

# Role of Hydrogen Peroxide Produced by Baker's Yeast on Dough Rheology<sup>1</sup>

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## ABSTRACT

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Baker's yeast, *Saccharomyces cerevisiae*, has a well-known effect on dough rheology during breadmaking. During a 3-hr fermentation, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) produced by yeast (0.76%, fwb) increased from 1.09 to 2.32 μmol/g of flour. The spread test, a measure of a dough's rheological properties, showed that yeast had an effect on dough rheology similar to that of H<sub>2</sub>O<sub>2</sub>, an oxidant that makes flour-water dough more elastic. In additional experiments (spread test and H<sub>2</sub>O<sub>2</sub> measurement), glucose oxidase, an enzyme that produces H<sub>2</sub>O<sub>2</sub>, gave results similar to those with yeast.

The fact that catalase, an enzyme that destroys H<sub>2</sub>O<sub>2</sub>, reversed the rheological effect of added H<sub>2</sub>O<sub>2</sub> but did not reverse the effect of either yeast or glucose oxidase suggested that either wheat flour contains an inhibitor to catalase or H<sub>2</sub>O<sub>2</sub> was not the active component. A series of experiments, including use of defatted flour, remixing, and mixing dough under nitrogen, all indicated that catalase was inhibited by peroxides in the lipid fraction of flour. These results also suggested that H<sub>2</sub>O<sub>2</sub> is responsible for the effects of yeast and glucose oxidase on dough.

Fermentation is one of the most important processes in breadmaking. In addition to the production of carbon dioxide, fermentation changes the rheology of the dough dramatically. The dough becomes drier, more elastic, and less viscous. Hosney et al (1979) reported that yeast was the major ingredient responsible for the rheological changes in dough during fermentation. Hosney et al (1979) and Nagao et al (1981) showed that yeast had an oxidizing effect on dough similar to that of chemical oxidants. Several researchers (Hosney et al 1979, Kivett 1985, Manning et al 1988) found that fermentation time was required for yeast to have its oxidizing effect. Furthermore, Kivett (1985) reported that the water-soluble fraction of flour was necessary for yeast to exert its rheological effect. However, the mechanism by which yeast oxidizes the dough was not established clearly.

All biological systems generate H<sub>2</sub>O<sub>2</sub> from oxygen during growth (Izydorczyk et al 1990). Boveris (1978) found that yeast mitochondria generated H<sub>2</sub>O<sub>2</sub> and that yeast cytochrome c peroxidase utilized 53–55% of the H<sub>2</sub>O<sub>2</sub> produced. The excess H<sub>2</sub>O<sub>2</sub> diffused through the cell membrane and into the surrounding medium. He also reported that ethanol increased the rate of H<sub>2</sub>O<sub>2</sub> diffusion and thereby enhanced the amount of H<sub>2</sub>O<sub>2</sub> in the medium.

Addition of certain oxidants such as H<sub>2</sub>O<sub>2</sub>, potassium bromate, ascorbic acid, and azodicarbonamide to wheat flour affects dough rheology. Those oxidants, when used at optimum levels, produce a more elastic dough and improve bread quality (larger loaf volume and better crumb grain).

The objective of this study was to determine whether H<sub>2</sub>O<sub>2</sub> was produced and accumulated in a yeasted dough system and to determine whether H<sub>2</sub>O<sub>2</sub> is the agent by which yeast has its oxidizing effect on wheat flour dough.

## MATERIALS AND METHODS

### Materials

The flour was a commercial bread flour donated by Cargill, Inc., Wichita, KS. It contained 10.5% protein (N × 5.7) and 0.45% ash (both on a 14% moisture basis) and 13.2% moisture. Instant active dry yeast (Fleischmann's Yeast Inc., Fenton, MO) was used. Catalase

(EC 1.11.1.6, bovine liver) was obtained from Sigma Chemical Co. (St. Louis, MO). One unit of catalase decomposes 1.0 μmol of H<sub>2</sub>O<sub>2</sub> per minute at pH 7.0 at 25°C. Glucose oxidase (EC 1.1.3.4) was provided by Novo Nordisk A/S (Novo Alle, Denmark). One unit of glucose oxidase oxidizes 1.0 μmol of β-D-glucose to gluconic acid and H<sub>2</sub>O<sub>2</sub> per minute at pH 5.1 at 35°C. All chemicals were at least reagent-grade.

### Extraction of Water Solubles

Flour-water doughs (made from 100 g of flour) containing 0.76% (fwb) yeast were mixed to optimum (determined subjectively by an experienced baker) in pin mixers. Doughs were fermented at 30°C and 86% rh for 0, 60, 120, and 180 min. The doughs then were dispersed (1:5 or 1:10 flour-to-water ratio) in a blender (Osterizer) at low speed for 5 min. The slurry was centrifuged at 1,000 × g for 15 min. The sediment was discarded. The supernatant was collected and centrifuged again at 1,000 × g for 15 min. The supernatant was decanted and taken as the water-soluble fraction. The hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content of the water-soluble fraction was measured.

In separate experiments, glucose oxidase (600 U) was used in place of yeast; defatted flour was used in place of regular flour; and the yeast level was increased to 2%. In all cases, the extraction procedure was as described above.

### Flour Defatting Procedure

Flour (400 g) was extracted with 2,500 mL of petroleum ether in a Soxhlet extractor for 24 hr. Extracted flour was dried overnight under a stream of air. If the moisture content of the defatted flour was <12%, it was rehydrated to ≈12% moisture in a fermentation cabinet at 30°C and 86% rh.

### H<sub>2</sub>O<sub>2</sub> Measurement

The colorimetric assay of Sinha (1972) was used to determine the H<sub>2</sub>O<sub>2</sub> content in the dough. Water-soluble fraction (1 mL) was added to 2 mL of reagent containing potassium dichromate and acetic acid (1:3, v/v). The mixture was heated to 80°C for 10 min in a water bath (model 70, Fisher Scientific, Pittsburgh, PA) and then cooled to room temperature. The absorbance was measured at 570 nm in a spectrophotometer (1001+, Fisher Scientific Instrumental Service Division, Pittsburgh, PA). The H<sub>2</sub>O<sub>2</sub> content of the sample was determined from a standard curve.

### Spread Test

The spread test (Hosney et al 1979) was used to measure changes in the rheological properties of flour-water doughs made with or without yeast and from regular or defatted flour. Doughs were mixed to optimum development and molded immediately (0 min)

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or fermented for 180 min at 30°C and 86% rh. Fermented doughs were punched after 105 and 155 min and molded after 180 min of fermentation. The width and height of molded doughs were measured with a digital caliper (Mitutoyo Corp., Kawasaki-shi Japan) 60 min after molding to determine the spread ratio (width/height).

### Mixing Under Nitrogen

When doughs were mixed under nitrogen, the gas in the flour and other dry ingredients first was exchanged five times by alternately subjecting the material (placed in a desiccator) to a vacuum and replenishing the desiccator's atmosphere with nitrogen (Lillard et al 1982). All solutions were made with water that had been boiled for 30 min and stored under nitrogen. The desiccator and mixer were placed in a large plastic bag that was inflated by a constant stream of nitrogen. After mixing under nitrogen, the spread ratios were measured as described above.

### Procedure to Remix Dough

Flour-water doughs were mixed to optimum development and fermented for 15 min at 30°C and 86–90% rh in a fermentation cabinet. These doughs then were remixed for 15–30 sec (sufficient time to incorporate the liquid) to incorporate the H<sub>2</sub>O<sub>2</sub> and then were not fermented (0 min) or fermented for 180 min. The spread ratios were measured as described above.

### pH Measurement of Doughs

The pH level of doughs was measured immediately after mixing and after fermentation with a surface electrode as described by Miller et al (1994). Doughs were fermented in covered bowls at 30°C and 86% rh for 3 hr.

### Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS Institute, Cary, NC). Significantly different means were separated using least significant difference. All reported results are the averages of at least three replicates.

## RESULTS AND DISCUSSION

### Effect of Yeast and H<sub>2</sub>O<sub>2</sub> on Wheat Flour Dough Rheology

Hydrogen peroxide is a fast-acting oxidant. The spread test was used to measure the changes in dough rheology of flour-water doughs containing different levels of H<sub>2</sub>O<sub>2</sub>. A large spread ratio indicates a viscous dough, while a small spread ratio indicates an elastic dough. The spread ratio of flour-water dough decreased as the level of H<sub>2</sub>O<sub>2</sub> was increased from 0.00 to 4.00 μmol/g of flour (Fig. 1). The spread ratio decreased dramatically even when a low level of H<sub>2</sub>O<sub>2</sub> (0.25 μmol/g of flour, 8.5 ppm, fwb) was added. These results agree with the finding of Dahle and Sullivan (1963), who showed that 10–20 ppm H<sub>2</sub>O<sub>2</sub> had a strong effect on dough rheology.

Changes in the spread ratio of doughs containing either H<sub>2</sub>O<sub>2</sub> or yeast as a function of fermentation time are shown in Fig. 2. The spread ratio of the flour-water dough increased with time, indicating

that the dough became more viscous. The spread ratio of dough containing 2.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour was substantially lower immediately after mixing (0 min) than that of flour-water dough and increased with fermentation time. This increase presumably was caused by the same mechanism that occurred in the flour-water dough. When 0.76% yeast was added to the flour-water dough, the spread ratio decreased during fermentation and was similar to that of dough containing 2.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour at 3 hr of fermentation (Fig. 2). Yeast clearly had an oxidizing effect on dough similar to that of chemical oxidants, which confirmed the findings by Hoseney et al (1979) and Nagao et al (1981).

### H<sub>2</sub>O<sub>2</sub> Produced by Yeast

The amount of H<sub>2</sub>O<sub>2</sub> in a flour-yeast dough was measured as a function of fermentation time. The amount of H<sub>2</sub>O<sub>2</sub> increased from 1.09 to 2.32 μmol/g of flour during 3 hr of fermentation (Table I). The substantial increase in H<sub>2</sub>O<sub>2</sub> content of the flour-yeast dough indicated that yeast generates H<sub>2</sub>O<sub>2</sub> during fermentation. The H<sub>2</sub>O<sub>2</sub> content of a flour-water dough increased slightly from 1.02 to 1.21 during a 3-hr rest. The slight increase in the H<sub>2</sub>O<sub>2</sub> content of the flour-water dough may have been due to H<sub>2</sub>O<sub>2</sub> produced by microorganisms or other peroxides present in the flour that were detected by the colorimetric procedure.

### Effect of Catalase on Dough Rheology

Kruger (1976) reported that the catalase activity of hard red wheat flour ranged from 40 to 80 U/g of flour, with a mean of 56 U/g of flour. Catalase decomposes H<sub>2</sub>O<sub>2</sub> into oxygen and water. However, our data showed that H<sub>2</sub>O<sub>2</sub> continued to accumulate in dough during a 3-hr fermentation.

To determine the effect of added catalase in a dough system, various levels of catalase were added to flour-water doughs containing 2.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour. After 180 min of fermentation, the spread ratio of doughs containing 2.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour increased from 2.15 (no catalase) to 3.19 (12,600 U of catalase) (Table II). The spread ratio of a dough containing 2.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour and 12,600 U of catalase with no fermentation was 2.61, which was equivalent to that of the flour-water dough (2.62). With 180 min of fermentation, the spread ratio of doughs containing 2.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour and 12,600 U of catalase was 3.19, which approached the control (no additive)

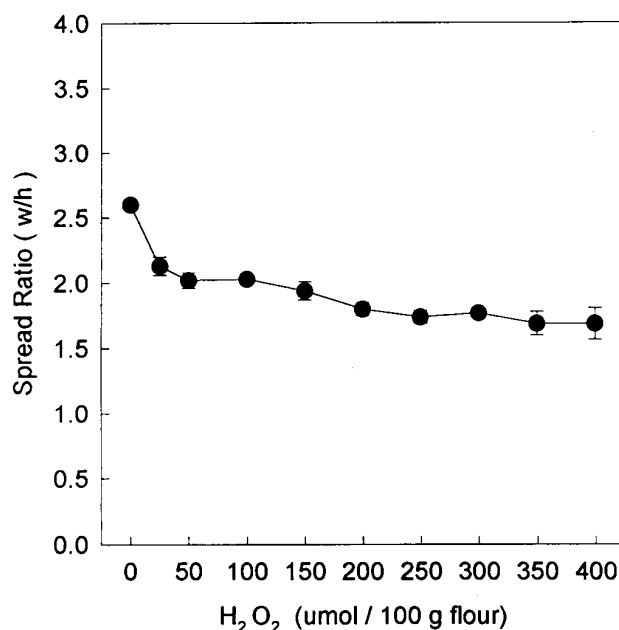


Fig. 1. Effect of hydrogen peroxide on spread ratio of flour-water dough immediately after mixing.

TABLE I  
Hydrogen Peroxide Content of Flour-Water Doughs and Flour-Yeast Doughs at Different Times

Treatment	Fermentation Time (hr)	H <sub>2</sub> O <sub>2</sub> (μmol/g of flour) <sup>a</sup>
Flour	0	1.02 ± 0.01
	1	1.05 ± 0.06
	2	1.16 ± 0.06
	3	1.21 ± 0.04
Flour + 0.76% yeast	0	1.09 ± 0.05
	1	1.28 ± 0.02
	2	1.76 ± 0.04
	3	2.32 ± 0.10

<sup>a</sup> Mean ± standard deviation.

value of 3.25. This shows that when added together, sufficient catalase can destroy the H<sub>2</sub>O<sub>2</sub> before it affects dough rheology.

A similar result might be expected for yeast if its oxidizing effect was caused by the H<sub>2</sub>O<sub>2</sub> it produces. Surprisingly, the addition of catalase at levels up to 63,000 U did not completely overcome the effect of yeast on the spread ratios of fermented doughs containing yeast (Table III). Possibly, entities produced by yeast or in the flour inhibited catalase activity.

### Factors Affecting the Spread Ratio of Wheat Flour Dough Containing Yeast and Catalase

*pH.* Carbon dioxide and a number of organic acids (e.g., acetic acid, lactic acid) are formed during yeast fermentation and can be found in dough and bread (Johnson et al 1958, Wiseblatt 1960, Reed and Pepler 1973). These compounds decrease the pH of fermented doughs. The pH of flour-water dough decreased slightly from 6.03 to 5.86 after 180 min of resting. However, the pH of flour-yeast dough decreased from 6.03 to 5.09 during 180 min of fermentation. Enzyme activity is known to be a function of pH. The optimum pH for the bovine liver catalase used in our experiment was ≈7.0, with a stated range of high activity from 5.3 to 8.0. The activity of catalase is reported to fall off sharply below 5.3 (Galston 1955). Therefore, 1% calcium carbonate was added to maintain the pH of the flour-yeast dough at 5.58. Even with a constant pH, changes in spread ratios of flour-yeast dough with or without catalase (52,500 U) were similar (2.84 vs. 2.76 at 0 min and 2.18 vs. 2.21 at 180 min). Apparently, the lower pH of fermented doughs was not the critical factor affecting catalase activity.

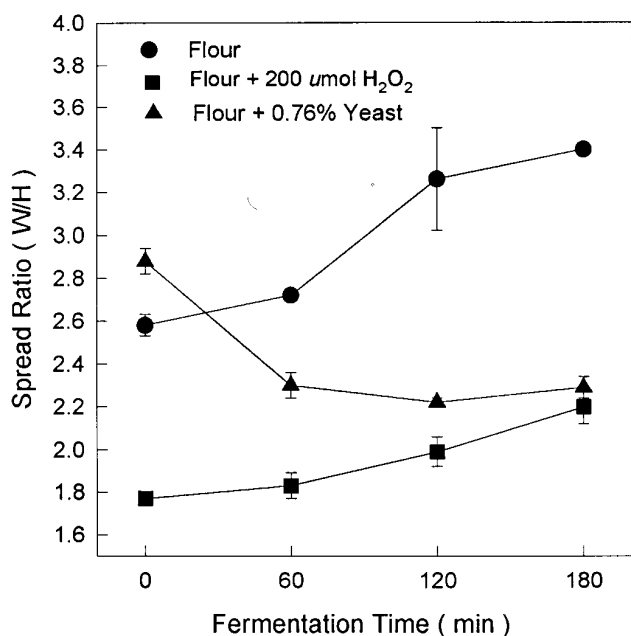


Fig. 2. Effect of yeast and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on spread ratio of doughs during fermentation

TABLE II  
Spread Ratio of Flour-Water Doughs Containing Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>, 2.00 μmol/g of flour) and Catalase

Treatment	Fermentation Time (min)	
	0	180
Flour	2.62b <sup>a</sup>	3.25a
Flour + H <sub>2</sub> O <sub>2</sub>	1.77e	2.15d
+ catalase (504 U)	1.91e	2.37c
+ catalase (1,260 U)	1.93e	2.48b,c
+ catalase (5,040 U)	2.46b,c	3.11a
+ catalase (12,600 U)	2.61b	3.19a

<sup>a</sup> Means with the same letter are not significantly different ( $P < 0.05$ ).

*Remixing.* A flour-water dough was mixed to optimum, allowed to rest for 15 min, and then remixed for 15 sec. The spread ratio of the remixed flour-water dough increased slightly at the beginning of fermentation (2.96 vs. 2.60 at 0 min) (Table IV). A flour-water dough containing 12,600 U of catalase was mixed to optimum, allowed to rest for 15 min, then 4.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour added, and the dough was remixed for 30 sec. The spread ratio (Table IV) of dough first mixed with catalase and then remixed with H<sub>2</sub>O<sub>2</sub> showed insignificant increases that were similar in magnitude to those found for remixed flour (1.91 vs. 1.69 at 0 min and 2.19 vs. 1.92 at 180 min). No effect was attributable to the action of catalase. However, 12,600 U of catalase completely overcame the effect of 4.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour when they were added at the same time (Table IV). Thus, mixing catalase with flour appeared to destroy the enzyme's activity. This suggests that the rheological action of yeast could occur through the H<sub>2</sub>O<sub>2</sub> it produces.

*Defatting.* Flour contains an active lipoxygenase (Faubion and Hosoney 1981), which requires polyunsaturated free fatty acids and oxygen for its activity. The lipid peroxides formed as the result of lipoxygenase activity may competitively inhibit catalase. Extracting of flour with petroleum ether removes the free fatty acids. The amount of H<sub>2</sub>O<sub>2</sub> in dough made with defatted flour decreased to 0.66 μmol/g of flour compared to 1.05 μmol/g of flour in the dough made from regular flour. This shows that our colorimetric procedure measured not only H<sub>2</sub>O<sub>2</sub>, but presumably also lipid peroxides produced by unsaturated free fatty acids and lipoxygenase in flour.

The H<sub>2</sub>O<sub>2</sub> contents of both flour-water and flour-yeast doughs made from regular flour were higher than those of flour-water and flour-yeast doughs made from defatted flour. The H<sub>2</sub>O<sub>2</sub> content increased from 0.66 to 0.92 μmol/g of flour during fermentation when 2% yeast was added to the defatted flour. The difference in H<sub>2</sub>O<sub>2</sub> content between the regular flour and defatted flour both with and without yeast was 0.39 μmol/g of flour. The addition of yeast increased the peroxide value by 0.26 μmol/g of flour in both cases. These results indicate that defatting flour removes lipid peroxides, but H<sub>2</sub>O<sub>2</sub> is still produced.

The spread ratios of dough made with defatted flour; dough made with defatted flour containing 0.76% yeast; and dough made with defatted flour, 0.76% yeast, and 63,000 U of catalase were determined (Table III). Addition of yeast decreased the spread ratio from 2.04 to 1.79 after 180 min of fermentation. The addition of catalase to dough made with defatted flour and yeast did not change the spread ratio from that of dough made from defatted flour without yeast (2.05 vs. 2.04) after 180 min of rest. Thus, catalase reversed the effect of yeast in doughs made with defatted flour. The fact that catalase is effective with defatted flour but not with regular flour suggests that something in the lipid fraction of flour inhibits the catalase. Presumably, this is the lipid peroxide produced by free fatty acids and lipoxygenase.

TABLE III  
Spread Ratio of Flour-Water Doughs Containing Yeast (0.76%) and Catalase (63,000 U) when Mixed Under Air or Nitrogen

Treatment	Fermentation Time (min)	
	0	180
Mixed in air		
Flour	2.53f <sup>a</sup>	3.20b,c
Flour + yeast	2.76e	2.01g
Flour + yeast + catalase	3.02c	2.47f
Mixed in nitrogen		
Flour	2.84d,e	3.35a
Flour + yeast	2.94d	2.60f
Flour + yeast + catalase	3.10c	3.25a,b
Defatted flour <sup>b</sup>		
Flour	2.28g	2.04g
Flour + yeast	2.43f	1.79h
Flour + yeast + catalase	2.33g	2.05g

<sup>a</sup> Means with the same letter are not significantly different ( $P < 0.05$ ).

<sup>b</sup> Mixed in air.

*Mixing under nitrogen.* Lipoxygenase requires oxygen in addition to free fatty acids to produce lipid peroxides (Hoseney et al 1980). Thus, to eliminate the effect of lipid peroxides, doughs were mixed under a nitrogen atmosphere. The changes in rheology of flour-water doughs, flour-yeast doughs, and flour-yeast doughs containing 63,000 U of catalase, all mixed under nitrogen, were measured (Table III). The spread ratios of flour-water dough or flour-yeast dough were lower (more elastic) when mixed in an air atmosphere than when mixed in a nitrogen atmosphere. At 0 min, the spread ratios of doughs were 2.53 (air) vs. 2.84 (N<sub>2</sub>) for flour-water dough and 2.76 (air) vs. 2.94 (N<sub>2</sub>) for flour-yeast dough. With 180 min of fermentation, the spread ratios were 3.20 (air) vs. 3.35 (N<sub>2</sub>) for flour-water dough and 2.01 (air) vs. 2.60 (N<sub>2</sub>) for flour-yeast dough. This shows the well-known effect of air on dough rheology (Bloksma and Bushuk 1988). When 63,000 U of catalase was added and the dough was mixed under nitrogen, the spread ratio of flour-yeast dough increased from 2.94 to 3.10 at 0 min and from 2.60 to 3.25 at 180 min. The latter spread ratio of the dough approached that of flour-water dough at 180 min (3.20), indicating that catalase reversed the effect of the H<sub>2</sub>O<sub>2</sub> produced by yeast when doughs were mixed under nitrogen. This is consistent with the assumption that lipid peroxides inhibited the activity of catalase.

### Effect of Glucose Oxidase on Dough Rheology

*Effect of hydrogen peroxide produced by glucose oxidase.* Glucose oxidase produces H<sub>2</sub>O<sub>2</sub>. Because glucose oxidase has a fairly high substrate specificity for β-D-glucose (Richter 1983), 0.1% D-glucose (fwb) was added to the dough to assure sufficient substrate for the enzyme. The amount of H<sub>2</sub>O<sub>2</sub> produced in a flour-water dough containing 0.1% glucose and 600 U of glucose oxidase increased from 1.93 to 2.84 μmol/g of flour during 3 hr of fermentation. This increase in H<sub>2</sub>O<sub>2</sub> content was similar to that found with flour-yeast doughs (Table I). Interestingly, the H<sub>2</sub>O<sub>2</sub> was produced very rapidly, as shown by the higher amount of H<sub>2</sub>O<sub>2</sub> found at 0 min (1.09 vs 1.93).

**TABLE V**  
Spread Ratio of Flour-Water Doughs Containing Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>, 4.00 μmol/g of flour) and Catalase (12,600 U)

Treatment	Fermentation Time (min)	
	0	180
Flour	2.60c <sup>a</sup>	3.26a,b
Flour (remix 1) <sup>b</sup>	2.96b	3.29a
Flour + H <sub>2</sub> O <sub>2</sub> <sup>c</sup>	1.69e	1.92d,e
+ catalase (remix 2) <sup>d</sup>	1.91d,e	2.19d
+ H <sub>2</sub> O <sub>2</sub> + catalase <sup>e</sup>	2.58c	3.19a,b

- <sup>a</sup> Means with the same letter are not significantly different ( $P < 0.05$ ).  
<sup>b</sup> Flour-water dough was mixed to optimum and fermented at 30°C, 86% rh, for 15 min, then remixed for 15 sec.  
<sup>c</sup> Flour-water dough containing H<sub>2</sub>O<sub>2</sub> was mixed to optimum and fermented at 30°C, 86% rh, for 15 min, then remixed for 15 sec.  
<sup>d</sup> Flour-water dough containing 12,600 U of catalase was mixed to optimum and fermented at 30°C, 86% rh, for 15 min, then H<sub>2</sub>O<sub>2</sub> was added and the dough remixed for 30 sec.  
<sup>e</sup> Added together in the initial mix.

**TABLE V**  
Spread Ratio of Flour-Water Doughs Containing Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>, 2.00 μmol/g of flour) and 600 U of Glucose Oxidase (GO)

Treatment	Fermentation Time (min)	
	0	180
Flour	2.69b <sup>a</sup>	3.38a
+ 0.1% glucose	2.65b	3.26a
+ 0.1% glucose + GO	1.72d	2.14c
+ H <sub>2</sub> O <sub>2</sub>	1.77d	2.20c

- <sup>a</sup> Means with the same letter are not significantly different ( $P < 0.05$ ).

The spread ratio of doughs containing glucose oxidase decreased substantially compared to those of flour-water dough (1.72 vs. 2.69 at 0 min) (Table V). These results were similar to those for doughs containing 2.00 μmol of H<sub>2</sub>O<sub>2</sub>/g of flour (1.72 vs. 1.77 at 0 min and 2.14 vs. 2.20 at 180 min). The results indicate that H<sub>2</sub>O<sub>2</sub> produced by glucose oxidase makes doughs more elastic.

*Effect of catalase on the rheology of dough containing glucose oxidase.* Catalase was added to flour-water doughs containing 600 U of glucose oxidase (Table VI) to decompose the H<sub>2</sub>O<sub>2</sub> produced by the enzyme. The spread ratio of doughs increased only slightly with increased catalase levels. This is similar to the effect of catalase in yeasted doughs. However, catalase did reverse the effect of added H<sub>2</sub>O<sub>2</sub> in flour-water doughs, provided that the H<sub>2</sub>O<sub>2</sub> and catalase were added at the same time (Table II).

*Factors affecting the rheology of dough containing glucose oxidase.* The spread ratio of dough made from defatted flour and glucose oxidase decreased dramatically from 2.18 to 1.62 immediately after mixing (0 min) (Table VII) and then remained constant during 3 hr of fermentation (1.62 vs. 1.60). Addition of catalase (44,100 U) to dough made from defatted flour containing glucose oxidase increased the spread ratio from 1.70 to 1.90 after 3 hr of fermentation, which was essentially equal to the spread ratio of defatted flour at 3 hr (1.90 vs. 2.0) (Table VII). These results are similar to those obtained with defatted flour containing yeast (Table III), except for the greater effect on the dough at 0 min. However, when regular flour was used in this experiment, the spread ratio of doughs containing glucose oxidase and 44,100 U of catalase was similar to that of doughs with no added catalase (1.73 vs. 1.72 at 0 min and 2.24 vs. 2.14 at 180 min) (Table VII). This shows that catalase had no effect on the spread ratio of doughs made with regular flour containing glucose oxidase. Overall, this indicates that the mechanism by which glucose oxidase affects dough rheology is similar to that by which yeast affects dough rheology.

## CONCLUSIONS

Yeast has an oxidizing effect on dough during fermentation. A decrease in the spread ratio and an increase in H<sub>2</sub>O<sub>2</sub> content of flour-yeast dough during a 3-hr fermentation suggested that the production of H<sub>2</sub>O<sub>2</sub> by yeast and the change in dough rheology were related.

**TABLE VI**  
Spread Ratio of Flour-Water Doughs Containing Glucose Oxidase (GO) and Catalase

Treatment	GO (U)	Catalase (U)	Fermentation Time (min)	
			0	180
Flour	...	...	2.69b <sup>a</sup>	3.38a
Flour+0.1% glucose	600	...	1.72e	2.14d
	600	630	1.73e	2.24c,d
	600	6,300	1.77e	2.30c
	600	44,100	1.81e	2.31c

- <sup>a</sup> Means with the same letter are not significantly different ( $P < 0.05$ ).

**TABLE VII**  
Spread Ratio of Flour-Water Doughs and Defatted Flour-Water Doughs Containing Glucose Oxidase (GO) and Catalase

Treatment	GO <sup>a</sup> (U)	Catalase (U)	Fermentation Time (min)	
			0	180
Defatted flour	...	...	2.18c <sup>b</sup>	2.00d
	600	...	1.62e,f	1.60f
	600	44,100	1.70e,f	1.90d
Flour	...	...	2.59b	3.32a
	600	...	1.72e,f	2.14c
	600	44,100	1.73e	2.24c

- <sup>a</sup> No glucose added

- <sup>b</sup> Means with the same letter are not significantly different ( $P < 0.05$ ).

Catalase reversed the effect of added H<sub>2</sub>O<sub>2</sub> if the two were added to flour-water dough together. However, addition of catalase to dough already containing H<sub>2</sub>O<sub>2</sub> (yeasted dough) did not cause this reversal. Apparently, catalase was inhibited by lipid peroxides formed in the lipid fraction of flour. These results suggest that the mechanism by which yeast oxidizes dough is by the production of H<sub>2</sub>O<sub>2</sub>. Similar results were obtained for dough containing glucose oxidase, which also produces H<sub>2</sub>O<sub>2</sub>.

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