Studies on a Raw-Starch Digesting Enzyme. I. Comparison to Fungal and Bacterial Enzymes and an Emulsifier in White Pan Bread[1,2]

T.-T. VALJAKKA,[3] J. G. PONTE, JR.,[4] and K. KULP[5]

Cereal Chem. 71(2):139–144

ABSTRACT

The effects of a raw-starch digesting enzyme (RSDE) in white pan bread were compared to those of commercial fungal and bacterial enzymes and an emulsifier (monoglyceride). The bacterial enzyme reduced the firming rate of bread, whereas the fungal enzyme and the RSDE decreased the initial bread firmness but did not affect the rate of firming. The emulsifier decreased both the initial firmness and the rate of bread firming. The RSDE increased bread crust color significantly, which in turn decreased the bread score. Furthermore, with higher levels of RSDE, key-holing (weakening of bread side-walls) occurred occasionally. With the combination of an enzyme and an emulsifier, bread was less firm than bread in which these additives were used separately, but none of the tested enzymes nor the emulsifier had synergistic interaction on bread firmness in white pan bread.

Amylases are used in baking to increase the level of fermentable sugars in bread doughs and to modify dough physical properties, both of which improve bread quality. Bacterial α-amylase is very effective in retarding bread firming rate, but unfortunately, it may produce a gummy and sticky bread crumb. Fungal and cereal α-amylases can also be used to decrease bread firmness, but they are not as effective as α-amylase from bacterial sources (Miller et al 1953). However, recent work by Martin and Hoseney (1991) showed that fungal α-amylase could be as effective as bacterial α-amylase.

Several studies have shown that starch plays a major role in bread firming (Kulp and Ponte 1981). Schoch and French (1947) suggested that bread firming was caused by heat-reversible aggregation of amylopectin. Amylose was mostly retrograded in cooled bread; hence, it could not contribute to bread staling in storage.

Factors other than starch recrystallization also may be involved in bread staling. Martin et al (1991) suggested that bread firming could be caused by interactions of starch and protein. During baking, starch granules swell and amylose is partially leached from granules. However, Inagaki and Seib (1992) showed that bread also firms without amylose in the starch, and they proposed that, during baking or aging of bread, interactions between swollen starch granules and the gluten matrix somehow occur. During aging of the bread, amylopectin recrystallizes, resulting in increased rigidity of the starch granule and decreased flexibility of the gluten matrix. All these changes seem to contribute to crumb firming.

Most α-amylase preparations digest native, ungelatinized starch at a very slow rate. At starch pasting temperature, only bacterial α-amylase is fully active for any appreciable length of time (Schultz et al 1952). A glucoamylase-amylase preparation able to digest native starch at a high rate was developed in Japan (Abe et al 1988a). It is an enzyme originating from the fungus *Aspergillus* K-27. This enzyme has 70% glucoamylase and 30% α-amylase

[1]Contribution 93156J from the Agricultural Experiment Station, Kansas State University, Manhattan.
[2]Presented in part at the AACC 75th Annual Meeting, Dallas, TX, October 1990.
[3]Graduate research assistant, Department of Grain Science and Industry, Kansas State University, Manhattan. Present address: Vaasan Leipomot Oy 0 0241, Helsinki, Finland.
[4]Professor, Department of Grain Science and Industry, Kansas State University, Manhattan.
[5]American Institute of Baking, Manhattan, KS.

activity, and it is able to hydrolyze native corn starch completely in a 24-hr incubation period. Mainly glucose was produced, but, at early stages of fermentation, oligosaccharides were also found (Bergmann et al 1988). The optimum pH for raw-starch digestion is pH 4.6–5.5, and heat activation of the enzyme begins at 60°C (Abe et al 1988a). In this raw-starch digesting enzyme (RSDE), degradation of raw starch granules is due mainly to the glucoamylase activity, while α-amylase exerts a synergistic action. Both enzymes are essential for maximum starch degradation. The glucoamylase component was relatively thermostable, retaining 90% of its activity after 1 hr at 60°C (Abe et al 1988b).

The overall objective of this study was to determine and study the effects of an RSDE on the properties, especially bread firming, of white pan bread. The effects were compared to those of an emulsifier and bacterial and fungal enzymes. Furthermore, the possible synergistic interactions between enzymes and emulsifier and their effects on bread firming were studied.

MATERIALS AND METHODS

Flour

Flour used in this study was unmalted and unbromated baker's flour obtained from Cargill (Wichita, KS). It contained 12.0% protein and 0.41% ash (14% mb). Water absorption (farinograph) was 59.8%; falling number was 404 sec; and starch damage was 5.4% of flour (Chopin SD4 starch damage analyzer, Chopin S.A., Paris).

Baking Additives

Enzymes. Three different amylase preparations were used. All exhibited at least two major enzyme activities. The fungal commercial α-amylase preparation (Rohm Tech, Malden, MA) had several enzyme activities. The high-temperature bacterial α-amylase preparation (Novo Nordisk Bioindustriales, Danbury, CT) had α-amylase and pullulanase activities. The RSDE (Dabiase, Daikin Industries, Osaka, Japan) was a fungal enzyme from *Aspergillus* K-27 and a mixture of glucoamylase and α-amylase. According to Hizukuri (personal communication 1993), the preparation contained small amounts of secondary activities such as cellobiase, carboxymethylcellulase, xylanase, pectinase, and low levels of protease. Use levels of enzymes and the emulsifier (Table I) were determined by test bakings. On the basis of these tests, low and high levels of enzymes and emulsifier were selected to obtain minimum and maximum use levels; the intermediate levels were the average between high and low levels.

Emulsifier. The emulsifier was a preparation of hydrated monoglycerides (American Ingredients, Kansas City, MO).

Physical Tests

AACC approved methods (1983) were used for flour protein (46-10), ash (08-01), amylograph (22-10), falling number (56-81B), farinograph (54-21), and bread moisture (44-18).

Sample Preparation

Five grams of wheat starch was incubated with the highest level of enzyme (Table I) in a citrate buffer (pH 5.35) for 4 hr. The starch-buffer slurry was centrifuged at 3,000 rpm for 10 min and washed four times with deionized water. Centrifugate from the last wash was air-dried and examined by scanning electron microscopy (SEM). Aluminum stubs were covered with double sticky tape, and the starch samples were applied to the sticky surface and coated with 200Å gold-palladium. The samples were then examined with a scanning electron microscope (Phillips 505) and photographed.

Baking Procedure

One-pound loaves of white pan bread were made following a typical test procedure for sponge-and-dough bread (formulas presented in Table II). Enzymes and an emulsifier were added to the sponge. Sponges were fermented for 4 hr at 27°C and 80% rh. After it was mixed, dough was fermented for 30 min at 27°C and 80% rh. Three loaves (539 g) were made from each dough. Loaves were proofed to height (2.5 cm above the top rim of pan) at 43°C and 90% rh. Loaves were baked for 20 min at 232°C. Loaf volume (measured by rapeseed displacement method) and weight were measured immediately after baking. Breads were cooled at room temperature for 90 min before being placed in polyethylene bags and stored at 22°C for up to seven days. Loaves were scored at day 1 after baking for crust and crumb color, break and shred, symmetry, grain, and texture.

Firmness

Firmness at day 1, 4, and 7 after baking was measured using a Voland-Stevens-LFRA texture analyzer. Loaf slices were 25 mm thick. The middle slice and the end slices were discarded. Six slices were analyzed from each loaf. The plunger diameter was 25 mm. Slices were compressed to 4 mm at a plunger speed of 1 mm/min. The firmness value is the load (*g*) required to compress the bread slice under the described conditions.

Interaction Studies

Possible interactions among each of the three enzymes and the emulsifier were studied separately. In each study, five different levels of emulsifier and enzyme were used in 13 combinations.

Statistical Methods

Statistical differences among treatments were analyzed using the general linear model (SAS 1979). Response surface analysis (Myers 1971) was used to study the interactions among enzymes and emulsifier.

RESULTS AND DISCUSSION

Physical Tests

Adding RSDE to flour decreased falling number, amylograph peak viscosity, and maximum peak temperature but did not affect

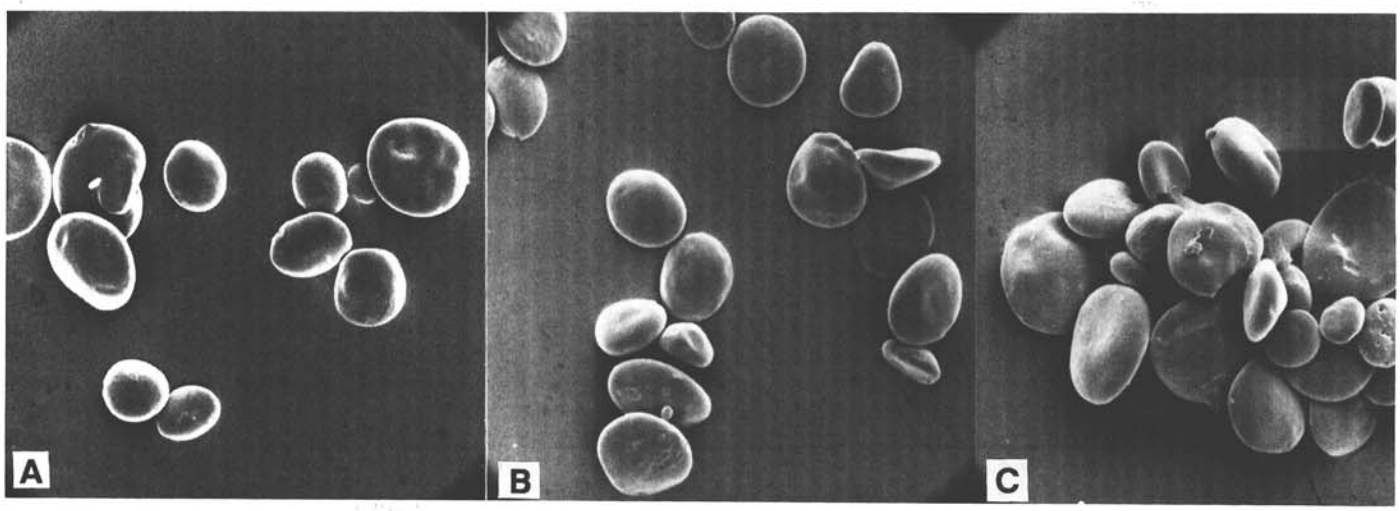

Fig. 1. Scanning electron micrographs of control starch (**A**) and starches treated with fungal (**B**) and bacterial (**C**) enzymes.

TABLE I
Levels of Enzymes and Emulsifier Used in Baking Tests

Additive	Level (% based on flour weight)		
	Low	Intermediate	High
Bacterial enzyme	0.05 (12.5 NU)[a]	0.15 (37.5 NU)	0.25 (62.5 NU)
Fungal enzyme	0.01 (50 SKB)[b]	0.015 (75 SKB)	0.20 (100 SKB)
Raw-starch digesting enzyme	0.02 (400 AUN)[c]	0.13 (2700 AUN)	0.25 (5000 AUN)
Emulsifier	0.5	1.0	1.5

[a]NU = amount of enzyme that breaks down 5.26 mg of soluble starch per hr at 37°C, pH 5.6, as assayed by Novo-Nordisk, Bioindustriales, Danbury, CT.
[b]SKB units as determined by method 22-01 (AACC 1983).
[c]AUN = the amount of enzyme that produces reducing power equivalent to 0.1 mg of glucose from 5 ml of 1.2% potato starch solution at pH 4.5 at 40°C in 1 min (Abe et al 1988b).

TABLE II
Dough Formula[a]

Ingredient	Sponge, %	Dough, %
Flour[b]	70	30
Water	38.4	26.6
Instant active dry yeast	1.0	...
Yeast food	0.25	...
Sucrose	...	6.0
Nonfat dry milk	...	3.0
Shortening	...	3.0
Salt	...	2.0

[a]Baker's percent.
[b]Total flour 1,000 g.

pasting temperature (Table III). These effects can be explained by the relatively high thermostability of the glucoamylose component of the RSDE (Abe et al 1988b).

The RSDE decreased farinograph water absorption, dough development time, and dough stability, as indicated by stability values and the mixing tolerance index (Table IV). A similar observation was made by Higashiura et al (1990), who also found decreased farinograph absorption with RSDE. This effect is difficult to explain because starch modification by the RSDE would be expected to increase rather than decrease water absorption. This change can be attributed to the presence of hemicellulases and their action on flour pentosans (Kulp 1968). According to the present data, the effect is of little significance in baking.

Scanning Electron Microscopy

Scanning electron micrograms of untreated starch and starches treated with bacterial and fungal enzymes are shown in Figure 1. The control starch and the starch treated with fungal enzyme appeared rather intact and without surface modification, whereas enzyme attack can be seen clearly in the bacterial enzyme-treated starch. These results agree with Kuricina et al (1987).

Scanning electron micrograms of RSDE-treated starch are shown in Figure 2. At lower magnification, enzyme attack can be seen as surface modifications. In micrographs taken at higher magnification, the mode of enzyme action can be seen more clearly.

Specific Loaf Volume

Tested enzymes and the emulsifier had very little effect on specific loaf volume. With the fungal enzyme and the RSDE,

TABLE III
Effects of Raw-Starch Digesting Enzyme (RSDE) on Falling Number and Amylogram

RSDE (%) in Flour	Falling Number, sec	Amylogram		
		Pasting Temperature, °C	Peak Viscosity, BU	Peak Temperature, °C
0	392	60.0	2,155	88.0
0.02	271	59.5	820	88.0
0.125	176	59.5	240	75.5
0.250	140	59.5	140	73.0

TABLE IV
Effects of Raw-Starch Digesting Enzyme (RSDE) on Farinogram (14% mb)

RSDE (%)	Water Absorption, %	Development Time, min	Stability, min	Mixing Tolerance Index, BU
0	59.8	9.0	17.50	10
0.02	57.5	8.0	14.50	25
0.125	55.5	2.75	12.25	30
0.25	55.5	2.0	10.50	35

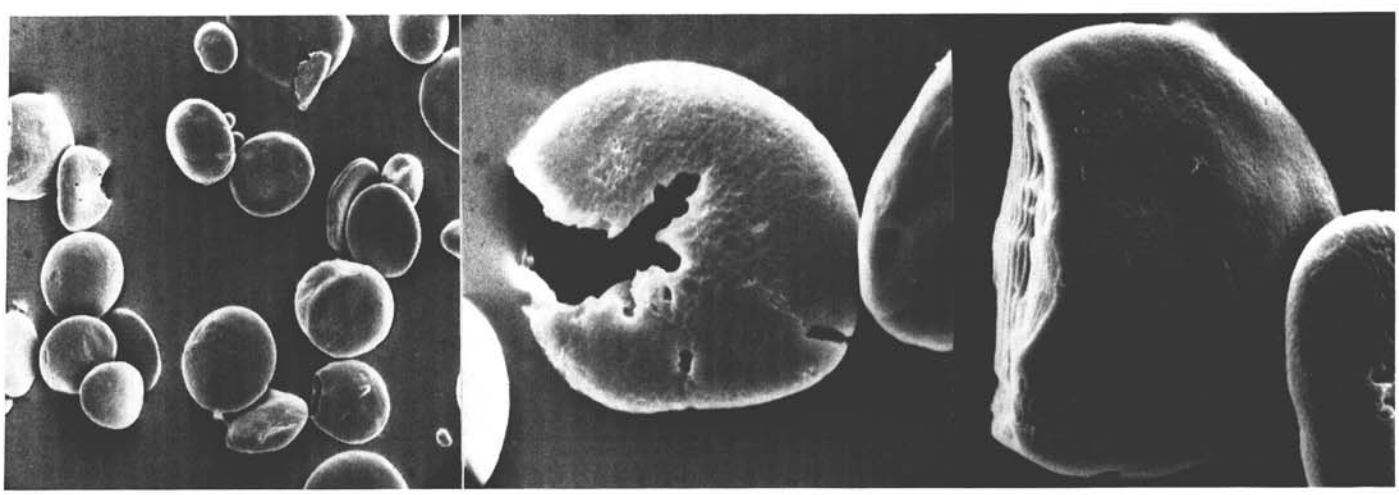

Fig. 2. Magnification by scanning electron micrograph of starch treated with raw-starch digesting enzyme.

specific loaf volume was increased slightly at certain levels, but the emulsifier and the bacterial enzyme had no effect. The small differences observed in specific loaf volume were not statistically significant.

Cauvain and Chamberlain (1988) showed that fungal α-amylase increased loaf volume and attributed this to longer dough expansion time in the oven. This effect of fungal α-amylase has been reported by Maninder and Jorgensen (1983) and Kuracina et al (1987). In the present study, an increase in specific loaf volume was obtained with an intermediate level of fungal α-amylase, but the difference, when compared to that of the control bread, was not statistically significant.

The fungal RSDE behaved in a manner similar to that of the conventional fungal enzyme. An increase in specific loaf volume was obtained with lowest level of RSDE, but loaf volume started decreasing with higher enzyme levels.

The emulsifier and bacterial enzyme did not affect specific loaf volume in this study, although previous reports indicated that bacterial enzyme did increase loaf volume (Rubenthaler et al 1965, Kuracina et al 1987).

Bread Quality

Increased levels of RSDE decreased bread score, as did low and high levels of fungal and bacterial enzymes (Fig. 3). Unlike the tested enzymes, the emulsifier did not affect bread score.

The decrease in bread score with RSDE was mainly due to the excessively dark crust color. Higher levels of RSDE increased crust color; fungal and bacterial enzymes did not affect crust color at tested levels. Some studies indicate that α-amylase increases crust color (Rubenthaler et al 1965, Maninder and Jorgensen 1983), although Kuracina et al (1987) could not show any increase in crust color caused by α-amylase.

The decrease in bread score with low and high levels of fungal and bacterial enzymes was partially due to a decrease in break and shred score and partially to a decrease in grain score.

Doughs treated with enzymes were slightly softer than control dough. However, this softening did not have any noticeable effect on dough-handling properties. Crumb grain in breads made with higher levels of enzymes was slightly open, which probably can be explained by the differences in dough softness.

Moreover, at higher levels RSDE had a tendency to produce weakness of loaf side-walls, generally referred to by bakers as *keyholing*. This effect was apparently caused by excessive starch digestion by the enzyme. Conventional α-amylases digest mainly damaged starch during sponge fermentation. Because the amount of damaged starch in flour is limited, conventional fungal α-amylases do not cause much keyholing. In this sense, RSDE behaved like flour with excessive amounts of damaged starch or flour milled from sprout-damaged wheats. Relatively heat-stable wheat α-amylase produces excessive starch degradation in the oven during baking.

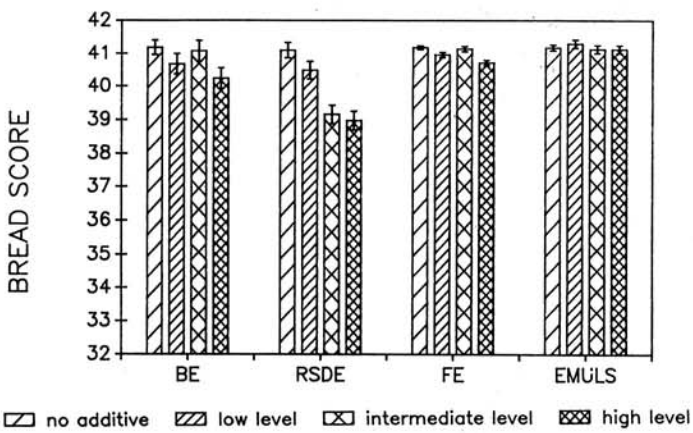

Fig. 3. Effect of tested enzymes and emulsifier on bread score. BE = bacterial enzyme; RSDE = raw-starch digesting enzyme; FE = fungal enzyme; EMULS = emulsifier.

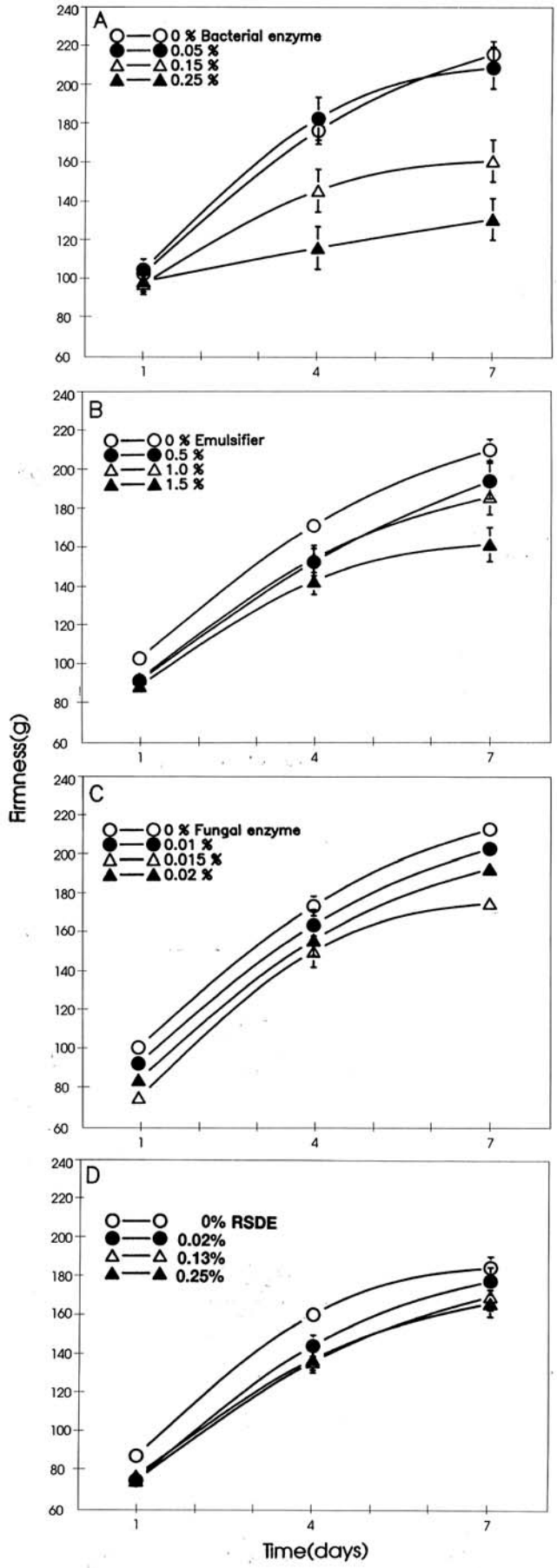

Fig. 4. Effect of baking additives at different levels on bread firmness. **A,** bacterial enzyme; **B,** emulsifier; **C,** fungal enzyme; **D,** raw-starch digesting enzyme (RSDE).

Moisture Content

Moisture content in breads varied from 36.4 to 38%. None of the tested baking additives affected bread moisture content significantly; therefore, moisture content differences were not factors in bread firmness measurements.

Firmness

All tested baking additives affected bread firmness. The bacterial enzyme did not affect the initial bread firmness, but reduced the firming rate in storage when used at the two higher test levels (Fig. 4A). The lowest level of enzyme did not affect bread firmness, but with intermediate and high levels of enzyme, bread firmness measured at days 4 and 7 after baking was reduced significantly, compared to that of the control of the same age. With the highest level of enzyme, bread firmness increased only slightly during the seven-day storage period. Unfortunately, the highest level of bacterial enzyme also produced sticky bread crumb, which is considered undesirable.

The reduction of firming rate by bacterial enzymes has been reported earlier (Miller et al 1953, Bussière and de la Guérivière 1974, Dragsdorf and Varriano-Marston 1980). The latter authors reported a decrease in initial bread firmness, but in the present study the bacterial enzyme did not have that effect.

Breads with emulsifier were initially less firm and exhibited slightly lower firming rates with storage time than did the control (Fig. 4B). Increased emulsifier levels further decreased bread firmness.

Ofelt et al (1958), and later Pisesookbuntern and D'Appolonia (1983), reported that monoglycerides retarded the rate of bread firming rather than decreasing the initial bread firmness. However, Schoch (1965) showed the opposite: monoglycerides decreased the initial bread firmness but did not affect the bread firming rate. In the present study, monoglycerides both decreased the initial bread firmness and retarded the rate of bread firming.

As reported earlier (Dragsdorf and Varriano-Marston 1980), the fungal enzyme decreased initial bread softness but did not affect bread firming rate. The least firm bread was obtained with 0.015% fungal enzyme (Fig. 4C). Because the enzyme-treated bread was initially less firm than the control bread, it remained less firm during the seven-day storage period.

The same trend in bread firmness can be observed with RSDE. Less firm bread was obtained with the enzyme initially, the bread remained less firm than the control bread, and the rate of firming was not affected (Fig. 4D). The lowest use level of enzyme produces most of the antifirming effect; additional levels of enzyme yielded little, if any, additional effect. The RSDE had an effect on bread firmness similar to that of the fungal enzyme. Thus, the raw-starch digesting property of an RSDE apparently does not give any extra benefits, in terms of bread firming, when compared with that of conventional fungal enzyme.

TABLE V
Statistical Data of Enzyme-Emulsifier Interaction Study on the Effect on Bread Firmness

	Probability Values		
Firmness	**BE[a]**	**FE[b]**	**RSDE[c]**
Day 1			
Cross-product	0.975	0.222	0.752
Effect of enzyme	0.682	0.176	0.527
Effect of emulsifier	0.129	0.018	0.029
Day 4			
Cross-product	0.464	0.622	0.895
Effect of enzyme	0.004	0.653	0.996
Effect of emulsifier	0.142	0.102	0.653
Day 7			
Cross-product	0.474	0.639	0.908
Effect of enzyme	0.009	0.296	0.471
Effect of emulsifier	0.084	0.004	0.156

[a]Bacterial enzyme.
[b]Fungal enzyme.
[c]Raw-starch digesting enzyme.

Because the RSDE did not decrease bread firmness more than the conventional fungal enzyme did, it is probable that the effect of α-amylase on decreasing bread firmness is not caused by its action time, but instead is caused by its action pattern. Schultz et al (1952) suggested that because bacterial α-amylase has higher heat stability, its residual activity in baked bread is able to prevent the recrystallization of starch by degrading it during bread storage.

However, Zobel and Senti (1959), and then Dragsdorf and Varriano-Marston (1980), showed increased crystallization with bacterial α-amylase. Zobel and Senti (1959) suggested that bacterial α-amylase cleaves linkages in the amorphous regions of starch, where they are most accessible to enzyme attack. This gives the crystallites greater freedom to move and results in a decreased rigidity of the system. On the other hand, according to the theory of Martin and Hoseney (1991), bacterial and fungal α-amylases produce small dextrins that interfere with starch-protein interaction and, thus, retard bread firming.

Interaction Studies

Monoglycerides decrease the swelling of starch. The microcrystals of monoglycerides adhere to the starch granules, and because water cannot penetrate these crystals, part of the starch granule surface is shielded from the action of water. This is observed as decreased swelling of starch granules (Van Lonkhuysen and Blankestijn 1976). Furthermore, studies showed that α-amylase cannot attack the clathrate formed between amylose and monoglycerides (Van Lonkhuysen and Blankestijn 1976). Reports also indicate that enzymes alone have very little effect on bread staling, and that emulsifiers alone increase bread softness. When bacterial α-amylase was added to the dough together with crumb softener emulsifiers, like monoglycerides, the firming rate was greatly reduced (DeStefanis and Turner 1981). Therefore, the interactions between enzymes and an emulsifier on bread firmness were studied in the present investigation.

However, no statistically significant interactions were found between the enzymes and emulsifier that affected bread firmness, as is evident from data in Table V. This observation failed to confirm earlier synergistic effect reported by Martin (1989) in pup loaves. Enzymes and the emulsifier decreased bread firmness. The bread obtained with the combination of enzyme and emulsifier was less firm than that obtained when these additives were used separately, but there was no interaction.

When a combination of bacterial enzyme and emulsifier was used, the bacterial enzyme had a major effect on firmness in breads measured at days 4 and 7 after baking. However, the emulsifier, in combination with the RSDE and the fungal enzyme, had a major effect on bread firmness. These results also indicate that the bacterial α-amylase and fungal α-amylase affect bread firmness differently.

ACKNOWLEDGMENTS

We would like to express gratitude to Susumu Hizukuri of Kagoshima University, Japan, for providing the raw-starch digesting enzyme sample and communicating analytical data of the enzyme.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 08-01, approved April 1961, revised October 1981; Method 22-10, approved May 1960, revised October 1982; Method 44-18, approved April 1961, reviewed October 1976 and October 1982; Method 46-10, approved April 1961, revised October 1976 and September 1985; Method 54-21, approved April 1961, revised October 1982; Method 56-81B, approval November 1972, revised October 1982, October 1988, and September 1992. The Association: St. Paul, MN.

ABE, J., BERGMANN, F. W., OBATA, K., and HIZUKURI, S. 1988a. Production of the raw-starch digesting amylase of *Aspergillus* sp. K-27. Appl. Microbiol. Biotechnol. 27:447.

ABE, J., NAKAJIMA, K., NAGANO, H., and HIZUKURI, S. 1988b. Properties of the raw-starch digesting amylase of *Aspergillus* sp.K-27: A synergistic action of glucoamylase and alpha-amylase. Carbohydr. Res. 175:85.

BERGMANN, F. W., ABE, J., and HIZUKURI, S. 1988. Selection of organisms which produce raw-starch degrading enzymes. Appl. Microbiol. Biotechnol. 27:443.
BUSSIERE, G. and DE LA GUÉRIVIERE, J.-F. 1974. Utilisation d'alpha-amylase et de glucamylase en technologie de panification industrielle. Ann. Technol. Agric. (Paris) 23:175.
CAUVAIN, S. P., and CHAMBERLAIN, N. 1988. The bread improving effect of fungal α-amylase. J. Cereal Sci. 8:239.
DE STEFANIS, V. A., and TURNER, E. W. 1981. Modified enzyme system to inhibit bread firming method for preparing same and use of same in bread and other bakery products. U.S. patent 4,299,848.
DRAGSDORF, R. D., and VARRIANO-MARSTON, E. 1980. Bread staling: X-ray diffraction studies on bread supplemented with α-amylases from different sources. Cereal Chem. 57:310.
HIGASHIURA, T., OBATA, K., HASEBE, T., and TOTAO, K. 1990. The bread improving effect of raw-starch degrading enzymes. In: International Symposium on Cereal and Other Plant Carbohydrates. Kagoshima University: Japan.
INAGAKI, T. and SEIB, P. A. 1992. Firming of bread crumb with cross-linked waxy barley starch substituted for wheat starch. Cereal Chem. 69:321.
KULP, K. 1968. Enzymolysis of pentosans of wheat flour. Cereal Chem. 45:339.
KULP, K. and PONTE, J. G., Jr. 1981. Staling of white pan bread: Fundamental causes. CRC Crit. Rev. Food Sci. Nutr. 15:1.
KURACINA, T. A., LORENZ, K., and KULP, K. 1987. Starch functionality as affected by amylases from different sources. Cereal Chem. 64:182.
MANINDER, K., and JORGENSEN, O. B. 1983. Interrelations of starch and fungal α-amylase in breadmaking. Starch/Staerke 35:419.
MARTIN, M. L. 1989. Rethinking bread firming. Ph.D. Diss. Kansas State University: Manhattan, KS.
MARTIN, M. L., and HOSENEY, R. C. 1991. A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. Cereal Chem. 68:503-507.
MARTIN, M. L., ZELEZNAK, K. J., and HOSENEY, R. C. 1991. A mechanism of bread firming. I. Role of starch swelling. Cereal Chem. 68:498-503.
MILLER, B. S., JOHNSON, J. A., and PALMER, D. L. 1953. A comparison of cereal, fungal, and bacterial alpha-amylases as supplements for breadmaking. Food Technol. 7:38.
MYERS, R. H. 1971. Response surface methodology. Allyn and Bacon: Boston, MA.
OFELT, C. W., MacMASTERS, M. M., LANCASTER, E. B., and SENTI, F. R. 1958. Effect on crumb firmness. I. Mono- and diglycerides. Cereal Chem. 35:137.
PISESOOKBUNTERN, W., and D'APPOLONIA, B. L. 1983. Bread staling studies. I. Effect of surfactants on moisture migration from crumb to crust and firmness values of bread crumb. Cereal Chem. 60:298.
RUBENTHALER, G., FINNEY, K. F., and POMERANZ, Y. 1965. Effects on loaf volume and bread characteristics of alpha-amylases from cereal, fungal, and bacterial sources. Food Technol. 19:239.
SAS. 1979. User's Guide. SAS Institute: Cary, NC.
SCHOCH, T. J. 1965. Starch in bakery products. Bakers Dig. 39(2):48.
SCHOCH, T. J., and FRENCH, D. 1947. Studies on bread staling. I. The role of starch. Cereal Chem. 24:231.
SCHULTZ, A. S., SCHOONOVER, F. D., FISHER, R. A., and JACKEL, S. S. 1952. Retardation of crumb starch staling in commercial bread by bacterial alpha-amylase. Cereal Chem. 29:200.
VAN LONKHUYSEN, H., and BLANKESTIJN, J. 1976. Influence of monoglycerides on the gelatinization and enzymatic breakdown of wheat and cassava starch. Starch/Staerke 28:227.
ZOBEL, H. F., and SENTI, F. R. 1959. The bread staling problem. X-ray diffraction studies on breads containing a cross-linked starch and a heat-stable amylase. Cereal Chem. 36:441-451.

[Received January 7, 1993. Accepted November 17, 1993.]