Effect of Different Enzymes on the Textural Stability of Shelf-Stable Bread

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ABSTRACT

Three enzyme systems (2 amylase-based and 1 protease-based) were tested in shelf-stable bread to determine effectiveness in preserving texture during storage for eight weeks. Each enzyme was tested in formulations without glycerol or with 6% glycerol. Bread samples were analyzed to determine physical properties (crumb density, crust-to-crumb ratio, rate of moisture distribution from crumb to crust), mechanical properties (modulus, and a parameter [C1] describing resistance to high levels of deformation obtained by fitting stress-strain data to a three-parameter function), and thermal properties (thermal stability and enthalpy of transitions) as a function of storage time. Mechanical properties were further analyzed to predict asymptotic firmness. Bread firmness after storage as evaluated in terms of modulus and C1 were lower in all enzyme-added systems, the effect of protease being the most significant. Enzymes had less effect on glycerol-containing systems with no apparent trend. The breads had complex thermal behavior and exhibited multiple transitions. Both amylase preparations in the presence of glycerol reduced the amount of starch recrystallization.

Military requirements for the shelf-stability of ration items are more stringent than those for commercial products. Army meal, ready-to-eat (MRE) products are generally expected to be shelf-stable and acceptable for up to 36 months of storage at 27°C (2001 revision of Joint Services Manual 4145.12). Specifically, the requirements for bread and sandwich products are textural and microbial stability for 18 months at 27°C (Military Service Requirement ADMN 02-11). While baked products are rendered microbiologically stable through control of water activity and pH, the texture of these items can degrade during storage.

MRE bread firms appreciably during storage (Barrett et al 2002). Firming is partially attributable to the migration of moisture from the crumb to the crust. Reduction of crumb moisture content increases glass transition temperature and decreases mobility. Other changes in the macromolecular structure of flour-based items including protein-starch linkages (Martin and Hoseney 1991; van Dam and Hille 1992; Martinez-Anaya and Jimenez 1997), retrogradation (Hug-Iten et al 2001), and gluten entanglement or development of a protein network (Ruan et al 1996; Barrett et al 2000a) can also contribute to textural changes.

Different strategies have been employed to delay firming of bread. Various additives such as sucrose ester and dough conditioners have been shown to reduce starch recrystallization and firming during prolonged storage and to improve sensory quality (Barrett et al 2002). Short-term textural benefits have been reported using starch-degrading enzymes (Akers and Hoseney 1994; Martinez-Anaya and Jimenez 1997; Gil et al 1999; Hug-Iten et al 2001). The effectiveness of α-amylases has been attributed to the inhibition or blocking of starch-protein complexation by dextrin products (Martin and Hoseney 1991; van Dam and Hille 1992) and to reduced amylopectin crystallization (Defloor and Delcour 1999; Hug-Iten 2001). However, limited information exists on longer term benefits of amylase incorporation, or, since protein interactions possibly contribute to firming, on the efficacy of protease enzymes.

This work compared the effectiveness of three different enzyme systems tested in standard MRE formula bread with and without addition of glycerol. Textural changes and moisture equilibration and migration over the course of eight weeks of storage were determined. Differential scanning calorimetry (DSC) was employed to monitor changes in melting transitions of recrystallized starch developing during storage.

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MATERIALS AND METHODS

Baking and Storage

Standard MRE round rolls (70 g) were produced in batches of 4,500 g according to the formulations with and without glycerol (Table I). The enzyme preparations were in either tablet or powder form and were suspended in water from which an aliquot was drawn. Manufacturers' recommended usage levels (if a range was specified, the midpoint was used) were added to the bread formulation. Dry weights of the enzymes used were 0.2 g/batch of U3, 0.4 g/batch of U200, and 1.0 g/batch of PR59 (Grinsted-Danisco, New Century, KS). U3 contains fungal α-amylase, maltogenic α-amylase, and fungal xylanase; U200 contains bacterial α-amylase, maltogenic α-amylase (20% less than U3), and bacterial xylanase; PR59 contains protease produced by selected strains of *Aspergillus oryzea*. Xylanase, which cleaves arabinoxylans, was reported to affect water distribution in the dough (Hilhorst et al 1999). Amylase activity was ≈75 and 30 units/mg of tablet for U3 and U200, respectively (Sigma assay procedure EC 3.2.1.1). One unit of enzyme is defined as the amount that liberates 1.0 mg of maltose from starch in 3 min at 20°C and pH 6.9.

Dry ingredients were premixed at low speed using a blender (Hobart H-600, Troy, OH). Shortening, and then water, or water plus glycerol, were added. Each batch was mixed at medium speed for ≈10 min, allowed to relax for 15 min, then formed into 70-g round rolls using a dough divider (Adamatic Fortuna A4-9670, Eatontown, NJ). The rolls were proofed at 95% rh and 32°C for 40 min and baked at 175°C for 20–25 min in a rotary oven (Hobart,

TABLE I
Bread Formulations

Ingredient (Supplier)	1 (%)	2 (%)
Flour (ConAgra)	54.2	51.1
Water	30.6	28.6
Shortening (ACH Food Co.)	9.0	8.5
Glycerol (KIC Chemicals)	0	6.0
Yeast (Saf-instant)	2.4	2.2
Salt (Morton)	1.4	1.3
Sucrose ester (Montello)	1.1	1.0
Gum arabic (Gum Technology)	0.5	0.5
Calcium sulfate (ADM Arkady)	0.3	0.3
Xanthan gum (Kelco)	0.3	0.3
Encapsulated potassium sorbate (Balchem)	0.1	0.1
Cream flavor (David Michael & Co.)	0.1	0.1

Troy, NY). The baked rolls were allowed to cool to <50°C before packaging individually in trilaminate pouches (polyethylene-foil-polypropylene) 13 cm × 20 cm (Cadillac Products, Troy, MI). One "FreshPax" oxygen scavenger (Multisorb Technologies, Buffalo, NY) was added to each pouch.

The rolls were frozen at –10°C for seven days (to maintain consistency because some were shipped frozen for DSC analysis), then maintained at 22°C for the duration of storage. Samples were removed from storage at 0, 0.5, 1, 2, 3, 4, 6, and 8 weeks to determine physical, thermal, and mechanical properties.

Physical Property Testing

At each analysis time, crumb density, crumb-to-crust ratio, crumb and crust moisture contents, and water activities were determined. Density measurements were obtained by weighing cored cylinders (2 cm diameter) of crumb trimmed to 2 cm height (six replicates). Crumb-to-crust ratio was determined by separating the crust and crumb using a razor blade (determination of separation was based on white vs. brown color) and weighing each component (three replicates). Crumb and crust moisture contents were determined by vacuum drying at 70°C for 16 hr (three replicates). Crumb and crust water activities were measured using a water activity unit (AquaLab 3TE, Decagon Devices, Pullman WA) (three replicates).

Mechanical Testing

Cylindrical samples (2 cm diameter and 2 cm height) were obtained near the center of the rolls. Samples (six replicates) were compressed at a velocity of 0.2 mm/sec to 50% strain using a texture press (TXT2 texture press, Texture Technologies, Scarsdale, NY). Force-deformation data were collected at 10 pt/sec with a computer interfaced with the texture press and were converted to stress (σ) Hencky's strain (ε) data. Hencky's strain is defined as

$$\varepsilon = -\ln [H(t)/H_0] \quad (1)$$

where $H(t)$ is sample height at any time during measurement and H_0 is the initial sample height.

The slope of the linear portion of the stress-strain data, defined as the modulus E, was calculated by linear regression using the data up to 10% deformation

$$E = \sigma/\varepsilon \quad (2)$$

Modulus is a measure of stiffness (Marin 1962).

The entire data set (up to 50% deformation) were additionally fitted to

$$\sigma = C1\ \varepsilon/[(1 + C2\ \varepsilon)(C3 - \varepsilon)] \quad (3)$$

where C1, C2, and C3 are constants. Constants for the fitted equation were calculated using a software program (v. 80, Sigmaplot, Chicago, IL).

Equation 3 has been used to fit stress-strain curves for "spongy" foods (Nussinovitch et al 1991; Swyngdau et al 1991; Barrett et al 2002) and conforms to the three-part relationship described by Gibson and Ashby (1988) for cellular plastics, which exhibit an initial linear elastic region, followed by a region of lower slope (caused by buckling of cell walls), followed by a region of sharply increasing slope (caused by densification but only observable at very high strain levels). While modulus E describes the resistance to a small deformation, C1 describes the overall rigidity of the material to a relatively large deformation.

Mechanical Data Evaluation

Two-sample comparisons (v. 10.2, Minitab Statistical Software, State College, PA) were used to determine significant differences between the control and each enzyme-containing sample using the averages of modulus and C1 values for bread samples stored from two to eight weeks. Bread samples with and without glycerol were evaluated separately.

To describe the relationship between firmness and storage time, modulus and C1 values (averaged across replicates for each storage time) were fitted to the Avrami equation, which has been used to describe crystallization kinetics during storage (Cornford et al 1964). For modulus, the equation is

$$(E_f - E_t)/(E_f - E_0) = \exp(-kt^n) \quad (4)$$

where, E_f is asymptotic modulus, E_t is modulus at any time during storage, E_0 is initial modulus, k is the rate constant for development of firming, and n is the Avrami exponent related to crystal nucleation and growth. Equation 4 was also used to evaluate the relationship between the firming parameter C1 and time.

Mechanical properties tests were also performed on rolls that had been packaged without crust and maintained for four weeks of storage, to assess crumb firming in the absence of moisture loss to the crust.

Moisture Equilibration Analysis

Data for wet basis moisture content of the crumb were fitted to the equation

$$(M - M_f)/(M_0 - M_f) = \exp(-kt) \quad (5)$$

where M is the moisture content at any time, M_0 is the initial crumb moisture, M_f is the final crumb moisture, k is the moisture migration rate constant, and t is time. The value k was used in a comparative assessment of the rate of moisture migration from crumb to crust for various samples tested.

DSC Analysis

A DSC (model 2920, TA Instruments, New Castle, DE) was used to record thermograms of crumb samples to provide comparison of the thermal behavior of bread with proteolytic or amylolytic enzymes or without any added enzyme in the formulation. Bread samples were received as frozen, thawed, and underwent DSC analysis on crumb samples after 0, 2, 4, and 8 weeks of storage at 22°C. Bread samples used for DSC analysis contained 6% glycerol.

Samples (40–50 mg) were placed in stainless steel pans and sealed. An empty pan was used as reference. For each sample, two DSC protocols were employed. The first was heating from 1°C to 180°C at 5°C/min; the second was partial scanning to 90°C, followed by rapid cooling to 1°C, and a second scan to 180°C at 5°C /min to separate the reversible and irreversible transitions observed in the initial scan. The second scan was subtracted from the first scan, and the enthalpy of the irreversible endotherm developed during storage was determined.

RESULTS AND DISCUSSION

Physical Properties

Crumb bulk density and crust proportion averaged over eight weeks of storage are shown in Table II. While crumb density was increased by glycerol addition for control and the protease-

TABLE II
Bread Physical Properties[a,b]

Formulation	Crumb Density (g/cm^3)	Crust Proportion (%)
Control, no glycerol	0.29 (0.03)	21 (3.0)
Control, glycerol	0.42 (0.03)	21 (2.5)
U3, no glycerol	0.34 (0.02)	23 (1.5)
U3, glycerol	0.32 (0.03)	27 (4.9)
U200, no glycerol	0.31 (0.02)	24 (2.5)
U200, glycerol	0.31 (0.02)	30 (4.1)
PR59, no glycerol	0.28 (0.03)	21 (1.5)
PR59, glycerol	0.35 (0.02)	21 (2.2)

[a] Averaged for all within-sample over 8 weeks of storage time.
[b] Standard deviations in parentheses.

containing bread, it did not change for amylase-containing bread. Crust proportion was higher in the amylase-added bread in comparison with control and protease-added bread ($P < 0.01$). The addition of glycerol increased the crust proportion further in amylase-added bread.

Crumb water activity for enzyme-containing bread without glycerol was 0.95 ± 0.01 at 0 time and 0.92 ± 0.007 after storage by averaging all data between 2–8 weeks. For glycerol-containing and enzyme-containing bread, the crumb water activity decreased from 0.90 ± 0.008 at 0 time to 0.86 ± 0.02 after storage (averaged between 2–8 weeks). Crust water activity for enzyme-added bread without glycerol was 0.85 ± 0.03 for 0 time and 0.90 ± 0.01 after storage (averaged between 2–8 weeks) and for enzyme-added bread with glycerol was 0.79 ± 0.03 for 0 time and 0.85 ± 0.02 after storage (averaging all data between 2–8 weeks). Glycerol addition reduced water activity both in crumb and crust, as expected. During storage, crumb water activity decreased and crust water activity increased for all bread samples, indicating moisture migration from crumb to crust.

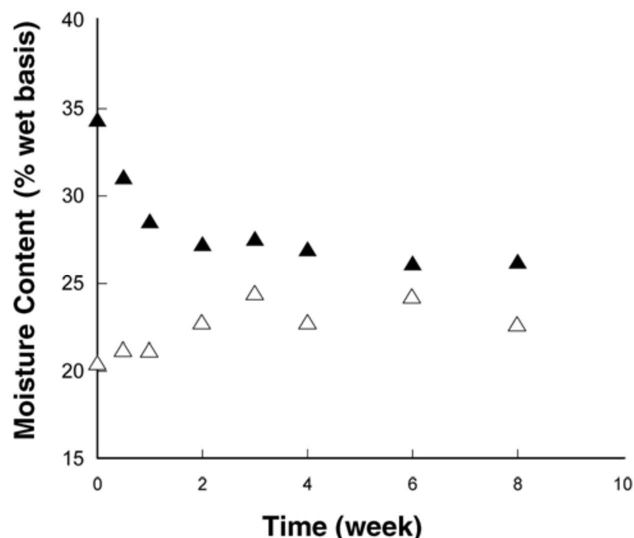

Fig. 1. Representative change in crumb moisture (▲) and crust moisture (△) as a function of storage time.

Moisture migration from crumb to crust during storage is also evident from the moisture content change of bread samples as a function of time (Fig. 1). Crumb moisture contents for all bread samples are given in Table III, with data averaged for samples stored between 2 and 8 weeks because after two weeks of storage, moisture content generally stabilized (Fig. 1). The moisture migration rate from crumb to crust, as evaluated in terms of the parameter k from Equation 5, varied among samples but was higher for the glycerol-containing bread (Table III), perhaps as a result of increased mobility due to the plasticization by glycerol. Moisture migration rates were also lower for samples containing amylolytic enzymes, possibly as a result of increased concentrations of dextrins.

Mechanical Properties

Modulus and C1 values of stored bread samples averaged between 2–8 weeks are shown in Table IV. For bread without glycerol, all the enzymes significantly (at the 0.05 level) lowered average C1 values during storage, with the protease PR59 producing the greatest effect. Modulus values for bread samples without glycerol were also lowered by the enzymes but only significantly by PR59. The more significant effect of enzyme addition on C1 rather than on modulus may be attributable to the fact that C1 represents the resistance to deformation over a relatively larger strain level (50% vs. 10% deformation). Softening that was manifest in the C1 measurements for the amylase-treated breads, in which proteins were presumably unaffected, may have been mostly attributable to changes in the cell wall that affected plastic deformation (collapse) characteristics rather than elastic (recoverable, macromolecular network) properties.

Softening by enzyme addition for bread samples containing glycerol, as evaluated by modulus and C1, was comparatively less

TABLE III
Crumb Moisture Content[a] as a Function of Storage Time, and Rate Constant for Moisture Migration from Crumb to Crust

Formulation	Storage Time (weeks) 0	0.5	1	Pooled Average 2–8 Weeks	k^b (day^{-1})	r
Control, no glycerol	40.0 (0.76)	35.0 (0.76)	28.0 (4.02)	31.3 (0.81)	0.41	0.90
Control, glycerol	37.0 (0.35)	30.0 (1.00)	29.0 (0.82)	29.3 (0.75)	0.61	0.96
U3, no glycerol	34.0 (0.30)	31.0 (0.43)	29.0 (0.94)	26.9 (0.81)	0.18	0.99
U3, glycerol	29.0 (0.97)	25.0 (0.17)	25.0 (0.28)	24.5 (0.21)	0.40	0.94
U200, no glycerol,	34.0 (0.09)	29.0 (0.14)	27.0 (0.27)	24.0 (0.61)	0.31	0.91
U200, glycerol	29.0 (1.20)	24.0 (1.20)	23.0 (0.85)	19.5 (0.76)	0.47	0.77
PR59, no glycerol	38.0 (0.70)	32.0 (2.50)	33.0 (0.74)	23.5 (1.30)	0.50	0.77
PR59, glycerol	35.0 (0.42)	30.0 (0.34)	29.0 (0.53)	28.5 (0.20)	0.56	0.97

[a] Mean values for three replicates; standard deviations in parentheses.
[b] From 0–8 week data fitted to Equation 5.

TABLE IV
Modulus[a] and C1[b] Values of Stored Bread Samples, Averaged from 2 to 8 Weeks

Sample	Modulus (kPa)	t-Ratio[c]	P[c]	C1 (kPa)	t-Ratio[c]	P[c]
Control, no glycerol	51.2	–	–	22.9	–	–
U3, no glycerol	47.3	0.71	0.48	14.0	2.21	0.03
U200, no glycerol	43.8	1.28	0.22	13.7	2.36	0.02
PR59, no glycerol	31.9	4.19	<0.01	9.33	4.23	<0.01
Control, glycerol	85.3	–	–	30.9	–	–
U3, glycerol	59.8	3.66	<0.01	29.8	0.22	0.83
U200, glycerol	84.0	0.05	0.96	26.0	1.15	0.26
PR59, glycerol	81.2	0.57	0.57	35.1	–0.80	0.43

[a] From Equation 2. Pooled data for 2–8 week (all replicates). Coefficient of variation for individual sample modulus values 32 ± 11%.
[b] From Equation 3. Pooled data for 2–8 week (all replicates). Coefficient of variation for individual sample C1 values was 38 ± 12%.
[c] Two-sample comparison between enzyme-containing batch and corresponding control batch.

pronounced and in most cases insignificant (Table IV). The only significant softening effect was the reduction in mean stored modulus of U3-added bread, which was not evident in the corresponding C1 measurement.

Asymptotic, or final, firmness parameters calculated by fitting modulus and C1 versus time data to the Avrami equation (Equation 4) are shown in Table V. Illustrative fits for modulus and C1 are displayed in Fig. 2A and B for selected enzyme-containing bread samples, with and without glycerol. The fitted parameters listed in Table V are consistent with those for average stored modulus and C1 values in that they show relatively greater effect of enzymes in no-glycerol bread with the most pronounced reduction within these samples resulted from the protease PR59 addition. The effectiveness of the protease is most likely due to interruption through direct cleavage of the developed protein-starch structure that occurred during storage. The contribution of the amylases may be assumed to be due to production of small dextrins that inhibit formation of protein-starch structure (Martin and Hoseney 1991). Xylanases, which were included in the amylase-based preparations, have been reported to affect original loaf volume but to have little independent effect on firming (Gil et al 1999).

The higher asymptotic modulus and C1 values in comparison with those of averaged values of stored bread samples suggests that firming of bread continues after two weeks, which is beyond the period of moisture equilibration. While the highest rate of textural change during the first two or three weeks of storage (Fig. 2A and 2B) corresponded to the period of moisture equilibration, physicochemical changes in the bread crumb, potentially due to macromolecular interactions, contributed to firming post moisture equilibration. This observation is supported by a comparison of modulus data for samples stored four weeks without crust with those for samples stored for the same period with crust. Bread without crust firmed appreciably, with the extent of firming somewhat lower than for rolls stored with the crust. Modulus averaged over four weeks for 0% glycerol bread samples without crust was 62% of the modulus for corresponding bread samples with crust ($P = 0.004$). For 6% glycerol bread samples without crust, the average modulus for four weeks was 82% of the modulus for corresponding bread samples with crust ($P = 0.04$). Firming, therefore, was attributable to both moisture migration and changes in the macromolecular configuration of the bread crumb.

The reduced effectiveness of the enzymes due to the presence of glycerol, in part, may be attributable to differences in the levels of moisture in the breads. Glycerol-containing rolls were formulated with a slightly lower level of water because the proportions of all ingredients, on a percent basis, were lowered. Consequently, after baking, the glycerol-containing samples had 3–5% less moisture than the breads without glycerol. Moreover, the effective moisture content (e.g., pertaining to localized moisture needed to plasticize macromolecules or to facilitate the action of the enzymes) may have been even lower than measured values. Baik and Chinachoti (2002) and Baik et al (2003) reported that high concentrations of glycerol in bread can phase-separate and competitively interact with moisture, thereby drawing water away from the macromolecular structure. Furthermore, Taub et al (1994) demonstrated through spectroscopic analyses that glycerol interacts primarily with the gluten rather than the starch phase in bread. A preferred association of glycerol with protein rather than starch is consistent with the particularly poor effect of the PR59 in glycerol-containing bread (in contrast to its significant benefit in bread without glycerol), possibly suggesting a direct inhibition by glycerol at cleavage sites. While glycerol has been reported to act as a plasticizer in flour systems due to its ability to lower glass

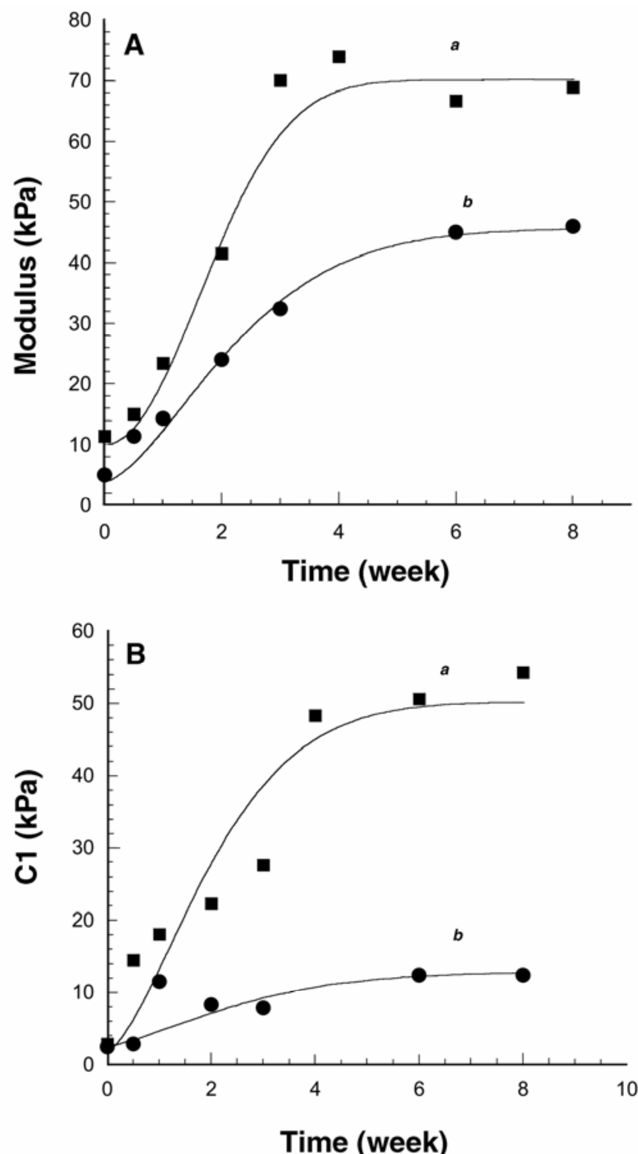

Fig. 2. Representative curves showing firming over time. **A,** Modulus, U200 with glycerol (*a*) and U200 without glycerol (*b*). **B,** C1, PR59 with glycerol (*a*) and PR59 without glycerol (*b*).

TABLE V
Asymptotic Parameters Obtained by Fitting Modulus and C1 Data to Equation 4

Sample	Asymptotic Modulus E_f, (kPa)	r^2	Asymptotic C1 $C1_f$, (kPa)	r^2
Control, no glycerol	110	0.90	40	0.99
U3, no glycerol	81	0.76	21	0.59
U200, no glycerol	48	0.99	18	0.80
PR59, no glycerol	34	0.80	13	0.52
Control, glycerol	89	0.80	51	0.91
U3, glycerol	81	0.86	46	0.33
U200, glycerol	70	0.99	32	0.98
PR59, glycerol	88	0.91	50	0.89

transition temperature (Jagannath et al 1999a,b) and, in some instances, to lower mechanical indices (Galal and Johnson 1976; Barrett et al 2000a,b), in other cases its hygroscopic nature has produced the opposite textural effect (Baik and Chinachoti 2002; Baik et al 2003).

In our previous work, observation of softening effect of glycerol on stored bread (Barrett et al 2000b) may be due to forming of rolls by extrusion, in which higher levels of shear were imparted to the dough and in which glycerol improved machinability. It is likely that the effect of glycerol on texture is system-specific, depending not only on glycerol and moisture contents but also on processing history and the presence of other microconstituents.

While the two amylolytic systems were roughly equivalent in efficacy (as assessed by similar averaged parameters for stored samples), it should be noted that U3, the stronger enzyme, was incorporated at half the level as U200 (manufacturer's recommended usage). Concentration effects on texture and the more subtle effects of all three enzyme systems on sensory properties will be addressed in future work.

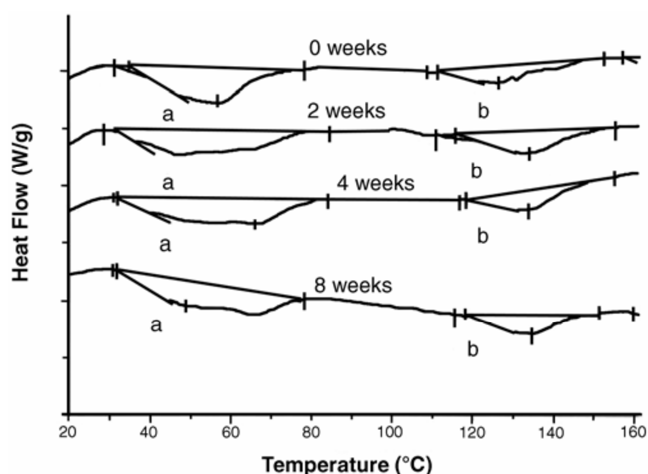

Fig. 3. Thermograms of control bread (with glycerol) as a function of storage time.

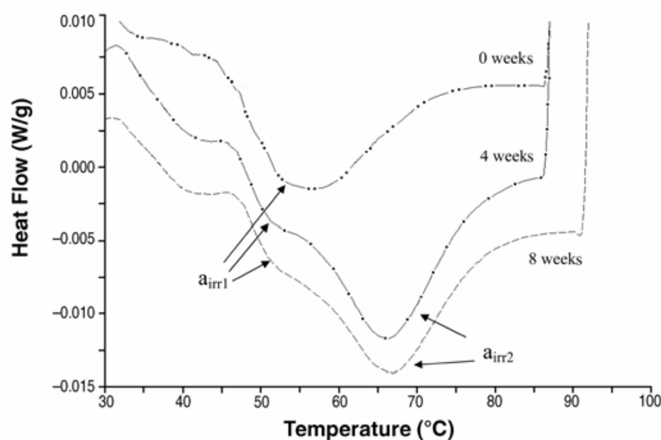

Fig. 4. Irreversible transitions in control bread (with glycerol) as a function of storage time.

Thermal Properties (DSC)

Figure 3 shows the DSC thermograms of control bread recorded by employing the first (single-heat) protocol. For all storage times, at least two endotherms were observed. The endotherm occurring at a peak temperature of ≈135°C was attributed to melting of amylose, based on Klucinec and Thompson's studies of high-amylose starch (1999). Our own DSC work on unbaked dough (*unpublished data*), shows a similar endotherm. Furthermore, partial scanning and rescanning revealed an endotherm at 100–130°C for both dough and bread samples. This endotherm is reversible and was attributed to an amlyose-lipid complex transition (Eliasson 2003) These results suggest 1) that amylose does not melt during baking, and 2) that endotherm *b* consists of two events occurring over a similar temperature envelope, reversible melting of an amylose-lipid complex and irreversible melting of amylose. The thermal stability (peak temperature) of endotherm *b* increased during the first two weeks of storage and then stabilized, which is consistent with and potentially due to moisture equilibration in the bread.

Endotherm *a* (data not shown) similarly appears to be a multicomponent event. The second scan (data not shown) reveals a reversible endotherm with a peak temperature of ≈48°C that most likely is attributable to melting of shortening. There were at least two irreversible components of this transition: one present at the beginning of storage with a peak temperature of 58°C and possibly attributable to ungelatinized starch (Roos 1995; Barrett et al 2002), and one that developed during storage. The peak temperatures for these endotherms were ≈64–65°C for all the enzyme-containing breads and 67°C for the control bread. These temperatures are slightly higher than reported values of ≈60°C for melting of amylopectin crystals formed due to retrogradation (Hug-Iten et al 2001). Discrepancies are most likely due to the use of partial scan and rescanning in this study, which served to separate individual events occurring in the bread matrix. The second irreversible component of the endotherm, obtained by subtracting the second scan from the first scan for all storage times (Fig. 4), and then by subtracting the 0 time thermogram from those for four and eight week samples is shown in Table VI as a function of storage time. The 0 time endotherm could have developed either immediately after baking or during frozen storage. The highest thermal stability and enthalpy of endotherm $a_{irrev,2}$ were observed for control bread. The enthalpy of endotherm $a_{irrev,2}$ was stabilized after four weeks for U3 and U200 samples. Incidentally, after eight weeks storage time, U200-containing bread samples had the lowest modulus (70 kPa) and C1 (32 kPa) values, which corresponded to the lowest enthalpy of starch crystallization (1.02 kcal/g) in glycerol-containing bread samples. On the other hand, control and PR59 samples showed increasing enthalpy even at eight weeks (although at a lower rate for PR59), which is similar to the trend observed for the C1 values of glycerol-containing bread. Crystallization results in glycerol-containing samples are consistent with firming as measured by textural analysis during storage.

CONCLUSIONS

Enzymes reduced the firming of shelf-stable bread over prolonged storage. The proteolytic enzyme was more effective in delaying firming than the amylolytic enzyme in bread without glycerol, suggesting an important role of the gluten in firming.

TABLE VI
Melting Enthalpy ΔH (Kcal/g) of Recrystalized Starch as a Function of Time and Enzyme Addition

Sample	0 Weeks	2 Weeks	4 Weeks	8 Weeks
Control, glycerol	0	–	1.47	1.62
PR59, glycerol	0	0.9	1.04	1.25
U3, glycerol	0	0.8	1.10	1.10
U200, glycerol	0	0.8	1.02	1.02

Proteolytic enzyme was less effective in glycerol-containing breads, possibly indicating a sensitivity to local availability of water for hydrolytic action. Amylolytic enzymes were effective in delaying starch recrystallization and firming.

Firming of bread crumb is most likely attributable to a number of contributing mechanisms, including reduced moisture due to moisture migration into the crust, association of macromolecules, and retrogradation.

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LITERATURE CITED

Akers, A. A., and Hoseney, C. 1994. Water-soluble dextrins from α-amylase treated bread and their relationship to bread firming. Cereal Chem. 71:223-226.

Baik, M.-Y., and Chinachoti, P. 2002. Effects of glycerol and moisture redistribution on mechanical properties of white bread. Cereal Chem. 79:376-382.

Baik, M.-Y., Dickenson, L. C., and Chinachoti, P. 2003. Solid-state ^{13}C CP/MAS NMR studies on aging of starch in white bread. J. Agric. Food Chem. 51:1242-1248.

Barrett, A. H., Tsoubeli, M., Maguire, P., Beck Tan, N., Conca, K., Wang, Y., Porter, W., and Taub, I. 2000a. Textural stability of intermediate-moisture extrudates: Effects of formulation. Cereal Chem. 77:784-790.

Barrett, A. H., Cardello, A. V., Mair, L., Lesher, L. L., Richardson, M., Briggs, J., and Taub, I. A. 2000b. Textural optimization of shelf-stable bread: Effects of glycerol content and dough-forming technique. Cereal Chem. 77:169-176

Barrett, A., Cardello, A., Maguire, P., Richardson, M., Kaletunc, G., and Lesher L. 2002. Effects of sucrose ester, dough conditioner, and storage temperature on long-term textural stability of shelf-stable bread. Cereal Chem. 79:806-811.

Cornford, S. J., Axford, D. W. E., and Elton, G. A. H. 1964. The elastic modulus of bread crumb in linear compression in relation to staling. Cereal Chem. 41:216-229.

Defloor, I., and Delcour, J. A. 1999. Impact of malodextrins and antistaling enzymes on the differential scanning calorimetry staling endotherm of baked bread doughs. J. Agric. Food Chem. 47:737-741.

Eliasson, A. 2003. Utilization of thermal properties for understanding baking and staling processes. Pages 65-115, in: Characterization of Cereals and Flours. G. Kaletunc and K. J. Breslauer, eds. Marcel Dekker: New York.

Galal, A. M., and Johnson, J. A. 1976. Effect of pectin and glycerol on bread staling. Baker's Dig. 50:20-22.

Gibson, L., and Ashby, M. F. 1988. Cellular Solids. Pergamon Press: Oxford.

Gil, M. J., Callejo, M. J., Rodriguez, G., and Ruiz, M. V. 1999. Keeping qualities of white pan bread upon storage: Effect of selected enzymes on bread firmness and elasticity. Z. Lebensm. Unters. Forsch. A. 208:394-399.

Hilhorst, R., Dunnewind, B., Orsei, R., Stegeman, P., van Vliet, T., Gurppen, H., and Schols, H. A. 1999. Baking performance, rheology, and chemical composition of wheat dough and gluten affected by xylanase and oxidative enzymes. J. Food Sci. 64:808-813.

Hug-Iten, S., Escher, F., and Conde-Petit, B. 2001. Structural properties of starch in bread and bread model systems: Influence of an antistaling α-amylase. Cereal Chem. 78:421-428.

Jagannanth, J. H., Jayaraman, K. S., and Arya, S. S. 1999a. Dynamic mechanical thermal analysis of chapata and phulka (Indian unleavened bread). Food Chem. 64:571-576.

Jagannanth, J. H., Jayaraman, K. S., and Arya, S. S. 1999b. Studies on glass transition temperature during staling of bread containing different monomeric and polymeric additives. J. Appl. Polym. Sci. 71:1147-1152.

Klucinec, J. D., and Thompson, D. B. 1999. Amylose and amylopectin interact in retrogradation of dispersed high-amylose starches. Cereal Chem. 76:282-291.

Marin, J. 1962. Mechanical behavior of engineering materials. Prentice Hall: Englewood Cliffs, NJ.

Martin, M. L., and Hoseney, R. C. 1991. A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. Cereal Chem. 68:503-507

Martinez-Anaya, M. A., and Jimenez, T. 1997. Functionality of enzymes that hydrolyse starch and non-starch polysaccharides in breadmaking. Z. Lebensm Unters Forsch. A. 205:209-214.

Nussinovitch, A., Steffens, M. S., and Chinachoti, P. 1991. Exponential model of the compressive stress-strain relationships of bread. Lebensm. Wiss. Technol. 24:266-269.

Roos, Y. H. 1995. Phase Transitions in Foods. Academic Press: San Diego.

Ruan, R., Almaer, S., Huang, V. T., Perkins, P., Chen, P., and Fulcher, R. G. 1996. Relationship between firming and water mobility in starch-based food systems during storage. Cereal Chem. 73:328-332.

Swyngdau, S., Nussinovitch, A., Roy, I., Peleg, M., and Huang, V. 1991. Comparison of four models for the compressibility of breads and plastic foams. J. Food Sci. 56:756-759.

Taub, I. A., Halliday, J. W., and Kim, Y.-K. 1994. Bread structure and stability: Rheological, calorimetric, spectroscopic, and microscopic characterization. Proc. Army Sci. Conf. 4:1391-1398.

Van Dam, H. W., and Hille, J. D. 1992. Yeast and enzymes in bread-making. Cereal Foods World 37:245-251.

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