was substantially increased without an increase in β-amylase, large quantities of dextrins were produced (Walden 1955).

Effect of Malted Barley Flour on Bread Firming

In the United States, 0.1–0.5% malted barley flour is normally added to flours used in yeast-leavened breads. Johnson and Miller (1948) showed that up to 6% added malted barley flour did not have a detrimental effect on bread quality or reduce bread firmness. In apparent contrast, Ponte et al (1963) baked bread containing 0.1 and 0.2% malted barley flour and measured firmness with a Baker compressimeter. Increased levels of malted barley flour lowered the firming rate.

The present study was undertaken to investigate the effect of starch hydrolysis on bread firming and incorporate the information obtained into a proposed mechanism of bread firming.

MATERIALS AND METHODS

Flours used were donated by Cargill (Wichita, KS). The flours had protein contents of 11.5–12.1% (14%, mb). All flours used firmed at essentially the same rate. Bacterial α-amylase was Dex-lo P from Gist-Brocades (King of Prussia, PA). β-Amylase type I-B from sweet potato, fungal α-amylase from *Aspergillus oryzae,* and pullulanase from *Enterobacter aerogenes* were purchased from Sigma Chemical Co. (St. Louis, MO). The monoglyceride was AM 341 from Grindsted Products, Inc. (Industrial Airport, KS). Other ingredients were as specified by AACC method 10-09 (AACC 1983).

Bread was baked according to the standard pup-loaf procedure of AACC method 10-09 (AACC 1983). Fermentation time was 180 min. Fermipan instant yeast (0.76 g) was used instead of compressed yeast, and malted barley flour was used in place of malt syrup.

Bacterial α-amylase was in powdered form, and the stated activity level was 55,000 SKB/g. It was dispersed in water to facilitate supplementation in bread. Dilution of the enzyme to 50 SKB/0.01 ml did not affect its activity, but dilution to 50 SKB/ml destroyed its activity. β-Amylase, 27,930 Sigma units (SU)/ml, was used as received or diluted to 1,000 SU/ml. (SU for β-amylase is defined as follows: One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 4.8 at 20°C.) Dilution did not affect the activity of β-amylase or fungal α-amylase.

Bread was stored in polyethylene bags at ambient temperature for five days unless otherwise noted. Firmness was measured as described in the companion article (Martin et al 1991).

The high-performance liquid chromatography (HPLC) system was a Varian (Palo Alto, CA) Model 5000 equipped with a 20-μl automatic injection loop. Detection was with a refractive index detector (ERMA Optical Works). The equipment had a Hewlett Packard Model 3392A integrator. A CH-30 column heater (Anspec Co. Inc., Milwaukee, WI) that maintained 85°C was used to detect oligosaccharides DP 2–9.

Residual maltose was separated from bread crumb and quantified by HPLC according to AACC method 80-04 (AACC 1983). A Bio-Sil amino 5S column, 250 × 4mm, equipped with an Amino 5S guard column (Bio-Rad Laboratories, New York), and packed with a 5-μm amino-bonded silica material, was used to separate glucose, fructose, lactose, maltose, and sucrose at room temperature.

After the bread was stored for five days in polyethylene bags, the crust was removed and the moist crumb was ground in a coffee grinder. Crumb moisture was determined by AACC method 44-18 (AACC 1983). A 10-g sample of bread crumb was placed in a 250-ml centrifuge bottle, and 100 ml of distilled water was added. The sample-water slurry was stirred with a magnetic stirrer for 25 min at room temperature. The sample was then centrifuged at 1,000 × *g* for 10 min. Supernatant was filtered twice—first through a Whatman No. 2 filter paper and then through a No. 5 filter—and refrigerated. Within the next 48 hr, the supernatant (10 ml) was placed in a 50-ml volumetric flask, diluted to volume with acetonitrile, and allowed to warm to room temperature. Samples were filtered through a 5-μm filter (Millipore) before injection onto the HPLC column. The mobile phase was a mixture of acetonitrile and water (80:20). An eluant flow rate of 1.0 ml/min was used. Column temperature was ambient, the refractive index detector was set at 35°C, and chart speed was 1 cm/min.

An Aminex HPX-42A column (Bio-Rad) packed with 4% cross-linked cation exchange resin in the silver form and equipped with a carbohydrate deashing guard column was used. The mobile phase was 100% distilled water. Eluant flow rate was 0.5 ml/min. The extraction procedure for analysis of dextrin chain length after debranching is similar to the procedure described above. Before dilution with acetonitrite, the supernatant was slurried with ethanol (40 ml), filtered, evaporated, and rehydrated. Pullulanase was used for debranching when specified.

RESULTS AND DISCUSSION

Effect of Monoglyceride on Thermal Stability of Amylases

Surfactants have been shown to stabilize and denature proteins. Denaturation temperatures of bacterial α- and β-amylases were measured by differential scanning calorimetry (DSC). DSC scans of bacterial α-amylase showed two endotherms with onset temperatures of 75 and 95°C. β-Amylase (Sigma) suspended in a 2.3*M* solution of $(NH_4)_2SO_4$ showed a single endothermic peak with onset at 74°C. Addition of monoglyceride to α- or β-amylase did not shift the onset temperature of the endotherms. These results indicated that the monoglyceride did not function by affecting the thermal stability of the amylases.

To determine whether monoglyceride affected the activity of β-amylase by other means, residual maltose in bread crumb was measured. Addition of monoglyceride to the bread formula did not affect the quantity of residual maltose present in the bread (2.8%, db). However, bread crumb supplemented with monoglyceride (1%) and β-amylase (50 SU per gram of starch) had less residual maltose (3.4%) than did bread crumb with β-amylase only (4.5%). Therefore, in a system with high levels of β-amylase, monoglyceride appeared to reduce β-amylase activity.

To further characterize the functionality of monoglyceride, bacterial α-amylase was added to bread supplemented with β-amylase and monoglyceride. Residual maltose content of bread crumb indicated that bacterial α-amylase overcame the effect of monoglyceride on β-amylase activity.

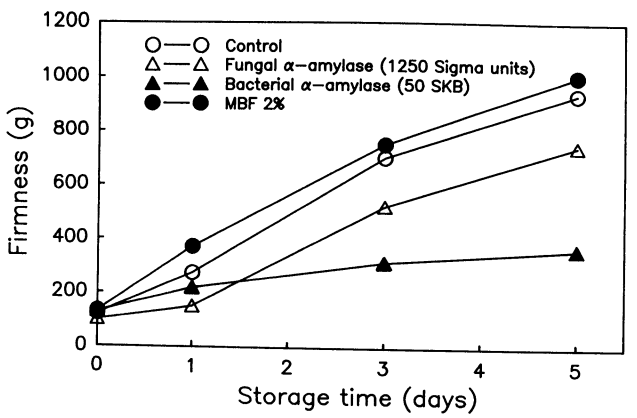

Fig. 1. Effect of malted barley flour (MBF), bacterial α-amylase, and fungal α-amylase on bread firmness.

Role of α-Amylases in Bread Firming

To further understand the mechanism of bread firming, breads supplemented with bacterial α-amylase, fungal α-amylase, or malted barley flour were compared (Fig. 1). Bread baked with a high level of bacterial α-amylase did not firm during five days of storage. Bread baked with 1,250 SU of fungal α-amylase firmed at a moderate rate. However, bread supplemented with malted barley flour (2%) firmed at essentially the same rate as did the control bread.

Window of Amylase Activity

A window of amylase activity exists between the temperature at which starch begins to swell and the temperature at which amylases are denatured. During this window of activity, amylases degrade starch. To confirm that a window of activity existed during baking, dextrins (DP 3–9) in water solubles extracted from dough and bread crumb were measured by HPLC. Doughs prepared with or without bacterial α-amylase (50 SKB units/loaf) and fermented for 5 hr did not contain any measurable amount of dextrins between DP 3 and 9. After baking, no dextrins between DP 3 and 9 were found in the water-soluble extract of bread (control formula). However, dextrins in the DP 3–9 range were detected in bread baked from flour supplemented with bacterial α-amylase. A profile of the dextrins extracted from bread crumb is shown in Figure 2. Clearly, a window for activity of amylases on starch existed during baking.

Effect of β-Amylase

Firmness and residual maltose contents of bread made with flour supplemented with various levels of β-amylase are shown in Table I. Addition of 1,000 SU of β-amylase per gram of starch resulted in 7.1% residual maltose, whereas 50 SU of β-amylase resulted in 4.2% residual maltose. Bread made from flour supplemented with 50–1,000 SU of β-amylase were similar in firmness after five days of storage.

Flour Plus α-Amylase

The effect of bacterial α-amylase at 10, 20, and 50 SKB units per loaf on bread firmness is shown in Figure 3. Bread baked from flour supplemented with 50 SKB units of bacterial α-amylase firmed at a rate of 81 g/day. Bacterial α-amylase at 100 SKB units per loaf effectively inhibited bread firming. Bacterial α-amylase is more thermostable than is cereal α-amylase; therefore, large quantities of cereal α-amylase were used to attempt to overcome the shorter window of activity. Bread baked from flour with either 1 or 2% malted barley flour added to the formula firmed at a faster rate than did bread baked without malt supplement (Fig. 4). The addition of 10% malted barley flour (about 100 times the normal level of addition) still firmed (data not shown). Therefore, it appears that the antifirming mechanism of bacterial α-amylase involved more than a higher degree of dextrinization of starch during a longer window of activity.

In an excess water system and with starch as a substrate, the method of attack of α-amylase has been described as essentially random cleavage of α-(1-4) glycosidic linkages. However, in a limited water system such as bread, the degradation of the starch would not be randomized throughout the granule. With limited swelling, the enzyme activity would be concentrated on the outside or near the surface of the granule. The enzyme is large and does not diffuse rapidly into a partially swollen starch granule. The substrate most easily hydrolyzed might be the starch molecules that had been leached or partially leached from starch granules.

Analysis of Residual Dextrins in Bread

Bread supplemented with bacterial α-amylase (100 SKB units per loaf) did not firm during five days of storage. In contrast, bread supplemented with malted barley flour firmed at a faster rate than did unsupplemented bread. Based on the inconsistency of these results and the "known" antifirming effects of α-amylase, we sought to understand why malted barley flour did not have an antifirming effect. The residual dextrins of DP 3–9 produced by the enzyme systems were studied. Profiles of dextrins extracted from bread supplemented with bacterial α-amylase and malted barley flour are shown in Figures 5 and 6, respectively. Dextrins of DP 3–7 were found in the extract from bread supplemented with bacterial α-amylase. No extractable dextrins of DP 3–9 were found in bread supplemented with 2% malted barley flour. The quantity of dextrins in the void volume indicated that the dextrins produced by the malted barley flour were larger than DP 9. Dextrins of DP 3–9 also were present in bread supplemented with fungal α-amylase (data not shown).

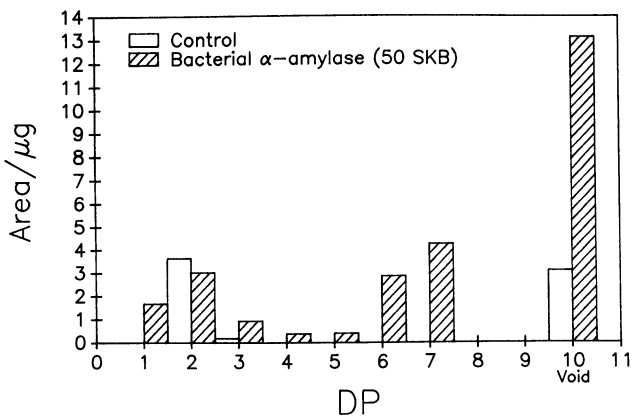

Fig. 2. Profile of water-soluble dextrins extracted from unsupplemented bread crumb and bread supplemented with bacterial α-amylase. DP = degree of polymerization.

TABLE I
Effect of β-Amylase on Residual Maltose and Bread Firmness

Treatment	Firmness on Day 5 (g)	Residual Maltose (%, db)
Flour	1,210	2.6 ± 0.2[a]
+ β-amylase (50 SU)[b]	1,020	4.2 ± 0.2
+ β-amylase (200 SU)	965	5.4
+ β-amylase (1,000 SU)	1,015	7.1

[a]SD, n = 3.
[b]Sigma units: One unit will liberate 1 mg of maltose from starch in 3 min at pH 4.8 at 20°C.

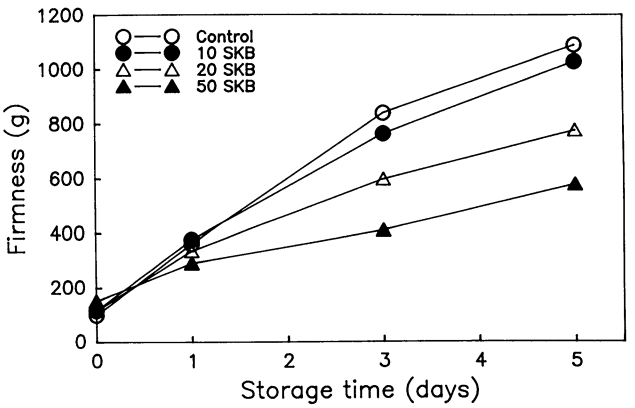

Fig. 3. Effect of bacterial α-amylase on bread firmness.

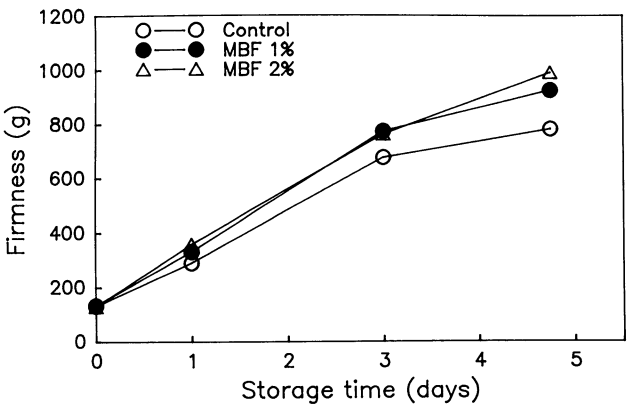

Fig. 4. Effect of malted barley flour (MBF) on bread firmness.

Relevance of a Debranching Enzyme

α-Amylase produces branched dextrins of random size. If α- and β-amylases and a debranching enzyme were all active in a bread system, the lack of residual dextrins in a flour + malt system would be explained. Results would depend on the relative heat stability of the different enzymes.

Pullulan was hydrolyzed with pullulanase. HPLC analysis showed the presence of dextrins of many sizes. Thus, pullulanase attack was essentially random, and the dextrins produced were resolved on the HPLC column.

Malted barley flour was added to pullulan to determine if malted barley flour contained a debranching enzyme specific for α-(1-6) glycosidic linkages. After incubation at 30°C for 24 hr, HPLC of the water-soluble extracts did not show an increase in maltotriose. Therefore, the supposition that the malted barley flour contained a debranching enzyme was not supported.

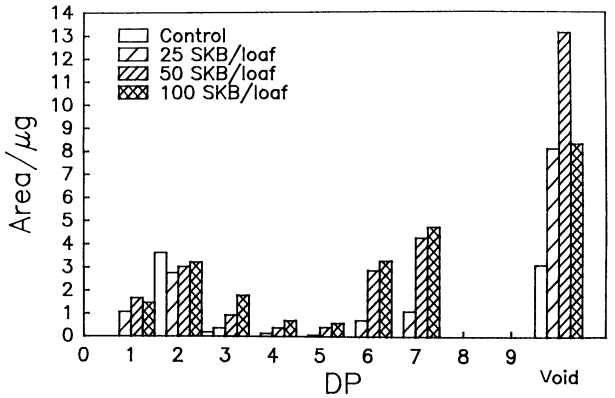

Fig. 5. Profile of dextrins extracted from bread supplemented with bacterial α-amylase. DP = degree of polymerization.

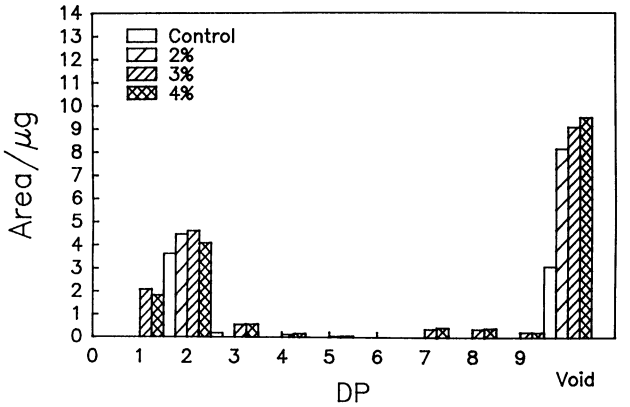

Fig. 6. Profile of dextrins extracted from bread supplemented with malted barley flour (2–4%). DP = degree of polymerization.

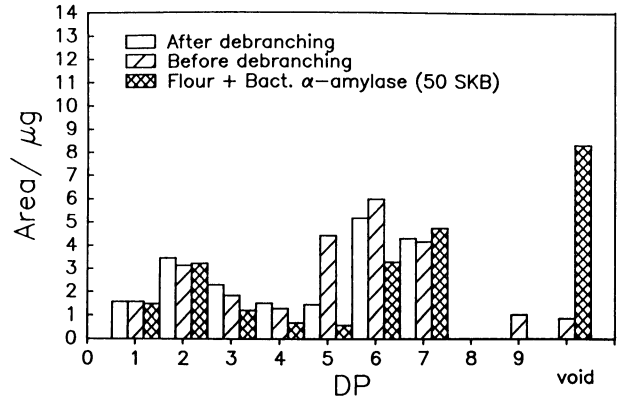

Fig. 7. Profile of dextrins extracted from bread crumb after precipitation of large dextrins with ethanol and resolubilizing with water. DP = degree of polymerization.

Pullulanase was added to the water-soluble extract from bread crumb with malted barley flour added. The HPLC column that was used resolved low molecular weight dextrins (DP less than 9). Ethanol was used to precipitate the higher molecular weight dextrins from the water-soluble extract of bread. HPLC analysis showed that residual dextrins of DP 5 and 6 increased after ethanol precipitation. The ethanol-soluble fraction after rehydration in water was hydrolyzed with pullulanase (1%). This had only a minor effect on the profile of dextrins. Dextrins greater than DP 8 were eliminated and those of DP 5 increased. Quantities of other dextrins were essentially unchanged (Fig. 7). The lack of a significant increase in low molecular weight dextrins indicated that most of the water-soluble dextrins of less than DP 8 were not branched. Therefore, the antifirming effect of bacterial α-amylase vs. the lack of effect of malted barley flour might be related to the size of the dextrins produced and not the presence of branched dextrins.

Effect of Pullulanase on Bread Firming

Bread supplemented with pullulanase (10 SU) firmed at a faster rate than did unsupplemented bread. The firming profile of bread supplemented with pullulanase is shown in Figure 8. Debranching amylopectin yielded a wide distribution of dextrin chain lengths from 12 to more than 90 glucose units (Kobayashi et al 1986). Based on the above, it appears that high molecular weight dextrins do not have an antifirming effect on bread crumb. Instead, the high DP dextrins may entangle and/or form a hydrogen bond with protein fibrils, thus effectively cross-linking the gluten. Consequently, the firming rate is increased.

A Model of Bread Firming

The fact that bread supplemented with malted barley flour (2%) did not contain low molecular weight dextrins and did not retard firming, whereas bread supplemented with either fungal or bacterial α-amylase contained low molecular weight dextrins and retarded firming, suggests that the low molecular weight dextrins indeed have antifirming properties. It appears that the different sources of α-amylase produce fragments of different sizes. Dextrins of an intermediate size are effective in decreasing bread firmness.

These results can be applied to the mechanism of bread firming presented in the companion article (Martin et al 1991). A model of the mechanism of bread firming and the effect of dextrins on the mechanism are presented in Figure 9. Because maltose is a small molecule relative to larger dextrins, it may diffuse away from the interface between starch and protein. If maltose molecules are not at the interface, interference with the entanglements between starch and protein or protein/protein does not occur. Large dextrins produced by malted barley flour and pullulanase enhance the rate of bread firming. The most reasonable explanation at present is that the larger dextrins act to cross-

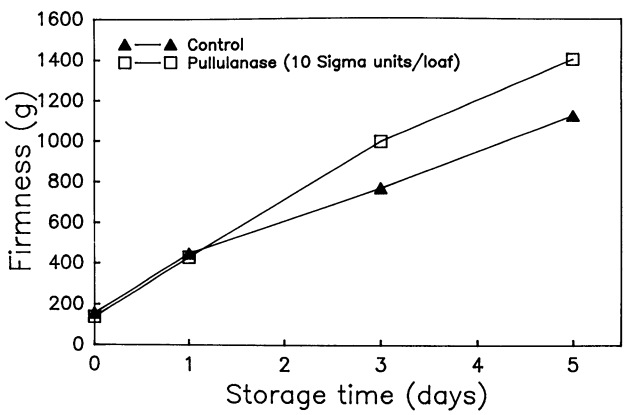

Fig. 8. Effect of pullulanase on bread firmness.

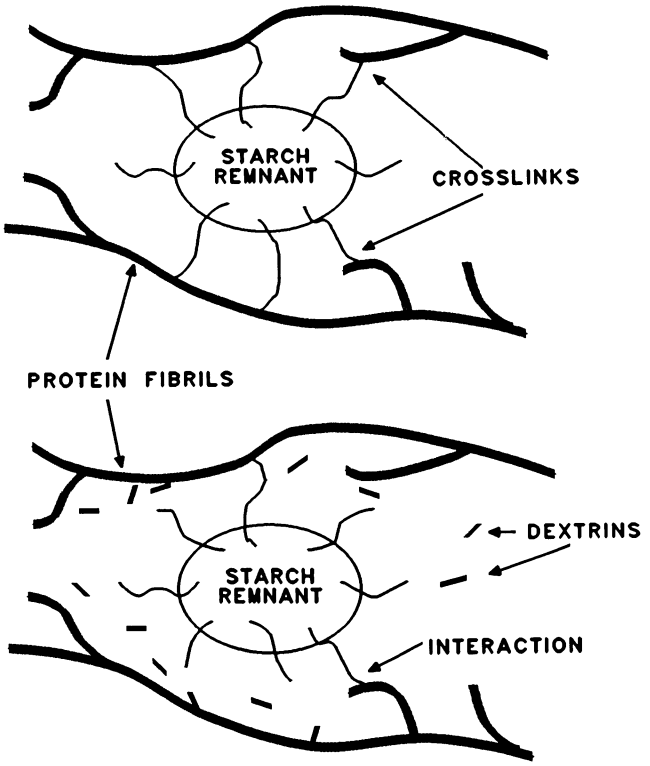

Fig. 9. Mechanism of bread firming and the antifirming role of dextrins.

link the protein fibrils, thus bread becomes firmer during storage.

The firming mechanism suggested herein accounts not only for the well-known antifirming effect of shortening and monoglycerides (restricting the swelling of starch) and the effect of certain amylases (production of certain dextrins), but also for a number of other factors. For example, the fact that bread firms more rapidly at a lower temperature is consistent with the effect of temperature on polymer interactions. The fact that bread can be refreshened with mild heating is explained by the heat-liable nature of hydrogen bonds and the effect of heat on polymer entanglements. Also of interest is the fact that reheated bread (as well as "brown-and-serve" bread) firms at a rapid rate. This can be explained by the diffusion of dextrins away from the starch-protein interface during storage and the fact that no new dextrins are produced during reheating. With no dextrins present, the bread firms at a rapid rate.

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