Studies on a Raw-Starch Digesting Enzyme. II. Replacement of Sucrose in White Pan Bread[1,2]

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ABSTRACT

The effects of a raw-starch digesting enzyme (RSDE) on breads containing no, low, and normal levels of sucrose were studied. The RSDE increased glucose formation in doughs, which, in turn, increased crust color at all sucrose levels and loaf volume in bread with no sucrose. The RSDE decreased bread firmness but did not affect bread firming rate. In no- or low-sucrose doughs, the lowest level of RSDE produced a loaf volume comparable to that of control bread (6% sucrose), but the highest level of enzyme was needed to produce a crust color comparable to that of control bread. However, the highest level of RSDE tended to produce keyholing (weakening of bread side walls). Thus, RSDE is most beneficial in breads where high loaf volume but fairly light crust color is desired.

The effects of α-amylase in baking have been studied intensively, while the role of glucoamylase in baking has received less attention. Glucoamylase (EC 3.2.1.3., 1,4-α-D-glucan glucohydrolase) is an exo-acting enzyme that cleaves glucose molecules from the nonreducing ends of starch or glycogen. Glucose produced is in the β-configuration. Glucoamylase is able to hydrolyze both α-1,4- and α-1,6-glucosidic linkages so that, theoretically, the end product of starch digestion is 100% glucose. Most commercial glucoamylase preparations are from either *Aspergillus* sp. or *Rhizopus* sp. (Ueda 1988).

Many fungal glucoamylases are also able to hydrolyze raw starch. They are composed of at least two components, commonly referred as glucoamylase I and glucoamylase II. Glucoamylase I has strong debranching action and is more active on raw starch than is glucoamylase II (Ueda 1988). Commercial glucoamylase preparations usually also contain α-amylase, which exerts a synergistic effect with glucoamylase on starch hydrolysis (Yamamoto 1988).

In white bread, loaf volume comparable to that achieved with a normal sucrose level (6%) was obtained at a reduced sucrose level (2%) by adding glucoamylase or glucoamylase and α-amylase together to the dough (Pomeranz et al 1964). In no-sucrose doughs, loaf volume comparable to that of the control bread could not be obtained with any glucoamylase and α-amylase combination. Furthermore, α-amylase increased loaf volume less than did the glucoamylase or glucoamylase and α-amylase combinations. Bussière and Guévière (1974) reported that glucoamylase was able to produce the same amount of carbon dioxide as sucrose did (up to 6%).

A raw-starch digesting enzyme (RSDE) was developed in Japan. Its origin is the fungus *Aspergillus* sp. K-27. It has 70% glucoamylase and 30% α-amylase activity; its optimum for raw-starch digestion is pH 4.6–5.5; and it is inactivated by heat at 60°C (Abe et al 1988). It is produced commercially as Dabiase (Daikin Industries, Osaka, Japan)

The objective of this study was to determine and study the effects of this RSDE on the properties of white pan bread baked with normal and reduced sucrose levels. Furthermore, a specific objective was to ascertain whether sucrose in white pan bread can be partially replaced by the RSDE.

MATERIALS AND METHODS

Materials

Untreated baker's flour was used in this study. Flour analytical data are given in Valjakka et al (1994).

Dabiase RSDE has 70% glucoamylase and 30% α-amylase activity. The enzyme was added to the sponge at three levels: 0.02, 0.13, and 0.25% (based on flour weight). These levels were selected on the basis of preliminary baking experiments (Valjakka et al 1994).

In bread without sucrose, the effects of RSDE were compared to those of other α-amylase preparations: a commercial bacterial α-amylase (Novo Nordisk, Danbury, CT) and a fungal α-amylase (Rohm Tech, Malden, MA).

Baking Procedure

One pound loaves of white pan bread were made following the Kansas State University test procedure for sponge-and-dough bread as described in Valjakka et al (1994). In this study, three different sucrose levels were used: normal (6% sucrose), low (2% sucrose), and none (0% sucrose).

Gassing Power

The amount of gas produced was measured using a gasograph (Demaray Scientific Instruments, Pullman, WA). Ten grams of dough was placed in a bottle and fermented for 120 min in the gasograph; then the amount of released gas was recorded.

Moisture Content

Whole bread moisture content was determined at day 1 after baking (AACC 1983).

Firmness

Firmness was determined at day 1, 4, and 7 after baking, using the Voland-Stevens-LFRA texture analyzer as described in Valjakka et al (1994).

Agtron Color

Crust color was measured using an Agtron model M-500-A with a wide-area viewer M-300-A (Magnuson Engineers, San Jose, CA). Color was measured at three different positions on the top of a whole loaf (right, center, and left) using the red mode. Disc 12 (gray) was used for 0% calibration and disc 75 (white) for 100% calibration.

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Residual Sugars

Residual sugars (glucose, fructose, disaccharides, and maltotriose) were determined in bread crumbs containing no or an intermediate level (0.13%) of RSDE at all sucrose levels. Analyses were performed on a Hitachi 655A-12 high-performance liquid chromatography (HPLC) system, equipped with an ERC-7510 refractive index detector. The guard column was μBonda-pak C18 Guard-PAK, and the separation column cation exchange column in Na^+ form. Eluent was deionized water. Flow rate was 0.5 ml/min.

In preparing the sample for HPLC, moist bread crumb was ground with a coffee grinder and air-dried. Ten grams of air-dried sample was mixed with 100 ml of distilled water and stirred for 30 min. Thereafter, the sample was centrifuged at 3,000 rpm for 10 min. Water-solubles were filtered through glass-fiber filter paper. A 10-ml aliquot was mixed with 40 ml of ethanol to precipitate proteins and gums left in the water-solubles. After ethanol precipitation, the mixture was evaporated to dryness. The sediment was rehydrated with 10 ml of distilled water and filtered through glass-fiber filter paper and membrane filter (0.2 μm) to obtain the sample for HPLC. The temperature of the separation column was 85°C.

The standard solution was prepared in deionized water (milligrams per 100 ml): glucose (98.7 mg), fructose (73.4 mg), maltose (52.3 mg), lactose (79.6 mg), and maltotriose (39.2 mg). The retention times for maltotriose, disaccharides, glucose, and fructose were 5.8, 6.7, 8.5, and 9.4 min, respectively.

Statistical Methods

Statistical differences between treatments were analyzed using the general linear model (SAS 1979).

RESULTS AND DISCUSSION

Proof Time

In no-sucrose doughs, RSDE significantly decreased proof time (Table I). This was caused by increased glucose formation followed by increased yeast fermentation. In low and normal sucrose doughs, the RSDE did not affect proof time significantly, although RSDE increased proof time slightly at normal sucrose level. This was probably because increased glucose formation increased osmotic pressure in the dough, slowing fermentation.

TABLE I
Effects of Raw-Starch Digesting Enzyme (RSDE) on Proof Time at Different Sucrose Levels[a]

RSDE, %	Proof Time, min		
	0% Sucrose	2% Sucrose	6% Sucrose
0	80 a	56	60
0.02	61 b	59	60
0.125	52 c	56	68
0.25	53 c	57	69

[a] Different letters indicate statistically significant difference at the 5% alpha level.

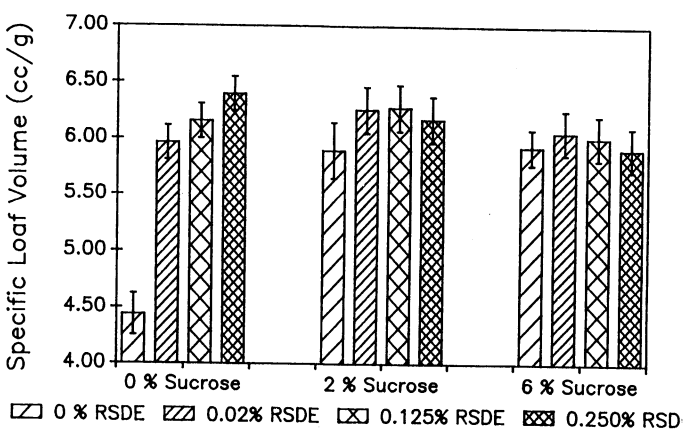

Fig. 1. Effect of sucrose and raw-starch digesting enzyme (RSDE) on specific loaf volume.

Specific Loaf Volume

In no-sucrose doughs, RSDE increased specific loaf volume significantly. The lowest level of enzyme gave a considerable increase in specific loaf volume, resulting in a loaf volume comparable to that of the control bread (Fig. 1). Further increases in the level of RSDE resulted in an additional increase in specific loaf volume, although it was no longer statistically significant.

In earlier studies on no-sucrose doughs (Pomeranz et al 1964, Rubenthaler et al 1965), a loaf volume comparable to that of 6% sucrose bread could not be obtained with any glucoamylase and α-amylase combination or with these enzymes separately. In their experiments, none of the enzymes added enhanced the dough proof time to a normal duration.

In the present study, separate trials were conducted to evaluate the effects of bacterial and fungal commercial enzymes in no-sucrose doughs. Both enzymes, especially the fungal enzyme, increased loaf volume, but not as much as the RSDE (Table II). The effect of RSDE during dough fermentation can be seen in proof time. Although bacterial and fungal enzymes increased loaf volume, they did not decrease proof time of no-sucrose dough (Table II). A significant decrease in proof time was obtained only with RSDE. In no-sucrose bread, RSDE produced fermentable sugars during fermentation; the proof time and loaf volume were comparable to those of normal-sucrose (control) bread.

Low-sugar and normal-sugar doughs were similarly affected by the RSDE: an increase in the level of enzyme increased specific loaf volume to a certain point, after which it started decreasing (Fig. 1). At the 2 and 6% sucrose levels, the differences in specific loaf volume were not statistically significant.

Gassing Power

In no-sucrose doughs, RSDE significantly increased the amount of gas produced during dough fermentation (Table III). In the 2 and 6% sucrose doughs, the enzyme did not significantly affect the amount of gas produced during dough fermentation, probably because these doughs had an adequate supply of fermentable sugars.

Bread Crust Color

Both the amount of sucrose and the amount of RSDE significantly affected crust color measured by Agtron (Fig. 2). At all sucrose levels, crust color significantly increased with increasing levels of RSDE. In no-sucrose and low-sucrose breads, a crust

TABLE II
Effects of Different Enzymes on Specific Loaf Volume and Proof Time of No-Sucrose Bread[a]

Sample	Specific Loaf Vol. (cm^3/g)	Proof Time (min)
Control	3.64 a	85 a[b]
Bacterial enzyme	5.07 b	85 a
Fungal enzyme	5.93 b	85 a
Raw-starch digesting enzyme	6.93 c	54 b

[a] Different letters indicate statistically significant difference at 5% alpha level.
[b] Not proofed to height in 85 min.

TABLE III
Effects of Sucrose and Raw-starch Digesting Enzyme (RSDE) on Gassing Power of Dough[a]

RSDE, %	Sucrose Level, %		
	0	2	6
0	172 a	211	195
0.02	203 b	218	187
0.125	214 c	221	172
0.25	225 c	206	184

[a] Different letters indicate statistically significant difference at the 5% alpha level.

color comparable to that of control bread (6% sucrose, no enzyme) was obtained with the highest level (0.25%) of RSDE. At the low sucrose level, the intermediate level of RSDE gave acceptable crust color.

The intermediate level of RSDE increased the amount of residual glucose in no-sucrose bread crumb from 0.05 to 1.2% (dwb). Although this increased crust color, it was not sufficient to produce a crust color comparable to that of the control bread (6% sucrose).

Enzyme-treated no- and low-sucrose breads had more fructose than did no- and low-sucrose control breads, in which fructose was partially used for yeast fermentation. Thus, higher fructose content in these enzyme-treated breads affected crust color. Theoretically, the increase in crust color could be attributed to the presence of reducing sugars other than glucose and fructose. However, HPLC analysis of RSDE-treated breads disproved this possibility, because no other sugars than glucose could be detected.

Bread Quality

The RSDE decreased water absorption, and because all doughs were made with fixed water absorption, doughs made with the enzyme were softer than doughs made without it. However, only small differences occurred in appreciable effects on dough-handling properties. Breads made with higher levels of enzyme had a slightly open crumb grain (Table IV), which probably could be explained by dough softness.

Increased levels of the RSDE had a tendency to produce keyholing, which was probably due to excessive starch degradation by the enzyme. Keyholing occurred generally with the highest level of enzyme and was less frequent in normal-sucrose breads than it was in no- and low-sucrose breads. The occurrence of keyholing can also be seen as a decrease of symmetry scores (Table IV).

In normal-sucrose breads, bread score decreased with increasing levels of RSDE (Fig. 3). The decrease in bread score was mainly due to the increase in crust color. In low-sucrose breads, best scores were obtained with low and intermediate levels of enzyme. The occurrence of keyholing at the highest enzyme level decreased bread score in low-sucrose bread. In no-sucrose breads, increasing the amount of the RSDE increased bread score (Fig. 3 and Table IV). The increase was caused mainly by the increase in crust color.

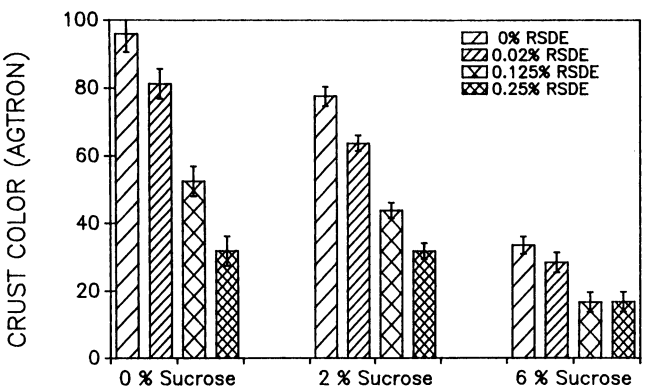

Fig. 2. Effect of sucrose and raw-starch digesting enzyme (RSDE) on bread crust color measured by Agtron. Reduced reflection indicates darker color.

TABLE IV
Effects of Intermediate Level of Raw-Starch Digesting Enzyme (RSDE) on Bread Quality at Different Sucrose Levels[a]

Level	Crust color[b]	Grain[b]	Symmetry[b]	Bread score[c]
0% Sucrose				
0 RSDE	5.0 a	6.8	6.9	38.7
0.125% RSDE	6.5 b	6.3	6.5	39.7
2% Sucrose				
0 RSDE	5.6 a	6.3	7.1	39.0 a
0.125% RSDE	6.8 b	6.3	6.7	40.2 bc
6% Sucrose				
0 RSDE	7.0 a	6.6	7.0	41.1 a
0.125% RSDE	5.3 c	6.3	6.9	39.2 b

[a] Different letters indicate statistically significant difference at the 5% alpha level.
[b] Maximum 10.
[c] Maximum 60.

Moisture Content

The RSDE had significant effects on bread moisture content only in no-sucrose breads; enzyme-supplemented breads had lower moisture content than did nonsupplemented bread. Furthermore, moisture content decreased with increasing sucrose level: 39.8, 38.2, and 36.9% in no-, low-, and normal-sucrose breads, respectively.

Residual Sugars

Glucose, fructose, disaccharides, and maltotriose were detected in all tested samples (Table V). With the separation column used, the individual disaccharides could not be separated.

All tested bread samples gave a peak at 10.35 min that could not be identified. The retention time was closest to arabinose. This peak was obtained with yeast solution, flour, and all bread samples, but not with starch or starch treated with RSDE.

Starch was treated with RSDE to see what sugars are produced from starch by the enzyme. After 3 hr of incubation, the amount of maltose increased slightly, whereas a considerable increase in the amount of glucose was achieved. Sugars other than glucose or maltose could not be identified.

The amount of glucose increased with increasing sucrose levels and was higher in enzyme-supplemented breads than it was in nonsupplemented breads. The difference in the amount of residual glucose between nonsupplemented breads and supplemented breads was statistically significant at all sucrose levels (Table V). The increase in glucose was presumably affected by the glucoamylase action.

The increase in sucrose level also increased the amount of residual fructose in bread crumb. Furthermore, at reduced sucrose levels (0 and 2% sucrose), the fructose content was slightly higher in enzyme-supplemented bread than it was in nonsupplemented bread. This difference was statistically significant (Table V). Glucose is preferred to fructose in yeast fermentation when both sugars are present. It is possible that fructose was utilized by yeast because other fermentable sugars were lacking in nonsupplemented breads at reduced sucrose levels. It is noteworthy that the levels of glucose are higher than the levels of fructose in enzyme-treated sucrose doughs. This glucose-to-fructose ratio is the opposite of that encountered in breads produced with sucrose or high-fructose syrups. The reason for the higher level of glucose in breads supplemented with RSDE is probably that the glucose is generated from starch in the doughs by the enzyme at a higher rate than this sugar could be fermented by yeast.

The results for glucose and fructose in low- and normal-sucrose breads are in good accordance with Kai (1985), who reported

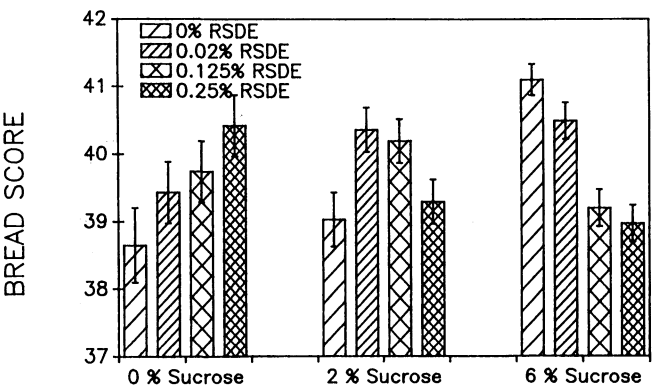

Fig. 3. Effect of sucrose and raw-starch digesting enzyme (RSDE) on bread score. Maximum 60.

TABLE V
Amount of Residual Glucose, Fructose, Disaccharides, and Maltotriose in Bread Crumb[b]

	0% Sucrose		2% Sucrose		6% Sucrose	
	0% RSDE[c]	0.125% RSDE	0% RSDE	0.125% RSDE	0% RSDE	0.125% RSDE
Glucose	0.052 a	1.242 b	0.102 a	1.832 b	1.330 a	3.085 b
Fructose	0.072 a	0.294 b	0.421 a	0.874 b	2.021	2.119
Disaccharides	2.037	2.418	2.064	1.989	1.723	1.719
Maltotriose	0.203 a	0.450 b	0.147 a	0.367 b	0.114 a	0.289 b

[a] Percent on dry weight basis.
[b] Different letters indicate statistically significant difference at the 5% alpha level.
[c] Raw-starch digesting enzyme.

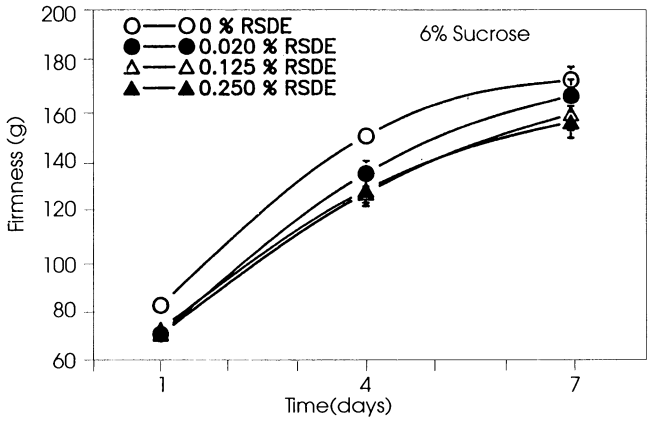

Fig. 4. Effect of sucrose and raw-starch digesting enzyme (RSDE) on bread firmness at normal-sucrose level.

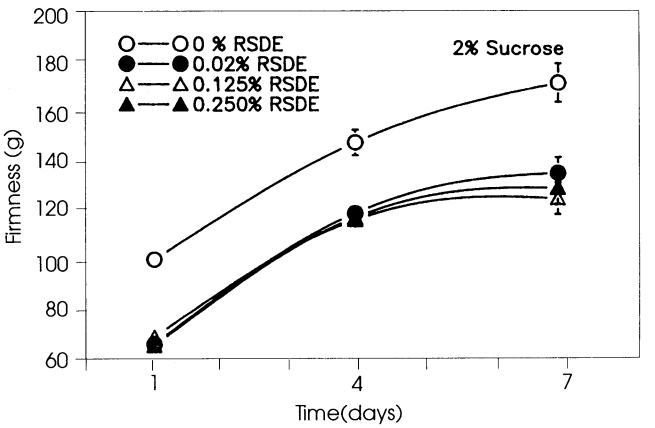

Fig. 5. Effect of sucrose and raw-starch digesting enzyme (RSDE) on bread firmness at low-sucrose level.

the amounts of glucose, fructose, and maltose in sponge-and-dough breads at different sucrose levels.

The amount of disaccharides was not affected by sucrose level nor by the enzyme treatment (Table V). Disaccharides were mainly lactose and maltose. Because the amount of nonfat dry milk added to all breads was equal, it can be assumed that the lactose content in all breads was equal. If all lactose added to dough remained in bread, the lactose content would be ≃1.5% (dwb). Thus, the maltose content in bread crumb was rather small, with lactose comprising most of the disaccharides. Kai (1985) reported that maltose content in sponge-and-dough bread at different sucrose levels (1–7%) was 0.3–0.4% (dwb).

Maltotriose content was higher in enzyme-supplemented breads than it was in nonsupplemented breads. Sucrose level did not affect maltotriose content (Table V).

Firmness

Adding RSDE to normal-sucrose dough decreased initial bread firmness measured at day 1 after baking, but it did not affect bread firming rate (Fig. 4). The enzyme-treated bread remained less firm than did the control bread throughout the storage time of seven days. In addition, an increase in the level of RSDE did not further decrease bread firmness, indicating that the maximum decrease in bread firmness can be obtained with low levels of RSDE.

In low-sucrose breads, the effects on bread firming were similar to those in normal-sucrose breads. The enzyme produced bread that was less firm initially, but it remained softer than control bread throughout the seven day storage time (Fig. 5). An increase in the level of enzyme did not affect firmness.

In no-sucrose breads, the lowest level of RSDE significantly reduced bread firmness (Fig. 6). This can be explained, at least partially, by the increase in loaf volume. A firmness value comparable to that of the control bread (6% sucrose, no added enzyme) was obtained with the lowest level of RSDE. Further increase in the level of RSDE did not affect bread firmness significantly, as was the case also in breads with higher sucrose levels.

In no-sucrose breads, RSDE also retarded the bread firming

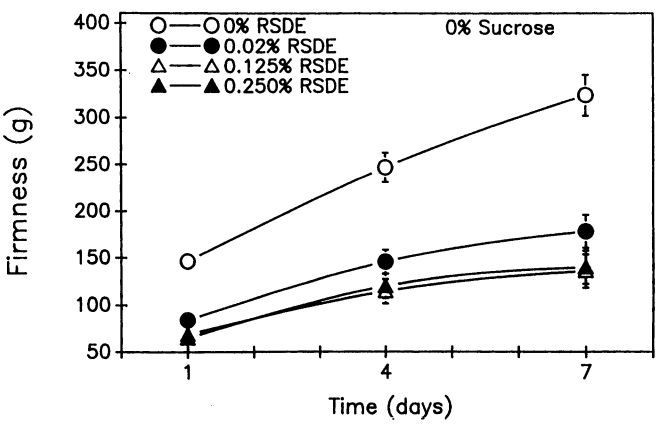

Fig. 6. Effect of sucrose and raw-starch digesting enzyme (RSDE) on bread firmness at no-sucrose level.

rate. All enzyme-treated breads firmed at a slower rate than did the control bread. Specific loaf volume affects bread firming rate (Axford et al 1968) and, thus, the differences in bread firming rate can be explained, at least partially, by the differences in specific loaf volume.

Because glucoamylase does not have effect on bread firmness (Bussière and Guérivière 1974), the effects of RSDE on bread firmness in the present study were probably due to the α-amylase activity.

Replacement of Sucrose by RSDE

RSDE was most beneficial in no- and low-sugar doughs. By generating glucose, it accelerated yeast fermentation and increased crust color. The effects were mainly due to glucoamylase activity, but α-amylase also had some functions. The α-amylase activity is needed to produce more substrate for glucoamylase and to further increase glucose formation. In some bread baking studies, glucoamylase has been found to produce glucose at a slow rate, and thus, a loaf volume comparable to that of normal-sucrose

bread could not be obtained in no-sucrose breads (Pomeranz et al 1964). Because the RSDE preparation digested raw starch, it had more available substrate than did conventional enzymes, which increased its effectiveness. Also, the presence of α-amylase in this enzyme preparation, and its synergistic effect with glucoamylase, affected glucose formation.

In no-sucrose and low-sucrose breads, loaf volume and a firmness value comparable to those of control bread (6% sucrose, no added enzyme) were obtained with the lowest level of RSDE. However, a crust color comparable to that of the control bread was not obtained except with the highest level of enzyme. Because the highest enzyme level may produce keyholing, it is advisable to use more sucrose (or other ingredients that affect crust color) and lower levels of enzyme. On the other hand, acceptable crust color was obtained at a low-sucrose level with an intermediate level of enzyme.

Although RSDE increased crust color, high crust color at reduced sucrose levels could not be achieved without side effects (keyholing). Thus, RSDE seems to be most beneficial in products for which high loaf volume and fairly light crust color are desired at low sucrose levels.

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