Firming of Bread Crumb with Cross-Linked Waxy Barley Starch Substituted for Wheat Starch[1]

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ABSTRACT

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White pan bread was baked from flour that had been fractionated and reconstituted using cross-linked waxy barley starch (5.9% amylose content) in place of prime wheat starch (28.3% amylose content). Compression measurements of crumb firmness showed that experimental bread firmed faster during storage at 25°C than control bread, even though the experimental bread had the lower crumb firmness 6 hr after baking. At the same time, the aged bread crumb of the experimental bread showed a higher enthalpy of melting than that of control bread crumb, except at 6 hr after baking. Furthermore, a 50% gel of cross-linked waxy barley starch in water recrystallized faster at 25°C than a 50% gel of wheat starch as measured by enthalpy of melting. These data suggest that amylose dilutes the effect of the amylopectin, the recrystallization of which is at least partially responsible for the firming of bread crumb.

Bread staling involves organoleptic and physicochemical changes, such as firming of crumb, declining flavor, increasing opacity of crumb, toughening of crust, and decreasing starch solubility (Willhoft 1973, Maga 1975, Knightly 1977, Kulp and Ponte 1981). Perhaps the most important change is firming of bread crumb.

Schoch and French (1947) suggested that amylopectin recrystallization is the main cause of firming of bread crumb on the basis of their study of the water-soluble starch extracted from bread crumb. Prentice et al (1954) supported the Schoch and French hypothesis using flour fractionation and starch replacement experiments with waxy corn and waxy sorghum. Several other investigators (Axford and Colwell 1967, Colwell et al 1969) also confirmed the hypothesis using differential thermal analysis. However, in more recent years, several researchers (Kim and D'Appolonia 1977, Ghiasi et al 1984, Rogers et al 1988) have suggested that mechanisms other than amylopectin recrystallization are involved in crumb firming of stored bread.

Barley starch was found to be interchangeable with wheat starch in bread baking (Hoseney et al 1971), but when wheat starch was replaced with waxy barley starch in reconstituted flour, the bread made from the reconstituted flour shrank excessively during cooling (Hoseney et al 1983). However, Wu (1988) succeeded in baking bread containing a high level (95%) of amylopectin by introducing cross-links into waxy barley starch. Such high-amylopectin bread may yield valuable information on the mechanism of crumb firming. If the Schoch and French hypothesis is operative, the rate and extent of crumb firming might be expected to be greater in the high-amylopectin bread than in regular bread, because amylose may dilute the effect of amylopectin (Tester and Morrison 1990).

The objectives of this study were to replace wheat starch with cross-linked waxy barley starch in reconstituted flour and determine the level of cross-linking that gives high-amylopectin bread volume comparable to that of control bread, to examine the effect of the cross-linked waxy barley starch on crumb firming, and to compare the rate of crumb firming of the high-amylopectin bread with the rate of amylopectin recrystallization in the crumb.

MATERIALS AND METHODS

Waxy Barley and Flour

Waxy barley (Wanubet), harvested in 1988, was obtained from Tom Blake, Department of Plant and Soil Science, Montana State University, Bozeman. Wheat flour (11.9%, protein N × 5.7) was provided by Cargill, Wichita, KS.

Assay Methods

Protein was determined by Kjeldahl nitrogen and moisture by oven-drying at 130°C for 1 hr (Methods 46-13 and 44-15A, respectively, AACC 1983).

Amylose in starch was estimated using iodine-binding capacity (Schoch 1964) of starch that had been defatted with 75% aqueous n-propanol (Takahashi and Seib 1988). Pure amylose was assumed to have an iodine-binding capacity of 20.0 g/100 g.

Isolation of Waxy Barley Starch

The isolation of waxy barley starch was done according to Wu (1988) with minor modification. Dehulled waxy barley kernels (500 g) were steeped with 0.2% sodium metabisulfite (1,000 ml) at 25°C for 24 hr. The softened kernels were ground with two volumes of water for 3 min in a blender (Waring, New Hartford, CT). The resulting slurry was screened and washed through a 10XX (132 μm) nylon bolting cloth. The residue was ground again with water in the blender and screened through the cloth. After the grinding and screening procedures were repeated a third time, the residue was discarded. The filtrates containing the suspended starch were combined and centrifuged at 1,000 × g for 10 min. The sedimented crude starch was suspended in 0.04M NaOH (500 ml) and centrifuged at 1,000 × g for 10 min. The dark layer (tailings) on top of the starch was carefully removed with a spatula and discarded. This alkali-washing step was repeated twice more with the starch. Then the starch was suspended in water (500 ml), neutralized with 1M HCl to pH 6, washed with distilled water three times, and dried in a convection oven at 40°C. The yield of starch was 39% based on grain, and the starch contained 11.0% moisture, 0.2% protein, and 5.9% amylose.

Cross-Linking of Waxy Barley Starch

Waxy barley starch (100 g) was suspended in water (185 ml), and the suspension was stirred 2 hr at 25°C. Sodium sulfate (2 g) was dissolved in the starch suspension, and the mixture adjusted to pH 11.5 with 1M NaOH. Phosphorous oxychloride (0.005, 0.02, and 0.05 g) was added using a microsyringe, the reaction mixture stirred at 25°C for 1 hr, and the reaction stopped by adding 1M HCl to pH 6. The cross-linked starch was recovered by centrifugation at 1,000 × g for 10 min, washed with distilled water several times, and dried in a convection oven at 40°C for 24 hr.

Fractionation of Wheat Flour

The flour fractionation procedure was a modification of that described by Dreese et al (1988). Flour (500 g) and distilled water (1,500 ml) were shaken vigorously for 2 min. The resultant slurry was centrifuged at 1,350 × g for 20 min, and the supernatant

containing the water solubles was immediately lyophilized. The pellet (gluten and starch) was mixed to a dough in a slow-speed pin mixer for 4 min. The dough was massaged in distilled water (100 ml) by hand to release starch from the cohesive mass of gluten into the wash water. The wash water was decanted. This dough-washing step was repeated 14 more times, and the gluten mass was frozen and lyophilized. The combined wash water was centrifuged at $1,350 \times g$ for 20 min, and the supernatant containing water solubles was frozen and lyophilized. The loosely packed layer (starch tailings) on top of the tightly packed bottom layer (prime starch) was carefully removed using a spatula, and both layers were frozen and lyophilized. The two freeze-dried, water-soluble fractions were combined.

Reconstitution of Flour

Before the flour was reconstituted, prime starch, starch tailings, and gluten fractions were ground (using a Wiley mill, Thomas Scientific, Swedesboro, NJ) through a 40-mesh (375 μm) screen, and the water-soluble fraction was broken into small pieces. The control flour was reconstituted from the prime wheat starch, starch tailings, gluten, and water solubles at the proportions obtained upon fractionation. Experimental flours were reconstituted using each of three cross-linked waxy barley starches in place of prime wheat starch. Rehydration of freeze-dried fractions was done in two steps to reconstitute flours. Starch tailings and prime wheat starch or cross-linked waxy barley starch were blended and rehydrated to about 12% moisture at 32°C and 90% relative humidity in a fermentation cabinet. The rehydration was done on a 1-cm layer of starch over approximately 6 hr. The rehydrated starch tailings and starch were blended with gluten and water solubles, and the final mixture was rehydrated in the fermentation cabinet to a moisture content of 13.4%.

Breadmaking

Pup loaves were baked from 100 g of flour (14% moisture basis) according to the standard pup loaf procedure (Method 10-10B, AACC 1983), except that nonfat dry milk was added at 4% of flour weight. Microloaves were baked from 10 g of flour (14% moisture basis) following the procedure of Shogren and Finney (1984). At least two replicate pup loaves and five replicate microloaves were baked for each treatment. Loaf volume was measured by rapeseed displacement immediately after baking.

Crumb Firmness

After baking, the bread was allowed to cool 1 hr before it was placed in a polyethylene bag. Crumb firmness was measured during storage at 25°C. An Instron universal testing machine (Instron, Canton, MA) equipped with a 36-mm diameter plunger and a 2,000-g load cell was used to compress a bread slice (25 mm thick) that had been cut from a pup loaf. Firmness was expressed by the force in grams required to compress each slice 4 mm (16% compression). The chart speed was 25 cm/min, and the crosshead speed was 5 cm/min. For a microloaf, the diameter of the plunger was changed to 8 mm. Firmness values were the average of at least five measurements per treatment.

Differential Scanning Calorimetry

Starch gels were prepared for differential scanning calorimetry analysis. Starch (3.5 mg) and water (3.5 or 10.5 mg) were sealed in pans, and the pans heated in a differential scanning calorimeter (DSC) (Perkin-Elmer DSC-2, Norwalk, CT) at 10°C/min from 7 to 127°C at a sensitivity of 0.5 mcal/sec (Zeleznak and Hoseney 1987). The pans were cooled to 27°C, stored at 25°C, and then heated again in the DSC to melt recrystallized amylopectin. The enthalpy of the endotherm, which was observed between 35 and 75°C, was calculated with a Data Acquisition, Retention and Examination System for Differential Scanning Calorimetry. The software was obtained from Industrial Technology Research Institute, Cambridge, UK.

The level of recrystallized amylopectin in bread crumb also was estimated using differential scanning calorimetry (Zeleznak and Hoseney 1987). Bread crumb was taken immediately after measurement of crumb firmness, frozen, and lyophilized. The dried crumb was ground using a mortar and pestle, and the ground crumb (3.5 mg) was heated in the DSC with water (7.0 mg). At least two measurements were taken for each treatment.

Swelling Power of Starch

Swelling power was determined using a modification (Takahashi and Seib 1988) of the method of Leach et al (1959). A mixture of starch (0.5 g) and water (25 ml) was held at 95°C for 40 min in a glass centrifuge tube fitted with a screw cap. During heating, each mixture was stirred gently using a magnetic stir bar to prevent clumping of the starch. Each mixture was centrifuged at $1,000 \times g$ for 15 min, and the supernatant was separated from the sediment by decantation. The volume of the supernatant was recorded, and the carbohydrate in the supernatant was determined using the phenol-sulfuric acid method (Dubois et al 1956). The wet weight of the sediment was recorded. Swelling power was calculated as follows: Swelling power (g/g) = wet wt of sedimented gel/(wt of dry starch − wt of soluble starch in supernatant).

RESULTS AND DISCUSSION

Fractionation of Wheat Flour

The bread wheat flour was fractionated into the four components shown in Table I. Recovery was 94.5% of dry solids and 95.0% of protein. The prime wheat starch contained 28.3% amylose compared with 5.9% for waxy barley starch.

Enthalpy of Melting Amylopectin Crystals in Starch Granules of Wheat and Waxy Barley

The melting of crystallites in starch granules is observed as the first endotherm in the DSC when starch is heated in excess water (Zobel et al 1988). The crystalline fraction is widely accepted to be amylopectin (Imberty et al 1991). In this investigation, the enthalpy of melting amylopectin crystals in wheat starch at 75% moisture was estimated to equal 2.54 cal/g (10.5 J/g), whereas that in all of the cross-linked and native waxy barley starches averaged 3.31 cal/g (13.8 J/g, Table II). It is noteworthy that cross-linking with up to 0.05% POCl₃ did not affect the enthalpy of melting amylopectin crystals in waxy barley starch. Moreover, when the enthalpies were normalized to an equal amylopectin basis (amylopectin content 71.7% for prime wheat starch and 94.1% for waxy barley starch), the enthalpies became almost equal (3.54 and 3.52 cal/g of amylopectin, respectively). The enthalpies indicate that the content (quantity) and perfection (quality) of

TABLE I
Compositional Data and Yields of Flour Fractions

Fraction	Yield[a] (%)	Moisture (%)	Protein[a] (%)
Wheat flour	...	13.4	13.7
Water solubles	6.1	5.3	24.9
Gluten	13.5	1.7	77.3
Starch tailings	14.1	1.3	5.4
Prime wheat starch	60.8	0.6	0.5

[a] Dry weight basis.

TABLE II
Enthalpies of Melting Amylopectin Crystals in Starch Granules in the Presence of 75% Moisture

Starch	POCl₃ (%)	Enthalpy[a] (cal/g)
Prime wheat	...	2.54 ± 0.05
Native waxy barley	...	3.26 ± 0.10
Waxy barley	0.005	3.30 ± 0.12
Waxy barley	0.02	3.35 ± 0.09
Waxy barley	0.05	3.34 ± 0.07

[a] Calories per gram can be converted to joules per gram by multiplying by 4.18.

amylopectin crystals are nearly equal in the wheat and waxy barley starch granules. Both unmodified and cross-linked waxy barley starches had gelatinization temperatures in 75% water that were 2–4°C above that of wheat starch. However, the difference in gelatinization temperatures may be attributed to differences in the structure of the amorphous phase, rather than to crystallite perfection (Shi and Seib, in press). The concepts of quantity (enthalpy) and quality (melting temperature) of amylopectin crystals have been discussed by Tester and Morrison (1990).

Amylopectin Recrystallization in 50% Starch Gel

Three cross-linked waxy barley starches and prime wheat starch were heated to 127°C with an equal weight of water and stored at 25°C. To interpret the amylopectin recrystallization in their 50% starch gels, it was assumed from the results of Shi and Seib (in press) that the amylopectin crystallites in both of the retrograded starches were of approximately the same perfection, especially since amylopectin from wheat and waxy barley starches gave almost identical distributions of unit chains between chain length 6 and 45 (Y. C. Shi and P. A. Seib, unpublished data). As already pointed out, the crystallites in native wheat and waxy barley starch gave enthalpies of melting that were equal based on their amylopectin levels.

Figure 1 shows that the rate of amylopectin recrystallization in the wheat starch gel was slower than that in any of the three
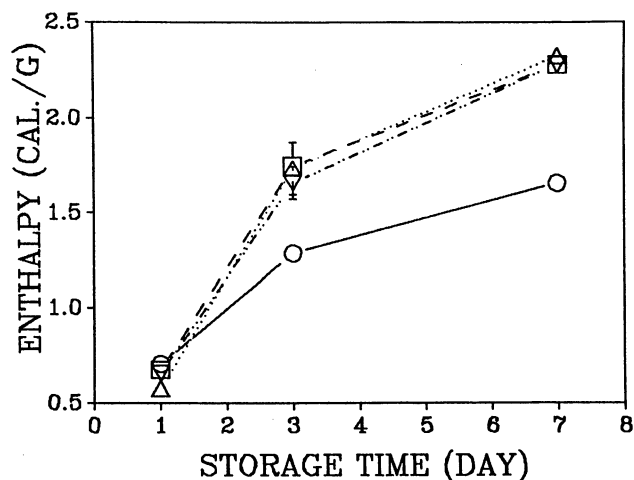

Fig. 1. Amylopectin recrystallization, measured as enthalpy of melting, in 50% starch gels stored at 25°C. Error bar represents standard deviation. Wherever the error bar is missing, the data points are superimposed. O = Control. △, □, ▽ = Waxy barley starch cross-linked with 0.005, 0.02, and 0.05% POCl$_3$, respectively.

gels of the cross-linked waxy barley starches, all of which showed approximately the same rate. Nevertheless, crystallinity after three days of storage appeared nearly proportional to the amylopectin content of the starches. In other words, at seven days, wheat starch showed an enthalpy of melting (ΔH_m) of 1.66 cal/g, whereas waxy barley had ΔH_m 2.30 cal/g. The ratio of ΔH_m for retrograded wheat starch to that of retrograded waxy barley starch was 0.72, whereas their amylopectin ratio was 0.77.

Effect of Cross-Linked Waxy Barley Starch on Loaf Volume

In baking experiments, the microloaves from the experimental flours gave loaf volumes (62.8–65.7 cm^3) that were not significantly different from that (68.2 cm^3) of the control flour at a 5% confidence level. All loaves were baked at 70% absorption. That absorption was optimum for experimental flours, but 8% above that for the control flour. Loaf volume of the control bread did not change between 62 and 70% absorption, and an equal moisture content was found in the breads made from the control and the experimental flours.

Because the loaf volumes and moisture levels of the experimental and control breads were nearly equal, we concluded that firmness tests could be conducted using the cross-linked waxy barley starch in place of wheat starch.

Effect of Cross-Linked Waxy Barley Starch on Crumb Firming

Microloaves were baked from the reconstituted control and the three experimental flours made with the cross-linked starches shown in Table II. The average loaf volumes (50.6–52.3 cm^3) of all the breads were statistically the same. Moreover, the moisture content at the center of all the loaves was identical at 41.2 ± 0.2%. The experimental flour bread crumb containing any of the cross-linked waxy barley starch firmed faster than the control bread crumb (Fig. 2). The experimental flour containing starch cross-linked with 0.02% POCl$_3$ gave bread that showed the largest difference in crumb firming from the control flour, so it was chosen for further experiments using pup loaves.

The average loaf volumes of pup loaves made from the reconstituted control flour (875 cm^3) and the experimental flour containing starch cross-linked with 0.02% POCl$_3$ (841 cm^3) were not statistically different at a 1% confidence level. The moisture contents at the center crumb of both loaves were equal: 44.7 ± 0.1%. Upon aging of loaves at 25°C, the experimental bread showed a faster crumb firming than the control bread, even though the initial firmness (6 hr after baking) of the experimental bread was lower (Fig. 3).

Amylopectin Recrystallization in Bread Crumb

The enthalpy of melting recrystallized amylopectin in bread crumb was higher for the experimental bread containing starch
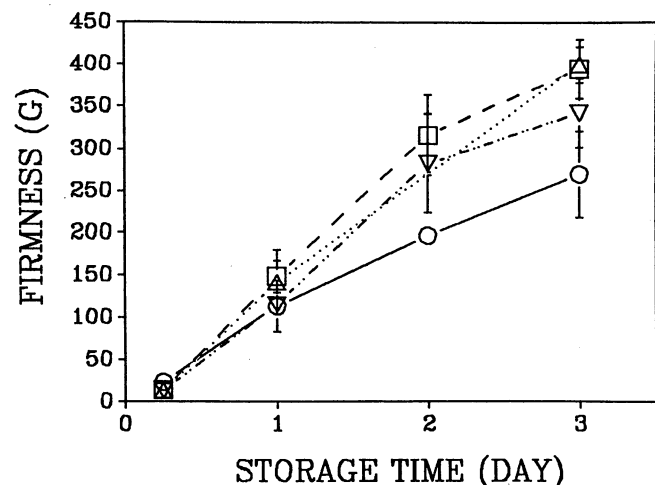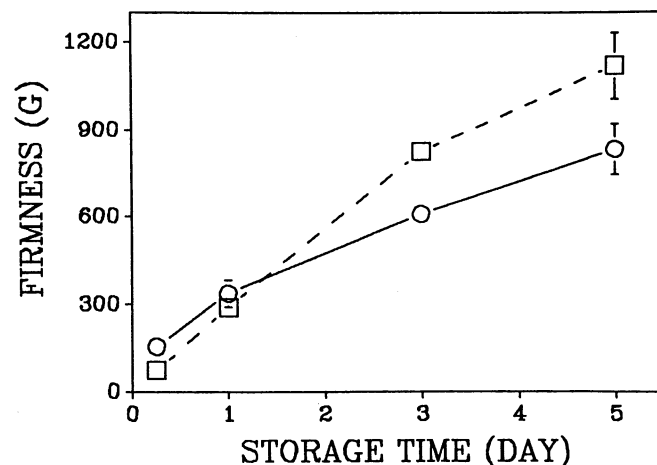

Fig. 2. Effect of cross-linked waxy barley starch on crumb firming of microloaves. O = Control. △, □, ▽ = Waxy barley starch cross-linked with 0.005, 0.02, and 0.05% POCl$_3$, respectively.

Fig. 3. Effect of cross-linked waxy barley starch on crumb firming of pup loaves. O = Control; □ = waxy barley starch cross-linked with 0.02% POCl$_3$.
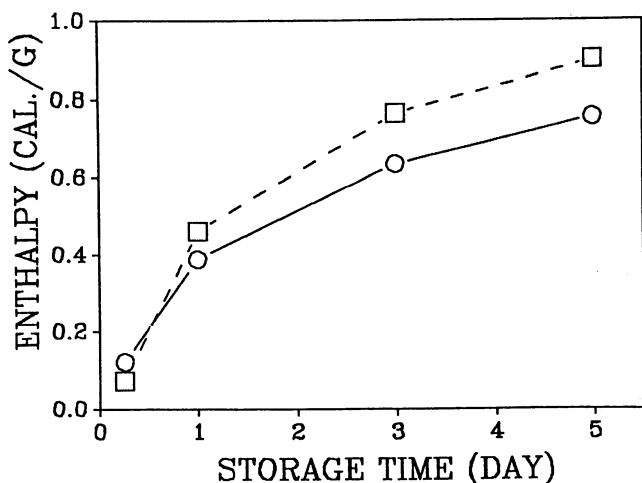
Fig. 4. Amylopectin recrystallization, measured as enthalpy of melting, in bread crumb stored at 25°C. The bread crumb was freeze-dried and heated with two parts of water in the differential scanning calorimeter. O = Control; □ = waxy barley starch cross-linked with 0.02% POCl₃.

TABLE III
Swelling Power of Starches at 95°C

Starch	POCl₃ (%)	Swelling Power (g/g)
Prime wheat	...	12.87 ± 1.70
Waxy barley	0.005	19.15 ± 0.31
Waxy barley	0.02	14.28 ± 0.21
Waxy barley	0.05	11.64 ± 0.16

cross-linked with 0.02% POCl₃ than for the control bread, except at 6 hr after baking (Fig. 4). As observed in 50% starch gels, the crystallinity after one day of storage was nearly proportional to the amylopectin content in the breads, which was 72% of the starch in the control and 91% in the experimental bread. The amylopectin content of 91% for experimental flour was calculated by considering the reconstitution ratio (81/19) of waxy barley starch and wheat starch tailings, the respective amylopectin contents of waxy barley and wheat starches (95 and 72%), and the starch level in the tailings (80%).

In general, amylopectin recrystallized faster in the bread containing the higher level of amylopectin (Fig. 4). The bread crumb containing the higher level of amylopectin also firmed at a faster rate than the crumb with the normal level of amylopectin (Fig. 3). These data suggest that amylose may play a passive role in the firming of bread crumb, or that cross-linking promotes recrystallization of amylopectin by keeping the molecular chains in close proximity. It is clear that recrystallization of amylopectin is associated with crumb firming.

Starch Swelling and Crumb Firming

Martin et al (1991) suggested that the degree of starch swelling during baking correlated with firming of bread crumb. Highly swollen starch granules were postulated to have more surface area to interact with the gluten matrix in bread. To examine this relationship, the swelling power of the starches used in the crumb firming experiments on microloaves was measured (Table III). Prime wheat starch showed the lowest swelling power among the four starches, except for the waxy barley starch cross-linked with 0.05% POCl₃, which indicated a swelling power nearly equal to that of the wheat starch. As expected, the swelling power of the cross-linked waxy barley starches declined as the level of cross-linking increased. The swelling power of the starches (Table III) and the respective firming (Fig. 2) appear to agree with Martin's suggestion. The more highly swollen the starch granules were in the bread crumb, the higher the rate of crumb firming. The order of swelling power of the starches (waxy barley

[WB] with 0.005% POCl₃ > WB with 0.02% POCl₃ > WB with 0.05% POCl₃ ≤ prime wheat control; Table III) is almost the same as the order of crumb firming (WB with 0.005% POCl₃ = WB with 0.02% POCl₃ > WB with 0.05% POCl₃ > prime wheat control; Fig. 2).

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