EFFECTS OF GLUCOSE OXIDASE, HEMICELLULASE AND ASCORBIC ACID ON DOUGH AND BREAD QUALITY

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ABSTRACT

In this study, glucose oxidase alone or its combinations with hemicellulase or ascorbic acid were used in bread making. Glucose oxidase alone mainly decreased dough extensibility. It produced stiffer and less extensible dough. Combinations of glucose oxidase–hemicellulase presented lower extensibility and were more resistant to extension than glucose oxidase alone. When glucose oxidase–ascorbic acid combinations were used, the softening degree significantly decreased, regardless when added the lowest glucose oxidase in combination with ascorbic acid. Glucose oxidase–ascorbic acid combinations significantly modified dough resistance. The glucose oxidase alone significantly increased specific loaf volume. The Dallman value of loaves made with glucose oxidase alone was found higher than for control. The most dramatic effect of additives on specific loaf volume was observed when glucose oxidase–hemicellulase combinations were added. This effect has been ascribed to redistribution of water from the hemicellulose to gluten, which would render the gluten more extensible. Specific loaf volume showed a significant enhancement when glucose oxidase–ascorbic acid combinations were added, but this effect was not as good as glucose oxidase–hemicellulase. The effects of glucose oxidase and its combinations with ascorbic acid and hemicellulase on dough rheology and bread quality are highly dependent on the amount of enzyme and the original wheat flour quality.

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PRACTICAL APPLICATION

In practice, appropriate combinations of glucose oxidase with hemicellulase can be used as improvers in bread making, depending on their combination levels. This study will show the way to new research about glucose oxidase, ascorbic acid and hemicellulose.

INTRODUCTION

Dough conditioners have been developed to overcome deficiencies in the bread-making quality of the wheat gluten. The oxidizing agents ascorbic acid, azodicarbonamide and potassium bromate are the most commonly used (Rosell *et al.* 2003). Chemical oxidants are frequently added to flour to improve bread-making performance, although lately their relationship with cancer disease incidence is decreasing their use (Gujral and Rosell 2004).

The use of enzymes is the best alternative to chemical compounds because they are generally recognized as safe and do not remain active after baking. One of the enzymes that can confer strength to the dough is glucose oxidase. This enzyme acts through different catalytic mechanisms and may induce changes in the polymerized form of the glutenin subunits and may be transformed from soluble proteins into insoluble ones (Rosell *et al.* 2003).

Some of the literature reports have noted that the improvements in the properties of dough and baked bread can be attributed to the introduction of disulfide cross-links into the gluten network, because glucose oxidase was thought both to introduce protein cross-links into dough, and to improve dough and bread properties (Rasiah *et al.* 2005).

Glucose oxidase is an enzyme found in a number of fungal sources (Vemulapalli *et al.* 1998). It catalyzes the concomitant reduction of molecular oxygen to hydrogen peroxide that can either form disulfide bonds between proteins, or form tyrosine cross-links, whose role in the gluten structure was recently reported (Tilley *et al.* 2001; Hanft and Koehler 2006). Although H_2O_2 has been hypothesized to be the responsible factor, the mechanism for the dough-strengthening effect of glucose oxidase is not completely understood (Martin *et al.* 2005).

The use of glucose oxidase improves the loaf volume of bread and the crumb grain (Vemulapalli *et al.* 1998). It results in stronger and more elastic doughs with a dry surface (Vemulapalli and Hoseney 1998; Hanft and Koehler 2006). Addition of glucose oxidase to doughs reduced the -SH content of the sodium dodesil sulfate (SDS)-soluble protein fraction (Hilhorst *et al.* 1999) because hydrogen peroxide oxidizes, indirectly, the thiol groups of two cysteine residues to form disulfide bonds (Rasiah *et al.* 2005). This reaction on

bread dough induces the formation of a protein network with improved viscoelastic and structural properties, and therefore, betters performance for bread making (Wikström and Eliasson 1998; Fayle *et al.* 2000). However, it may also lead to the formation of arabinoxylan cross-linking or even a gel from the water-soluble arabinoxylan. There is controversy about the positive or negative effect of the latter. This effect can be corrected by the coaddition of an arabinoxylan-modifying enzyme (Martin *et al.* 2005).

The glutenin proteins subunits are tyrosine-rich, and these amino acids could participate in the formation of covalent unions, putatively involving tyrosyl residues in cross-links catalyzed by one or more peroxidases (Held *et al.* 2004). Dityrosine occurs in wheat flours and doughs, perhaps acting as a kind of stabilizing cross-link in the wheat gluten structure in addition to those provided by disulfide bonds (Tilley *et al.* 2001). It is usual to add oxidizing agents to doughs during mixing and baking. These compounds oxidize and modify certain amino acids and could influence the structure of the polymers, promoting their capacity to form aggregates and increasing the elasticity of the gluten. Dityrosines and isodityrosines are products of tyrosine oxidation. Thus, intramolecular and intermolecular cross-linked tyrosine might contribute to the structure of the gluten network (Pena *et al.* 2006).

The major nonstarch polysaccharides of wheat flour are pentosans. Pentosans originate from the endosperm, cell wall and bran of wheat grain (Wang *et al.* 2002). The pentosans are classified as hemicelluloses, being composed mostly of arabinoxylans (copolymers of the monosaccharides xylose and arabinose). These polysaccharides are found in small amounts in flour (2–3% of wheat flour, 5% of rye flour) (Denli and Ercan 2001) in both water-extractable pentosans and water-unextractable solids form. Arabinoxylans are the major constituent of nonstarch polysaccharide. Some of the arabinose side chains are esterified with phenolic acids, mostly ferulic acid. In solutions of water-extractable pentosans, this ferulic acid is involved in the oxidative cross-linking reaction of individual arabinoxylan to form a larger network (Wang *et al.* 2002). Phenolic compounds such as ferulic acid in the pentosans or tyrosine in proteins can also be oxidized by peroxidase, which is present in wheat flour. However, little is known about the reaction mechanism of glucose oxidase at the molecular level (Hanft and Koehler 2006).

Pentosans can indirectly effect gluten formation by competing for water. Pentosan-modifying enzymes can then act by reducing the water-binding capacity of pentosans thus inducing water redistribution. Another indirect effect of pentosans (soluble and insoluble) could be caused by their ability to form a network limiting the movement of glutenin proteins toward a larger aggregate. The beneficial action of pentosanase would then be exerted by breakdown of this pentosan network. A direct effect of pentosans envisages pentosans to become covalently linked or associated to glutenin proteins, thus hindering further

formation of gluten protein. Pentosan-modifying enzymes would then act by cleaving gluten-associated pentosans (Wang *et al.* 2002). Dough rheological characteristics (development time, consistency, extensibility, resistance to breakdown), loaf volume and texture are reported to be affected by pentosans. Modification of pentosans through the incorporation of microbial hemicellulase preparations (especially endoxylanases) in dough formulations represents one way of modifying their effects and thereby altering some of the quality characteristics of breads and other baked goods. They are reported to soften doughs and to exert favorable effects on dough handling, bread volume, texture and stability (Baillet *et al.* 2003).

The combination xylanase–glucose oxidase is widely used in baking to improve handling properties, bread volume, and crumb texture and structure. The synergistic action of glucose oxidase and hemicellulase/xylanase is due to a sort of sequential mechanism: during the first minutes of mixing, glucose oxidase favors protein–arabinoxylan linkages, then xylanase slowly transforms the heavily hydrated arabinoxylan carried by gluten into smaller oligomers, which improves the gas retention capacity of the gluten network (Martin *et al.* 2005).

Martin *et al.* (2003b) also concluded that pentosanase–glucose oxidase combination resulted in dough with improved extensibility yielding better gluten quality. That effect has been attributed to the H_2O_2 released from the glucose oxidase reaction.

Extensive hydrolysis of wheat dough pentosans by xylanase leads to slack, wet and sticky dough. When used at an appropriate level of addition, however, certain pentosan-modifying enzymes improve loaf volume and crumb structure of breads without giving rise to negative effects on dough handling and machinability. The release of arabinoxylans by xylanase, together with the preservation of the molecular size characteristics of the water-extractable pentosans, is considered important in improving dough and bread properties. Ferulic acid is involved in oxidative cross-linking of water-extractable pentosans. It is not unlikely that a similar combination reaction can occur between arabinoxylan-bound ferulic acid and for example cysteine side chains of gluten. Hence, either diferulic acid bridges or covalent linkages between ferulic acid and cysteine molecules, present in protein, can be formed (Wang *et al.* 2002).

Glucose oxidase has found limited use, in combination with ascorbic acid, as a flour oxidizing system. Ascorbic acid, normally a reducing agent, will act as an oxidizing agent when it is first oxidized to dehydroascorbic acid. This change is catalyzed either by ascorbic acid oxidase, an enzyme naturally present in flour, or indirectly by glucose oxidase that converts glucose to gluconic acid and hydrogen peroxide, with the latter then acting to bring about the oxidation of ascorbic acid. This system is actually more reliable than the one based on the ascorbic acid oxidase and can be used to replace inorganic

oxidizing agents (Pyler 1988). The purpose of this study was to determine the effects of glucose oxidase and its combinations with ascorbic acid and hemicellulase on dough rheology and bread quality.

MATERIALS

Commercial wheat flour (good quality bread flour) was obtained from the Toru Flour Milling Co., Ltd (Bandirma, Turkey) with 11.8% protein (dry weight), 14% moisture, 0.65% ash (dry weight), 27.2% wet gluten, 91% gluten index. Commercial compressed yeast (3%, w/w) and salt (1.5%, w/w) were added to the flour. Glucose oxidase (E.C. 1.1.3.4.) (10,000 GODU/g) was purchased from NOVOZYMES Biotech, Inc., Bagsvaerd, Denmark. L(+)-ascorbic acid (GR Merck, Brussels, Belgium) was used as an oxidant. Hemicellulase (E.C. 3.1.1.73) (6,000 fungal xylanase units [FXU]/g) was purchased from Ekin Food Co., Ltd (Ankara, Turkey). Used dosages of the additives are given in Table 1.

METHODS

Chemical and Rheological Analysis

Moisture, ash, protein (Nx5.7) and wet gluten contents were determined using ICC Standard Methods No. 110/1, 104/1, 105/2, 106/2, respectively (Anon 1976, 1984, 1990, 1994). Gluten index value was carried out according to ICC Standard Methods No. 158 (Anon 1995). Farinograph and extensograph were carried out according to ICC Standard Methods No. 115/1 and 114/1, respectively (Anon 1992a,b).

TABLE 1.
DOSAGES OF ADDITIVES

Sample	Dosage (mg/kg flour basis)	Sample code
Control	–	C
Glucose oxidase (E.C. 1.1.3.4.)	2	G1
	4	G2
	6	G3
Glucose oxidase + ascorbic acid	2 + 30	G1A
	4 + 30	G2A
	6 + 30	G3A
Glucose oxidase + hemicellulase (E.C. 3.1.1.73)	2 + 50	G1H
	4 + 50	G2H
	6 + 50	G3H

Bread-making Procedure

Each sample was baked in duplicate according to the AACC quick-knead procedure (method 54-10) (Anon 1971). In all experiments, water determined according to farinograph value was put in the mixer and then 1,000-g flour was added. Yeast, salt, glucose oxidase, ascorbic acid and hemicellulase were put in the mixer and mixed. The control dough had no glucose oxidase, ascorbic acid and hemicellulase. Dough was kneaded (1,400 rpm) for 1 min. After kneading, the dough was fermented in a fermentation room, which had temperature and humidity-adjusting features (30C and 80–85% relative humidity) for 30 min. After the first fermentation, the dough was rounded. After waiting for 30 min (second fermentation), it was given form and then deposited in baking pans. In the final proofing, the dough was fermented for 55 min and then baked for 20 min at 250 ± 5C in an oven with vapor injection. The loaves were cooled to ambient temperature and sealed in plastic bags for storage at 20C. After 2 h, the loaves volumes were measured, and after 6 h they were weighed. The internal properties of the bread samples were determined using the method of Pelshenke *et al.* (1964). After that, cross-section and external appearance photographs of bread samples were taken.

Statistical Analysis

The SD was calculated by analysis of variance using Minitab Statistical Package (Anon 1998). Furthermore, Duncan's multiple range test was used to determine the differences between variances by using MSTAT Statistical Package (Anon 1980).

RESULTS AND DISCUSSION

Farinograph and Extensograph Properties

Rheological properties of wheat doughs containing different levels of additives are summarized in Table 2. Water absorption showed a significant enhancement when glucose oxidase was added alone. This result is in agreement with that of Prabhasankar *et al.* (2004). The addition of it did not significantly modify the mixing tolerance number and the softening degree. Dough extensibility significantly decreased when glucose oxidase was added alone. The effect of glucose oxidase was especially clear from the decrease in extensibility. It produced stiffer and less extensible dough. This result agrees with previous findings of Martin *et al.* (2003a,b, 2005), Prabhasankar *et al.* (2004) and Bonet *et al.* (2006). The effect of glucose oxidase on dough properties is thought to be the result of increased S-S formation and/or arabinoxylan cross-linking (Martin *et al.* 2005). According to Mitami *et al.* (2003),

TABLE 2.
THE EFFECTS OF DIFFERENT ADDITIVES ON FARINOGRAPH AND EXTENSOGRAPH PROPERTIES

Sample	Dosage (flour basis)	Water absorption	Development time	Stability	Mixing tolerance number	Softening degree	Maximum resistance	Extensibility	Ratio number
	(mg/kg)	(%)	(min)	(min)	(BU)	(BU)	(BU)	(mm)	(BU/mm)
C	–	61.4^{f}	2.0^{bc}	9.9^{d}	30^{bc}	45^{b}	200^{h}	141^{a}	1.42^{i}
G1	2	62.2^{e}	1.6^{de}	9.8^{d}	30^{bc}	40^{bc}	240^{g}	128^{b}	1.88^{h}
G2	4	62.4^{de}	1.9^{bc}	10.7^{c}	40^{ab}	40^{bc}	305^{e}	116^{c}	2.63^{f}
G3	6	62.9^{c}	1.5^{de}	7.1^{f}	40^{ab}	60^{a}	350^{d}	116^{c}	3.02^{d}
G1A3	2 + 30	63.4^{b}	1.4^{e}	11.0^{b}	30^{bc}	40^{bc}	230^{g}	138^{ab}	1.67^{i}
G2A3	4 + 30	62.6^{d}	2.4^{a}	11.5^{a}	20^{c}	30^{c}	290^{f}	126^{b}	2.30^{g}
G3A3	6 + 30	63.4^{b}	1.7^{cd}	10.5^{c}	20^{c}	35^{c}	340^{d}	118^{c}	2.88^{e}
G1H3	2 + 50	63.8^{a}	2.1^{b}	9.6^{d}	40^{ab}	50^{ab}	550^{c}	93^{e}	5.91^{c}
G2H3	4 + 50	63.2^{b}	2.1^{b}	9.3^{e}	40^{ab}	50^{ab}	590^{b}	98^{d}	6.02^{b}
G3H3	6 + 50	63.2^{b}	1.7^{cd}	9.6^{d}	50^{a}	50^{ab}	620^{a}	94^{e}	6.60^{a}

$^{a–i}$ Means with different superscripts in columns indicate significant difference ($P \leq 0.001$).
BU, Brabender Unit.

dough mixed with single glucose oxidase increases the hardness, which indicates that the extensibility of gluten is quite poor and the starch granules cannot be sufficiently covered with the gluten matrix. The work of Martin *et al.* (2003a) suggests that this effect of glucose oxidase on dough properties is thought to be the result of gluten S-S formation and/or pentosan cross-linking. Vemulapalli *et al.* (1998) determined that doughs made with glucose oxidase had significantly more elastic properties than doughs made without any oxidant. In a study made by Rosell *et al.* (2003), only a slight decrease could be observed in the extensibility of dough prepared with glucose oxidase.

Combinations of glucose oxidase–hemicellulase significantly increased the water absorption (Table 2). These combinations did not significantly modify the stability and the softening degree. It presented lower extensibility and was more resistant to extension than glucose oxidase alone. These results are in contrast with those of some previous studies. Martin *et al.* (2003b), while studying the effects of pentosanase and oxidases on the characteristics of doughs and the glutenin macropolymer, reported that addition of pentosanase plus glucose oxidase presented greater extensibility and was less resistant to extension than glucose oxidase alone. The difference could be due to the lower amount of enzyme used in this study (at least 10 times lower).

When glucose oxidase–ascorbic acid combinations were used, the water absorption and the stability significantly increased, the softening degree significantly decreased, regardless when added the lowest glucose oxidase in combination with ascorbic acid (Table 2). Glucose oxidase–ascorbic acid combinations significantly modified dough resistance.

Bread-making Results

Bread-making results are given in Figs. 1–5. The glucose oxidase alone significantly increased specific loaf volume (Fig. 1). These results are in accordance with those of Gujral and Rosell (2004). Vemulapalli and Hoseney (1998) have also noted that glucose oxidase is known to improve the loaf volume and results in a drying of the dough. Cross section and external appearances of bread samples made with glucose oxidase alone are shown in Fig. 2. The Dallman value calculated from external properties of loaves made with glucose oxidase alone was found higher than for the control (Fig. 1). The effects of additives on Dallman value are shown in Fig. 3. Hilhorst *et al.* (1999) also determined that addition of xylanase or peroxidase improved crumb structure whereas glucose oxidase had a negative effect.

Bonet *et al.* (2006) concluded that despite some types of deficiencies in bread-making quality of wheat flour being overcome by glucose oxidase treatment, it should be stressed that an overdosage yields a detrimental effect on the handling characteristics of dough and the quality of the resulting bread.

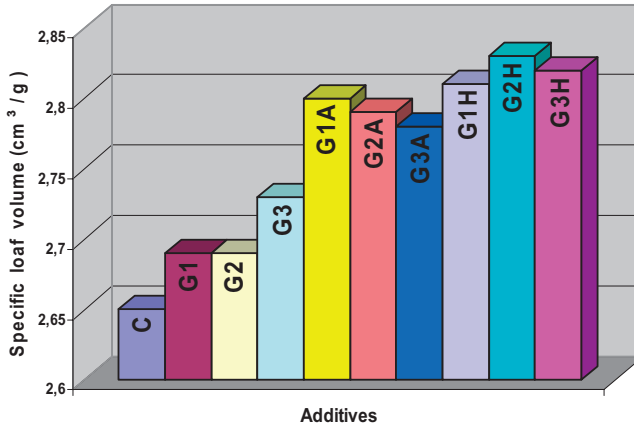

FIG. 1. THE EFFECTS OF ADDITIVES ON SPECIFIC LOAF VOLUME

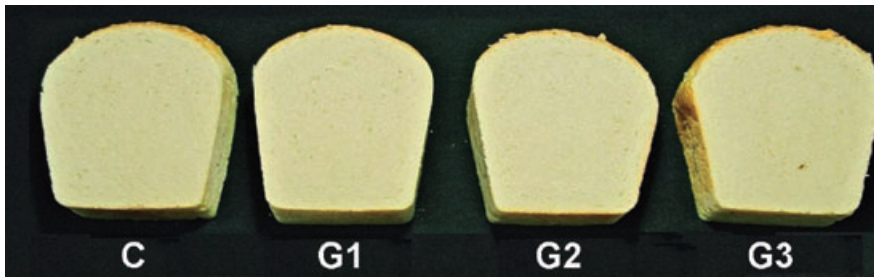

FIG. 2. CROSS SECTIONS AND EXTERNAL APPEARANCES OF CONTROL AND BREAD SAMPLES MADE WITH GLUCOSE OXIDASE ALONE

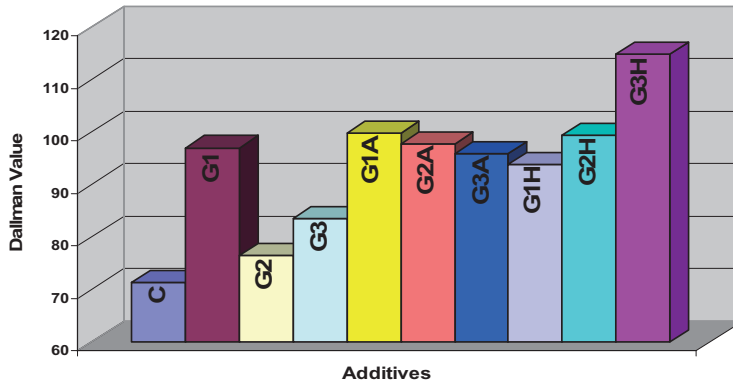

FIG. 3. THE EFFECTS OF ADDITIVES ON DALLMAN VALUE

FIG. 4. CROSS SECTIONS AND EXTERNAL APPEARANCES OF CONTROL AND BREAD SAMPLES MADE WITH GLUCOSE OXIDASE–HEMICELLULASE COMBINATIONS

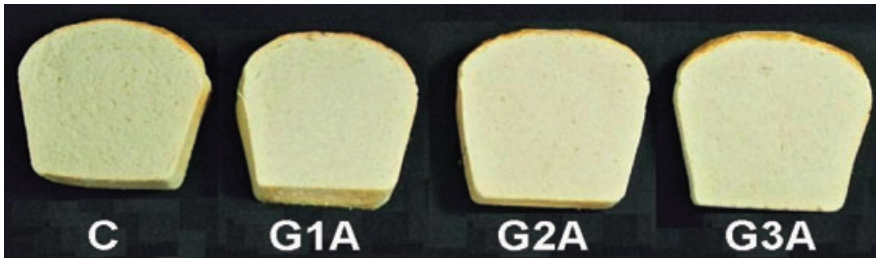

FIG. 5. CROSS SECTIONS AND EXTERNAL APPEARANCES OF CONTROL AND BREAD SAMPLES MADE WITH GLUCOSE OXIDASE–ASCORBIC ACID COMBINATIONS

The specific loaf volume showed a significant enhancement when glucose oxidase–hemicellulase combinations were added (Fig. 1). The most dramatic effect of additives on specific loaf volume was observed when glucose oxidase–hemicellulase combinations were added (Fig. 1). Conversely, Mitami *et al.* (2003) determined that these enzyme combinations could not improve the specific volume with the increasing glucose oxidase levels. In the work of Hilhorst *et al.* (1999), glucose oxidase–xylanase gave the increase in volume as xylanase. This effect has been ascribed to redistribution of water from the hemicellulose to gluten, which would render the gluten more extensible. Cross section and external appearance of bread samples made with glucose oxidase–hemicellulase combinations are also shown in Fig. 4. As can be seen in Fig. 3, the addition of glucose oxidase–hemicellulase significantly increased the Dallman value. These combinations gave the highest Dallman values of this work.

Specific loaf volume showed a significant enhancement when glucose oxidase–ascorbic acid combinations were added (Fig. 1), but this effect was not as good as glucose oxidase–hemicellulase. These combinations signifi-

cantly increased the Dallman value as compared to the control (Fig. 3). Cross section and external appearance of bread samples are also shown in Fig. 5.

CONCLUSION

The purpose of this study was to investigate the effects of glucose oxidase alone and its combinations with ascorbic acid and hemicellulase on dough rheology and bread quality.

Glucose oxidase alone mainly decreased dough extensibility. It produced stiffer and less extensible dough. Water absorption showed a significant enhancement when glucose oxidase was added alone. Combinations of glucose oxidase–hemicellulase significantly increased the water absorption. These combinations did not significantly modify the stability and the softening degree. It presented lower extensibility and was more resistant to extension than glucose oxidase alone. When glucose oxidase–ascorbic acid combinations were used, the water absorption and the stability significantly increased, the softening degree significantly decreased, regardless when added the lowest glucose oxidase in combination with ascorbic acid. Glucose oxidase–ascorbic acid combinations significantly modified dough resistance.

The glucose oxidase alone significantly increased specific loaf volume. The Dallman value calculated from external properties of loaves made with glucose oxidase alone was found higher than for control. The specific loaf volume showed a significant enhancement when glucose oxidase–hemicellulase combinations were added. The most dramatic effect of additives on specific loaf volume was observed when glucose oxidase–hemicellulase combinations were added. This effect has been ascribed to redistribution of water from the hemicellulose to gluten, which would render the gluten more extensible. These combinations gave the highest Dallman values of this work. Specific loaf volume showed a significant enhancement when glucose oxidase–ascorbic acid combinations were added, but this effect was not as good as glucose oxidase–hemicellulase. These combinations significantly increased the Dallman value as compared to the control.

The effects of glucose oxidase alone and its combinations with ascorbic acid and hemicellulase on dough rheology and bread quality are highly dependent on the amount of enzyme and the original wheat flour quality.

As a result, the best bread properties were obtained in glucose oxidase–hemicellulase combinations. The highest specific loaf volumes were also determined in G2H (4 mg/kg of glucose oxidase–50 mg/kg of hemicellulase), G3H (6 mg/kg of glucose oxidase–50 mg/kg of hemicellulase) and G1H (2 mg/kg of glucose oxidase–50 mg/kg of hemicellulase). The combination of glucose oxidase–hemicellulase gave more regular crumb structure than other

applications. Therefore, in practice, appropriate combinations of glucose oxidase–hemicellulase can be used as improvers in bread making, depending on their combination levels.

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