
No oxidant	2.87a <sup>c</sup>	1.89a
20 GU	2.06b	1.61b
20 GU + 2.4% corn oil	2.10b	1.64b
20 GU + 4.0% Tenox	2.10b	1.87a

<sup>a</sup> Spread ratio was calculated as width over height (with higher values indicating more spread) after 0 and 90 min of fermentation.

<sup>b</sup> GU = glucose oxidase units.

<sup>c</sup> Values followed by the same letter in the same column are significantly different ( $P < 0.05$ ).



**Fig. 3.** Effect of glucose oxidase (20 GU) and Tenox (4.0%) on viscoelastic properties of commercial flour B and water doughs at 1% strain as measured with a dynamic rheometer using a cone and plate. If error bars are not visible, they are smaller than the symbol.

similar to those of doughs made with only glucose oxidase (20 GU). Thus, the corn oil in Tenox had no effect on the spread ratio of doughs. Tenox (4.0%) did not reverse the oxidative effect of glucose oxidase in unfermented doughs (0 min). However, after a 90-min fermentation, Tenox reversed the oxidative and drying effects of glucose oxidase. The doughs containing glucose oxidase (20 GU) and 4.0% Tenox had spread ratios similar to those of doughs without any oxidant.

The rheological properties were compared for doughs made with glucose oxidase (20 GU) and 4.0% Tenox versus doughs made with only 20 GU (control). At low frequencies, doughs made with glucose oxidase and Tenox had significantly lower  $G'$  values than doughs made with only glucose oxidase. The  $G''$  of doughs con-

taining glucose oxidase was similar to that of doughs containing both glucose oxidase and Tenox. Also at low frequencies, the  $\tan \delta$  values were affected significantly by the addition of Tenox. It decreased the elastic properties of the doughs relative to viscous properties (Fig. 3). In other words, Tenox reduced the drying and stiffening effects of glucose oxidase and made the doughs relatively softer. Presumably the mechanism by which Tenox softened the doughs was by preventing the formation of a gel.

## CONCLUSIONS

Glucose oxidase was found to be an effective oxidant in bread. Although it significantly improved loaf volume, it was lower than the loaf volume of bread made with  $KBrO_3$ . However, with the 90-min fermentation process, crumb grains with the two oxidants were comparable. With shorter fermentation processes (45 or 70 min), glucose oxidase still significantly improved loaf volume but was much less effective at improving the crumb grain. A suggested mechanism of glucose oxidase action is through its production of  $H_2O_2$ . Doughs made with glucose oxidase or  $CaO_2$  had higher  $G'$  and  $G''$  values and lower  $\tan \delta$  values. The rheological properties of doughs made with glucose oxidase and with  $CaO_2$  were similar. These results suggest that  $H_2O_2$  produced by glucose oxidase or  $CaO_2$  was responsible for the drying effect on doughs. The free radical scavengers (BHA and BHT) in Tenox reversed the drying effect of glucose oxidase and made the doughs relatively softer. Doughs containing both Tenox and glucose oxidase had spread ratios similar to those of doughs without any oxidant.

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