

Glucose Oxidase Effects on Gluten and Water Solubles¹

V. Vemulapalli^{2,3} and R. C. Hosney^{2,4}

ABSTRACT

Cereal Chem. 75(6):859–862

Hydrogen peroxide was responsible for the improving the effect of glucose oxidase in breadmaking. The mechanism by which H₂O₂ has its effect is not known. The objective of this study was to determine whether the H₂O₂ produced by glucose oxidase affected the gluten proteins or the water-soluble fraction of flour. Glucose oxidase had no effect on gluten protein as measured by protein solubility or the relative viscosity of soluble protein (solubilized using 1.5% w/v SDS). However, glucose oxidase did affect the water-soluble fraction. The sulfhydryl content of the water-

soluble fraction extracted from flour or dough decreased in the presence of glucose oxidase. Glucose oxidase also caused oxidative gelation of the water-soluble fraction extracted from flour. However, the viscosity of the water-soluble fraction extracted from fermented doughs containing glucose oxidase decreased when higher levels of glucose oxidase were used (≥ 5.0 units of glucose oxidase). Glucose oxidase appeared to have the same oxidizing action independent of whether the water-soluble fraction was boiled or not.

Glucose oxidase catalyzes the reaction: β -D-glucose + O₂ + H₂O \rightarrow D-gluconic acid + H₂O₂. In our previous work (Vemulapalli et al 1998), we found that H₂O₂ produced by glucose oxidase was responsible for the improving effect in breads. The use of glucose oxidase in combination with other enzymes and surfactants for the production of bread has been reported (Haarasilta et al 1989, Haarasilta and Vaesaenen 1989, Nakai et al 1995). The mechanism by which glucose oxidase improves bread is not fully understood.

Sasse (1918) found that the absorption of flour increased by 2.0–2.5% in the presence of 0.139% H₂O₂. Also, Patterson and McLaren (1918) stated that 0.033% H₂O₂ added to dough resulted in increased absorption. Durham (1925) reported that H₂O₂ increased the hydration capacity of flour by acting on the water-soluble fraction and not on the gluten protein. However, the detailed mechanism of action of H₂O₂ on the flour's water-soluble and water-insoluble fractions has not been reported. Therefore, the objective of this study was to determine the effects of glucose oxidase on gluten protein and the water-soluble fraction of flour.

MATERIALS AND METHODS

Commercial flour was obtained from Cargill (Wichita, KS). A sample of Larned, a cultivar of hard red winter wheat, was obtained from a seed producer. Larned 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 μ mole of β -D-glucose to gluconic acid and H₂O₂/min at pH 5.1 at 35°C. CaO₂ (marketed under the trade name CM Paniplus 240) was obtained from ADM Company (Olathe, KS). The KBrO₃ was obtained from Fisher Scientific (Fair Lawn, NJ). Fleischmann instant yeast (Fenton, MO) was used.

Extraction of the SDS-Soluble Fraction

Dough was made by mixing Larned flour (100 g, 14% moisture basis), salt (1.5 g), sugar, (6.0 g), nonfat dry milk (4.0 g), yeast (2.0 g), and optimum water (73%) for 3 min in a pin mixer (National, TMCO, Lincoln, NE). Dough (30 g, containing 18.75 g

of flour) was blended with distilled water in an Osterizer blender for 2.5 min at low speed, then sodium dodecyl sulfate (SDS, 5.06 g) was added to give a concentration of 1.5% (w/v) and a flour-to-SDS proportion of 1:18. The flour-SDS slurry was stirred with a magnetic stirrer for 1 hr at low speed to solubilize the gluten protein. The resulting slurry then was centrifuged at 15,000 \times g for 15 min. The supernatant was decanted carefully and again centrifuged at 15,000 \times g for 10 min. The supernatant was extracted from freshly mixed dough and dough fermented for 90 min. Some doughs were made without any oxidant (control) and others were made with the optimum level of glucose oxidase (5.5 GU) for breads made with Larned flour (Vemulapalli et al 1998).

Relative Viscosity, Protein Solubility, and Sulfhydryl Content of the SDS-Soluble Fraction

The relative viscosity of the supernatant was determined using a capillary viscometer (size 50, Cannon-Fenske). Viscosity was measured at a constant temperature (30 \pm 1°C). Each measurement consisted of pipetting 10 mL of solution into the viscometer tube and allowing a 5-min temperature equilibration period. The time required for a constant volume of solution to flow through the capillary was recorded. The relative viscosity was calculated as: flow time of supernatant/flow time of 1.5% (w/v) SDS solution.

The protein content in the dough or supernatant was determined by micro-Kjeldahl analysis (Approved Method 46-13, AACC 1995) with slight modifications. For digestion, 0.2 g of K₂SO₄ was used instead of 1.3 g, and 10 mL of sodium hydroxide-sodium thiosulfate solution was used for distillation instead of 8 mL. Approximately 0.5 g of dough or 2.5 g of supernatant was used for the protein determination. Protein reported is N \times 5.7. Protein recovery was calculated as: % soluble protein/% protein in dough.

The thiol (sulfhydryl [SH]) content was determined by Ellman's reagent (Beveridge et al 1974). The supernatant (1.0 mL) was added to 5.0 mL of 8M urea in tris-gly buffer (10.4 g of tris, 6.9 g of glycine, and 1.2 g of ethylenediaminetetraacetic acid [EDTA] dissolved in 1 L of distilled water) and 0.04 mL of Ellman's reagent (4.0 mg of 5,5'-dithiobis-2-nitrobenzoic acid dissolved in 1 mL of tris-gly buffer). A blank was made using 1.5% (w/v) SDS instead of sample. Adsorbance was measured at 412 nm using a spectrophotometer (Spectronic 601, Milton Roy Co., Rochester, NY). A standard curve made with glutathione (Sigma Chemicals, St. Louis, MO) was used to calculate the SH content of the sample.

SH Content of the Water-Soluble Fraction

Flour and water (1:3) were blended (1 min) in a 14-speed Osterizer blender at a low speed under a nitrogen atmosphere to eliminate the effect of oxygen. The flour-water slurry then was centrifuged at 1,000 \times g for 15 min. The water-soluble fraction was decanted, and the supernatant centrifuged at 15,000 \times g for 30 min. In a separate experiment, dough was made with flour (100 g, 14%

¹ Contribution No. 98-7-J from the Kansas Agricultural Experiment Station, Manhattan, KS 66506.

² Graduate research assistant and professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

³ Present address: Dominos Pizza, Ann Arbor, MI 48106.

⁴ Present address: R&R Research Services, Manhattan, KS 66502. Corresponding author. E-mail: r_and_r@kansas.net

moisture basis), salt (1.5 g), sugar (6.0 g), nonfat dry milk (4.0 g), yeast (2.0 g), and optimum water (73%) and fermented for 90 min. The water-soluble fraction from dough was extracted (1:3 flour-to-water ratio) by the procedure described above for flour.

Several experiments were performed to study the effect of glucose oxidase on the SH content of the water-soluble fraction. The water-soluble fraction extracted from flour was incubated (30 min) with various levels of glucose oxidase (2.5, 5.0, 7.5, 10.0, and 12.5 GU/100 g of flour). The SH content was then determined. In a separate experiment, the SH content of the water-soluble fraction extracted from Larned flour or complete dough was determined after incubating for 0, 0.5, 1.0, 1.5, and 2.0 hr with glucose oxidase (equivalent to 5.5 GU/100 g of flour). In yet another experiment, optimum levels of oxidants (10 ppm of KBrO_3 , 2.5 GU of glucose oxidase, and 15 ppm of CaO_2) for commercial flour were added during dough mixing, and the water-soluble fractions were isolated after 90 min of fermentation. The SH content of each of those fractions was determined using the procedure described above. For the determination of SH content in the water-soluble fraction of flour, distilled water was used as a blank.

Relative Viscosity of the Water-Soluble Fraction

Flour and water (1:3.25) were blended at low speed for 1 min in a 14-speed Osterizer blender. The flour-water slurry then was centrifuged at $1,000 \times g$ for 15 min. The water-soluble fraction was separated and again centrifuged at $1,000 \times g$ for 10 min to obtain a clear supernatant. Bread formula ingredients (except shortening) were added one at a time to the water-soluble fraction. The solutions were dispersed by stirring for 30 min on a stir plate. The dispersions then were centrifuged at $1,000 \times g$ for 10 min. The supernatant from 100 g of flour was incubated (30 min) with 5.5 GU. The supernatant without glucose oxidase was treated as the control.

In another experiment, various levels of glucose oxidase (1.5–10 GU) were added during dough mixing. The doughs were fermented for 90 min. After fermentation, the dough was blended with water (1:5 flour-to-water ratio) in an Osterizer blender for 1 min at low speed. The slurry was centrifuged at $1,000 \times g$ for 15 min. The water-soluble fraction was centrifuged again for 10 min at $1,000 \times g$ and used for viscosity measurement. The relative viscosity of the water-soluble fraction then was determined using the procedure described above. The relative viscosity was calculated as: flow time of water-soluble fraction/flow time of distilled water.

TABLE I
Composition and Properties of Flours^a

Flour	Moisture (%)	Protein (%)	Absorption (%)	Mixing Time (min)
Commercial	13.0	10.8	69	4.5
Larned	11.1	10.0	73	3.0

^a Bread dough made by a 90-min fermentation bake formula.

TABLE II
Effect of Glucose Oxidase on Protein Solubility, Relative Viscosity, and Sulfhydryl (SH) Content of Gluten Protein Extracted with SDS from Dough^a

Treatment	Protein Solubility (%)	Relative Viscosity	SH Content ($\mu\text{mol}/100 \text{ g}$)
Fermentation 0 min			
No oxidant	89.68b ^b	1.61a	0.79c
5.5 GU	90.10b	1.65a	0.70d
Fermentation 90 min			
No oxidant	91.66ab	1.68a	0.91a
5.5 GU	93.18a	1.76a	0.85b

^a Dough made with Larned flour (100 g, 14% moisture basis), salt (1.5 g), sugar (6.0 g), nonfat dry milk (4.0 g), yeast (2.0 g), water (73%), and 5.5 glucose oxidase units (GU).

^b Means in a column followed by different letters are significantly different ($P > 0.05$).

Flour Reconstitution

Flour (Larned) and water (1:10) were blended (1 min at 25°C) at a low speed in a 14-speed Osterizer blender. The slurry was centrifuged at $1,000 \times g$ for 15 min to separate the water-soluble and water-insoluble fractions. The water-soluble fraction was boiled to denature enzymes, cooled, and lyophilized. The water-insoluble fraction also was lyophilized. After lyophilization, the water-soluble fraction was crushed gently in a mortar with a pestle. The water-insoluble fraction was ground in a Thomas-Wiley laboratory mill (Arthur H. Thomas Co., Philadelphia, PA). The water-insoluble fraction then was rehydrated (12–13% moisture) in a fermentation cabinet (29°C, 95% rh) and mixed with the water-soluble fraction to make a reconstituted flour. A similar procedure was used to make the reconstituted flour containing the water-soluble fraction that was not boiled. Bread was baked with the reconstituted flour using Approved Method AACC 10-10B (AACC 1995).

The loaf volumes of all the breads were measured, and the internal and external characteristics were evaluated subjectively. A optimally oxidized crumb grain of bread is expected to have 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 an underoxidized crumb grain of bread, many small round cells are evident and slip plane is not distinct and the loaf has square corners and a pitted bottom. An overoxidized crumb grain of bread has large round cells with thick cell walls and a very prominent slip plane, often containing large open cells, and the loaf has round corners and a deep crease on the bottom.

TABLE III
Effect of Different Concentrations of Glucose Oxidase on the Sulfhydryl (SH) Content of a Water-Soluble Fraction Extracted from Larned Flour^a

Glucose Oxidase Units (GU)	SH Content ($\mu\text{mol}/100 \text{ g}$ of flour)
0.0	0.168a ^a
2.5	0.143b
5.0	0.131c
7.5	0.111d
10.0	0.076e
12.5	0.055f

^a Means in a column followed by different letters are significantly different ($P > 0.05$).

TABLE IV
Effect of Glucose Oxidase Incubation Time on the Sulfhydryl (SH) Content of Water-Soluble Fraction Extracted from Larned Flour^a

Glucose Oxidase Units (GU)	Incubated	SH Content ($\mu\text{mol}/100 \text{ g}$ of flour)
0.0	0.0 hr	0.151a ^a
5.5	0.0 hr	0.148a
5.5	0.5 hr	0.100b
5.5	1.0 hr	0.094b
5.5	1.5 hr	0.097b
5.5	2.0 hr	0.097b

^a Means in a column followed by different letters are significantly different ($P > 0.05$).

TABLE V
Sulfhydryl (SH) Content of Water-Soluble Fraction Extracted from Doughs Made with Different Enzymes or Oxidants^a

Treatment	SH Content ($\mu\text{mol}/100 \text{ g}$ of dough)
No oxidant	0.432a ^b
2.5 glucose oxidase units	0.363c
15 ppm of CaO_2	0.369c
10 ppm of KBrO_3	0.413b

^a Dough made with commercial flour A (100 g, 14% moisture basis), salt (1.5 g), sugar (6.0 g), nonfat dry milk (4.0 g), yeast (2.01 g), and water (69%).

^b Means in a column followed by different letters are significantly different ($P > 0.05$).

SH Content of the Boiled Water-Soluble Fraction

Flour (Larned) and water (1:5 ratio) were blended for 1 min at low speed in a 14-speed Osterizer blender under a nitrogen atmosphere. The slurry was centrifuged at $1,000 \times g$ for 15 min. The supernatant fraction was boiled and cooled for 1 hr. The water vapor lost during heating was added back. The boiled water-soluble fraction was centrifuged again ($15,000 \times g$) for 1 hr to remove protein. A similar procedure was used to extract a water-soluble fraction that was not boiled. The SH contents in the boiled and unboiled water-soluble fractions were determined using the procedure described above.

Statistical Analysis

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

RESULTS AND DISCUSSION

Effect of Glucose Oxidase on Gluten Protein

Approximately 90% of the gluten protein in dough was solubilized with 1.5% SDS (Table II). No significant differences occurred in solubility or relative viscosity of SDS-soluble protein extracted from freshly mixed doughs made with or without 5.5 GU. Similarly, no significant differences occurred in solubility or relative viscosity of SDS-soluble protein extracted from fermented (90 min) doughs made with or without 5.5 GU. The SH content of the SDS-soluble protein extracted from fermented dough was slightly higher than that of protein extracted from unfermented dough. Addition of glucose oxidase to doughs reduced the SH content of the SDS-soluble protein fraction. However, glucose oxidase and the H_2O_2 produced did not change solubility or the viscosity of extracted gluten protein (Table II). This experiment indicated that glucose oxidase does not directly act on gluten protein. Durham (1925) also reported that H_2O_2 did not act on gluten protein.

Effect of Glucose Oxidase on SH Content of the Water-Soluble Fraction

Because glucose oxidase did not act on the water-insoluble fraction, its effect on the water-soluble fraction was studied. The SH content of the water-soluble fraction (extracted from flour) decreased gradually but significantly with increasing additions of glucose oxidase (Table III). Incubation of the water-soluble fraction extracted from 100 g of flour with 5.5 GU for 30 min caused a significant reduction in the SH content (Table IV). Incubation of the water-soluble fraction extracted from dough made from 100 g of flour with 5.5 GU for 30 min also caused a significant reduction in the SH content (0.276 vs. 0.251). No additional reduction occurred in the SH content of water-soluble fractions extracted from flour or dough incubated for longer than 30 min. Doughs made with optimum levels (for baking) of glucose oxidase (2.5 GU) or CaO_2 (15 ppm) had significantly lower SH values in the water-soluble fractions, suggesting that H_2O_2 produced by glucose oxidase or CaO_2 was oxidizing the SH groups (Table V). In comparison to doughs without an oxidant, $KBrO_3$ caused only a slight but significant decrease in SH content. In general, the SH content of the water-soluble fraction extracted from flour was significantly lower than the SH content of the water-soluble fraction extracted from dough. Liao et al (1994) reported that doughs made with yeast and nonfat dry milk had higher SH contents in the water-soluble fractions than doughs made without those additives.

Effect of Glucose Oxidase on Relative Viscosity of the Water-Soluble Fraction Extracted from Flour

The possible involvement of the water-soluble pentosans in the improving action of glucose oxidase was studied by measuring the

viscosity of the water-soluble fraction. Glucose oxidase (5.5 GU) caused an increase in viscosity of the water-soluble fraction isolated from flour (Table VI). The H_2O_2 generated by glucose oxidase in the presence of peroxidase (native to flour) may have been responsible for the increase in viscosity (Hoseney and Faubion 1981, Crowe and Rasper 1988, Izydorczyk et al 1990). Addition of salt, sugar, or a combination of each to the water-soluble fraction not treated with glucose oxidase did not affect viscosity. Addition of nonfat dried milk alone or in combination with yeast increased the viscosity. In the presence of glucose oxidase, sugar (alone or in combination with salt) and nonfat dried milk increased viscosity. Addition of yeast to the combination decreased the viscosity to that of the water-soluble fraction without additives.

TABLE VI
Effect of Glucose Oxidase and Bread Ingredients on Relative Viscosity of the Water-Soluble Fraction Extracted from Larned Flour^a

Treatment	Without 5.5 GU	With 5.5 GU
WS	2.88f ^b	4.23bc
WS + salt	2.89f	4.24b
WS + sugar	2.99ef	5.20a
WS + salt + sugar	2.97ef	5.26a
WS + sugar + salt + NFDm	3.08e	5.30a
WS + sugar + salt + NFDm + yeast	3.26d	3.79c

^a GU = glucose oxidase units; WS = water-soluble fraction; NFDm = nonfat dry milk.

^b Means in and between columns followed by different letters are significantly different ($P > 0.05$).

TABLE VII
Relative Viscosity of the Water-Soluble Fraction Extracted from Doughs Made with Different Levels of Glucose Oxidase and H_2O_2 ^a

Treatment	Relative Viscosity
Glucose oxidase units (GU)	
No oxidant	2.41d ^b
1.5	2.54b
2.5	2.59a
5.0	2.49c
7.5	2.39d
10.0	2.23e
H_2O_2 (ppm)	
No oxidant	2.42a
0.05	2.38b
0.25	2.36c
2.50	2.35d
5.00	2.32e
15.00	2.12f

^a Dough made with commercial flour A (100 g, 14% moisture basis), salt (1.5 g), sugar (6.0 g), nonfat dry milk (4.0 g), yeast (2.01 g), and water (69%).

^b Means in a column followed by different letters are significantly different ($P > 0.05$).

TABLE VIII
Baking Breads with Reconstituted Flour (with and without glucose oxidase) by a 90-min Fermentation Process

Treatment	Loaf Volume (cm ³)	Cell Structure
Flour		
No oxidant	708c ^a	Underoxidized
30 ppm of $KBrO_3$	767a	Optimum oxidation
5.5 glucose oxidase units (GU)	742ab	Optimum oxidation
Reconstituted flour		
No oxidant	723cb	Underoxidized
5.5 GU	760a	Optimum oxidation
Reconstituted flour with boiled water solubles		
No oxidant	777a	Optimum oxidation
5.5 GU	770a	Overoxidized

^a Means in a column followed by different letters are significantly different ($P > 0.05$).

Effect of Glucose Oxidase on the Relative Viscosity of the Water-Soluble Fraction Extracted from Dough

Glucose oxidase up to a certain level (2.5 GU) increased the viscosity of the water-soluble fraction extracted from dough. Further addition of glucose oxidase caused a gradual and significant drop in the relative viscosity (Table VII). According to Painter and Neukom (1968), an excess of oxidant results in either no gel formation or a gel that forms but quickly dissolves. Addition of H₂O₂ caused a progressive decline in the relative viscosity of the water-soluble fraction extracted from dough (Table VII). Excess H₂O₂ generated by glucose oxidase may have decreased the viscosity of the water-soluble fraction extracted from fermented dough. The mechanism by which this occurs is not clear.

Effect of Boiling of the Water-Soluble Fraction on the Action of Glucose Oxidase

Breads made from Larned flour and reconstituted Larned flour without an oxidant were comparable in crumb grain and loaf volume (Table VIII). Likewise, breads made from Larned flour and reconstituted Larned flour both containing optimum levels of glucose oxidase (5.5 GU) were comparable in crumb grain and volume. Thus, the fractionation and reconstitution procedure did not affect the baking properties of the Larned flour.

The reconstituted Larned flour containing a boiled water-soluble fraction (to denature endogenous enzymes) was used to make bread with and without glucose oxidase. The bread made with reconstituted Larned flour containing a boiled water-soluble fraction had a larger loaf volume and better crumb grain than bread produced from the reconstituted control (Table VIII). The water-soluble fraction obviously had been changed by the boiling treatment, because the reconstituted flour containing the boiled fraction required less oxidation. Also, boiling the water-soluble fraction significantly decreased the level of SH groups in the water-soluble fraction (from 0.152 to 0.083). Addition of 5.5 GU/100 g of flour to reconstituted Larned flour containing a boiled water-soluble fraction resulted in bread with an overoxidized crumb grain (Table VIII). This phenomenon could have been due to the combined oxidation effects of boiling and addition of glucose oxidase. Glucose oxidase and boiling appeared to have similar effects on the SH content of the water-soluble fraction. The mechanism of action of glucose oxidase or boiling appears to be a decrease in the SH groups of the water-soluble fraction. Glucose oxidase had the same oxidizing action independent of whether the water-soluble fraction was boiled or not. This would suggest that the native peroxidase is not required for the oxidizing effect of glucose oxidase.

The above experiments clearly showed that glucose oxidase acts on the water-soluble fraction of flour. Durham (1925) also reported that the substance affected by H₂O₂ was in the flour's water-soluble fraction. Oxidation of water-soluble SH groups and an increase in viscosity appear to be the mechanisms by which glucose oxidase affects the rheological properties of the dough.

CONCLUSIONS

Glucose oxidase did not affect the protein solubility or relative viscosity of gluten proteins. However, glucose oxidase is known to improve the loaf volume and result in a drying of the dough. Treating the water-soluble fraction isolated from flour or dough with glucose oxidase reduced the SH content. Presumably this is the oxidation effect. Glucose oxidase also caused an increase in viscosity of the water-soluble fraction extracted from flour. This appears not to be related to the oxidation effect but may be important in relation to the dryness of the dough. The viscosity of the water-soluble fraction extracted from fermented dough decreased with increasing levels of glucose oxidase, suggesting that the water soluble pentosans had become less soluble or were degraded by the H₂O₂.

LITERATURE CITED

- American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9th ed. Method 10-10B approved November 1995; and Method 46-13 approved October 1976, revised October 1986, reviewed October 1994; The Association: St. Paul, MN.
- Beveridge, T., Toma, S. J., and Nakai, S. 1974. Determination of SH- and SS- groups in some food proteins using Ellman's reagent. *J. Food Sci.* 39: 49-51.
- Crowe, N. L., and Rasper, V. F. 1988. The ability of chlorine and chlorine-related oxidants to induce oxidative gelation in wheat flour pentosans. *J. Cereal Sci.* 7:283-294.
- Durham, R. K. 1925. Effect of hydrogen peroxide on relative viscosity measurements of wheat and flour suspensions. *Cereal Chem.* 2:297-305.
- Haarasilta, S., Pullinen, T., Vaeisaenen, S., and Tammersalo, K. I. 1989. A method of improving the properties of dough and the quality of bread. European patent 0338452A1.
- Haarasilta, S., and Vaeisaenen, S. 1989. Method for improving flour dough. European patent 0321811A1.
- Hoseney, R. C., and Faubion, J. M. 1981. A mechanism of oxidative gelation of wheat flour water-soluble pentosans. *Cereal Chem.* 58:421-424.
- Izydorczyk, M. S., Biliaderis, C. G., and Bushuk, W. 1990. Oxidative gelation studies of water-soluble pentosans from wheat. *J. Cereal Sci.* 11:153-169.
- Liao, Y. 1994. Studies on gel protein properties of wheat flour and dough. MS thesis. Kansas State University: Manhattan, KS.
- Nakai, K., Takami, K., Tanaka, N., and Takasaki, Y. 1995. Bread quality—Improving composition and bread producing process using the same. European patent 0686348A1.
- Painter, T. J., and Neukom, H. 1968. The mechanism of oxidative gelation of a glycoprotein from wheat flour. *Biochem. Biophys. Acta* 158:363-381.
- Patterson, C. J., and McLaren, L.M. 1918. Protein hydrolysis. *J. Am. Assoc. Cereal Chem.* 3:10-16.
- Sasse, A. R. 1918. Investigation of peroxide process. *J. Am. Assoc. Cereal Chem.* 3:20-22.
- Vemulapalli, V., Miller, K. A., and Hoseney, R. C. 1998. Glucose oxidase in breadmaking systems. *Cereal Chem.* 75:439-442.

[Received December 1, 1997. Accepted August 7, 1998.]