Enzyme-Resistant Starch. III. The Quality of Straight-Dough Bread Containing Varying Levels of Enzyme-Resistant Starch

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ABSTRACT

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Breads with varying levels of enzyme-resistant starch (RS) were obtained by replacing 24% of wheat flour (RS content 14.5%) with 4% vital wheat gluten and 20% of one of the following: corn starch (CS), high-amylose corn starch (HA), or extruded retrograded high-amylose corn starch (ERHA). RS levels were 44.1, 83.2, and 29.5%, respectively. Breads were produced by the Finney (1984) procedure (100.00 g of flour or 76.00 g of flour, 4.00 g of gluten, and 20.00 g of starch). All had excellent taste and shelf life, except for the CS breads. RS levels of bread were lower than could be predicted from the analytical data of the starting materials, which shows that some RS destruction occurs in the breadmaking process. Thus, one day after baking, the RS content of breads containing wheat flour, CS, HA, and ERHA was 0.0, 0.4, 7.7, and 8.4%, respectively. The latter breads showed the presence of retrograded amylose or resistant granules. After seven days of storage, the RS levels had increased to 4.0, 4.4, 10.2, and 11.0%, respectively. Differential scanning calorimetry measurements confirmed that the increase can probably be ascribed, at least in part, to increases in the levels of retrograded amylopectin. Bread volumes were 663.9, 654.3, 655.9, and 621.5 ml, respectively. The softest breads were those produced with ERHA; the least soft ones were those with CS; the breads with wheat flour and HA had intermediate levels of softness.

Enzyme-resistant starch (RS), the fraction of starch that escapes digestion in the small intestine of man, has been investigated extensively in the past few years (Berry 1986; Russell et al 1989; Siljeström et al 1989; Sievert and Pomeranz 1989, 1990; Sievert et al 1991; Gee et al 1992; Langkilde and Andersson 1992; Leloup et al 1992; Eerlingen et al 1993a,b).

Of particular interest to cereal chemists is research showing that bread contains limited quantities of RS. Siljeström and Asp (1985) investigated the influence of baking time and temperature and recipe variations on RS formation in wheat flour bread and reported RS contents of 0.3–1.0%. Englyst et al (1992) also found RS contents of 1.0% in white bread.

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Only limited quantities of RS are present in bread, which contributes to the fact that its impact on the breadmaking process and on bread quality is not very well understood. The present effort was therefore undertaken to gain more insight by formulating recipes that can be expected to yield increased levels of RS. A further goal was to contribute to the provision of RS-enriched diets for physiological studies within the framework of EURESTA (European Flair Concerted Action on Resistant Starch). Indeed, one of the problems associated with such studies is the poor availability of tasty RS-enriched foods.

MATERIALS

For breadmaking, Surbi commercial wheat flour (Cerestar, Vilvoorde, Belgium) with a protein content of 12.3%, vital wheat gluten (Amylum, Aalst, Belgium), Crisco shortening (Procter and Gamble, Cincinnatti, OH), and Fermipan yeast (Gist-Brocades, Delft, The Netherlands) were used. GL 03402 corn starch (Cerestar), Eurylon 7 high-amylose corn starch (Roquette, Lille, France) with an amylose content of 75%, and extruded retrograded high-amylose corn starch (EURESTA product, supplied by Cerestar) were also used. The latter product was prepared from Hylon 7 by extrusion, milling, subsequent storage at 40°C for 48 hr, drying and renewed milling. In the text, the cornstarch, high-amylose corn starch, and the extruded retrograded high-amylose corn starch are referred to as CS, HA, and ERHA, respectively.

Enzymes used for determination of RS were pepsin P6887 from porcine pancreas (Sigma Chemical Co., St. Louis, MO), pancreatin P1625 from porcine stomach mucosa (Sigma), and AMG amyloglucosidase from *Aspergillus niger* (300 AGU/ml, Novo Nordisk, Bagsvaerd Denmark). An enzyme cocktail was prepared following the procedure of Englyst et al (1992). Pancreatin (0.7500 g) was weighed into each of eight centrifuge tubes, and 10.0 ml of water and a magnetic stirrer were added to each tube. The pancreatin was suspended by vortex mixing followed by magnetic stirring for 10 min. After centrifugation at $1,500 \times g$ for 10 min, 7.0 ml of the supernatant was removed from each tube, and 2.9 ml of amyloglucosidase solution and 7.5 ml of water were added. Glucose determinations were made with GOD-PAP reagent from the Boehringer glucose test combination, 166391 (Boehringer, Mannheim, Germany).

The enzymes used in the isolation of dietary fiber (DF) were: pepsin P6887; Termamyl, a thermostable α-amylase from *Bacillus licheniformis* (120 KNU/g, Novo Nordisk); AMG; and protease P5147 from *Streptomyces griseus* (Sigma).

Phosphate buffers 1 (pH 6) and 2 (pH 7.5) used in the DF analysis were prepared by adding 56 ml or 409 ml, respectively, of $0.1M$ NaOH to 500 ml of $0.1M$ KH$_2$PO$_4$, and bringing the final volume to 1,000 ml.

All other chemicals were of analytical grade or better.

METHODS

Determining RS Content

RS content was determined according to the procedure of Englyst et al (1992) with slight modifications.

The samples (about 500 mg of starch or 800 mg of flour or 800 mg of freeze-dried crumb, in duplicate) were accurately weighed and incubated for 30 min at 37°C with 10.0 ml of a pepsin solution containing 7.5 mg of pepsin in $0.05M$ HCl (shaking water bath). Sodium acetate solution ($0.25M$, 10.0 ml) was then added to the samples. These samples were subsequently incubated with the enzyme cocktail (pancreatin and amyloglucosidase) for exactly 120 min in a shaking water bath at 37°C. Control (20.0 ml of sodium acetate buffer, $0.1M$, pH 5.2) and two standard solutions (20.0 ml of sodium acetate buffer with 0.5000 g of dried glucose, $0.1M$, pH 5.2) were incubated under the same conditions. After 120 min, 0.5 ml of the incubated mixtures was removed and added to 20.0 ml of 66% ethanol. After mixing and centrifugation ($1,500 \times g$, 5 min), the obtained supernatants (designated as G_{120}) were set aside for glucose determination. The remaining incubated mixtures were then vortex-mixed, placed

in a boiling water bath for 30 min, and vortex-mixed again. After cooling in ice water, 10.0 ml of $7.0M$ potassium hydroxide was added, and the mixtures were incubated at 0°C in a shaking ice water bath for exactly 30 min. An aliquot (1.0 ml) was then added to 10.0 ml of $0.5M$ acetic acid. Then 0.2 ml of an amyloglucosidase solution (prepared by mixing 1.0 ml of Novo amyloglucosidase preparation and 5.0 ml of water) was added to each tube, and the tubes were placed in a water bath at 70°C. After 30 min, the samples were incubated in a boiling water bath for 10 min. After cooling to room temperature, 25.0 ml of water was added to each sample. These samples (designated G_{tot}) were mixed and centrifuged ($1,500 \times g$, 5 min).

Glucose content of the G_{120} and G_{tot} samples was determined by adding 2.0 ml of GOD-PAP reagent to 0.1 ml of sample, standard or control (in duplicate) and mixing. The tubes were then placed in a water bath at 37°C for 20 min. The absorbance was measured against the control at 510 nm. Glucose content was calculated as:

$$\% \text{ Glucose} = A_t \times V_t \times C \times 100 \times (A_s \times W_t)^{-1}$$

where A_t is the absorbance (510 nm) of the test solution; V_t is the total volume of the test solution; C is the concentration (milligrams of glucose per milliliter) of the standard; A_s is the absorbance (510 nm) of the standard; and W_t is the weight (in milligrams) of the sample. For G_{120} samples: $V_t = 25.0$ ml + 1.0 ml per gram of sample (wet weight); $C = 20.0$. For G_{tot} samples: $V_t = 35.2$ ml + 1.0 ml per gram of sample (wet weight); $C = 14.2$. RS content (%) was calculated as:

$$RS = (G_{tot} - G_{120}) \times 0.9$$

Determinations were performed in quadruplicate (duplicate analysis on samples from two different breads) on bread crumb and in duplicate on wheat flour, CS, HA, and ERHA.

Determining DF Content

The determination of total DF was based on an enzymatic-gravimetric procedure (AOAC 1985). Samples ($\sim$1.00 g) were accurately weighed in centrifuge tubes with screw caps and incubated with 9.4 mg of pepsin in 12.5 ml of $0.05M$ HCl for 30 min at 37°C in a shaking water bath. Sodium hydroxide solution (1.25 ml, $0.5M$), phosphate buffer 1 (20 ml), and Termamyl (0.4 ml) were added with intermediary mixing. After 60 min of incubation at 100°C, the samples were cooled to room temperature and adjusted to pH 4.5 with a 2.0% phosphoric acid solution. Amyloglucosidase (1.0 ml) was added, and the samples were incubated for 60 min at 60°C (shaking water bath). After enzymic digestion, the samples were centrifuged ($1,000 \times g$, 10 min). The sediments were washed three times with distilled water. The residues were suspended in 25.0 ml of phosphate buffer 2. Protease solution (1.0 ml) containing 16.0 mg of protease in 100 ml of buffer was added. The residues were incubated for 2 hr at 42°C in a shaking water bath. Protease (1.0 ml) was added again, and the samples were incubated for another 2 hr and centrifuged ($1,000 \times g$, 10 min). The sediments were again washed three times with distilled water. The samples were filtered through a weighed, fritted crucible (no. 4 porosity) and dried overnight in an air oven at 80°C. After cooling to room temperature in a desiccator, the crucibles with the residues were weighed.

Determinations were performed in quadruplicate (duplicate analysis on samples from two different breads) on bread crumb and in duplicate on wheat flour, CS, HA, and ERHA.

Dough Rheology

Farinograms were recorded at 500-BU consistency for wheat flour and for the different flour-gluten-starch (76:4:20) mixtures. Mixograms were recorded at the 500-BU water-absorption levels.

Breadmaking and Bread Storage

Breads were produced by the Finney (1984) procedure using 100.0 g of flour as a control and using 76.0 g of flour, 4.00 g

of gluten, and 20 g of CS, HA, or ERHA (all at 14% mb). Shortening (3.00 g), yeast (0.76 g), sugar-salt solution (containing 6.00 g of sucrose and 1.50 g of sodium chloride), and water were added. The water-absorption levels were those for doughs of 500-BU consistency. Mixing times were read from the mixograms. After being mixed, doughs were transferred in a covered stainless steel bowl to a fermentation cabinet (National Mfg., Lincoln, NE) at 32°C, 90% rh. After 105 min, doughs were punched (3/16-in. sheeter gap), folded, and fermented again for 50 min. Doughs were punched again (3/16-in. sheeter gap) and then fermented a third time for 25 min, punched (5/16-in. sheeter gap), molded, and placed in a baking pan. Final proof time was 55 min. Loaves were baked for 24 min at 215°C.

Six loaves of the control and six of each of the substituted-flour samples were baked. After cooling (2 hr), the breads were weighed, and their volumes were determined by rapeseed displacement. The loaves were then stored in paper bags at 22°C for one, three, or seven days.

Crumb Firmness Measurements

Crumb firmness of the loaves stored one, three, or seven days at 22°C, was measured with a universal testing system (UTS Testsysteme Gmbh, D-7900 Ulm-Einsingen, Germany). A load cell with a maximum load of 200 N, a compression head of 21 mm, and a crosshead speed of 100 mm/min was used. A middle slice (25 mm thick) was cut from each loaf (duplicates) and used to measure the resistance against 25% compression. Crust was removed before measurement.

Immediately after measurement, the crumb of each loaf was frozen in liquid nitrogen and freeze-dried overnight. Dried bread crumb was crushed in a mortar and stored in plastic bags for differential scanning calorimetry (DSC) measurements and determination of RS, DF, and moisture content.

DSC Measurements

DSC measurements were performed with a Seiko DSC-120 calorimeter (Kawasaki Kanagawa, Japan). Indium and tin were used as standards. Freeze-dried crumb (~6 mg) was accurately weighed into aluminium pans, to which water (2× the sample weight) was added. An empty pan served as the reference sample. The crumb was heated from 20 to 130°C at a scanning rate of 4°C/min. The peak temperatures and enthalpies of the transitions were determined by Seiko software. All analyses were performed at least in triplicate.

Hot Stage Polarization Microscopy

ERHA was viewed under polarized light using a laboratory binocular microscope (Olympus BHS) with hot stage equipment (Mettler FP82HT and FP90 central processor)

Moisture Content

Moisture content was determined according to method 44-15A (AACC 1983).

Sensory Analysis

A trained panel of nine subjects evaluated the four kinds of bread for the quality attributes of crumb color, crumb structure, aroma intensity, aroma preference, taste intensity, taste preference, and mouthfeel.

Statistical Evaluation

The statistical analyses were performed using the general linear model (SAS 1987) including Tukey's studentized range test for pairwise comparisons (5% significance level).

RESULTS AND DISCUSSION

RS and DF Content of Flours and Starches

Table I shows the RS and DF content of the wheat flour and starches used in the breadmaking process. Using the RS procedure of Englyst et al (1992), we determined the sum of three types of RS: physically inaccessible starch, resistant starch granules, and retrograded starch. RS in wheat flour, CS, and HA consisted mainly of resistant starch granules, whereas RS in ERHA consisted of retrograded starch (retrograded amylopectin and retrograded amylose). HA had the highest total RS content. Much unlike CS and wheat starch (in wheat flour), gelatinization of HA is complete only at temperatures above 100°C, even in excess water (Colonna and Mercier 1985; R. C. Eerlingen, *unpublished data*), thus higher enzyme resistance of HA was expected. The fact that the RS content of ERHA was lower than that of HA could be attributed to the processing of the starch granules i.e., extrusion at 125°C (product temperature) with a starch-water ratio of 0.88 (w/w).

The DF (Table I) also included a fraction of RS; retrograded amylose and very resistant starch granules survived incubation with Termamyl at 100°C. Comparison of the RS and DF contents in Table I, shows that DF content was much lower. The conditions used to determine DF content were much more severe (Termamyl at 100°C and amyloglucosidase at 60°C) than those used for the determination of RS content (pancreatin and amyloglucosidase at 37°C). CS and starch in wheat flour are readily gelatinized at 100°C. This explains the negligible values of DF for these samples. DF contents of HA and ERHA show that a significant fraction of the starch was resistant to amylolysis under such severe conditions. Gelatinization of HA is complete only at temperatures above 100°C, as mentioned before, so the DF of HA probably consisted of very resistant starch granules or granule remnants. The DF of ERHA most likely consisted of retrograded amylose (retrograded amylopectin melts at 40–60°C). The very low yield of DF for wheat flour and CS indicates that the RS fractions of these samples contained a very limited quantity (if any) of very resistant structures as very resistant granules.

Dough Rheology

Table II lists the absorption characteristics of the different flour-gluten-starch combinations. While CS affected the absorption characteristics only slightly, HA and ERHA had a more drastic impact on moisture binding. Mixing times (Table II) and the shapes of the mixing curves were rather unaffected, except for ERHA, where much longer mixing times were noted.

RS and DF Content of Bread Crumb

The sum of three types of RS were measured, and the quantities of these three types of RS were influenced to different extents by processing (baking). Therefore, the RS yields of the bread

TABLE I
Resistant Starch (RS, % of dry matter) and Dietary Fiber Content (DF, % of dry matter) of the Wheat Flour and Starches Used in Breadmaking

Sample	RS	DF
Wheat flour	14.5	1.0
Corn starch	44.1	0.4
High-amylose corn starch	83.2	17.0
Extruded retrograded high-amylose corn starch	29.5	15.5

TABLE II
Mixograph Mixing Times (MT) and Farinograph Absorption Levels (BA) Used in Breadmaking

Mixtures, g[a]				
Flour	Gluten	Starch[b]	MT, sec	BA, %
100.0	0.0	0.0	180	59.2
76.0	4.0	20.0 g CS	180	61.2
76.0	4.0	20.0 g HA	180	65.9
76.0	4.0	20.0 g ERHA	360	70.5

[a] 14% moisture basis.

[b] CS = corn starch; HA = high-amylose corn starch; ERHA = extruded retrograded high-amylose corn starch.

crumbs could not be calculated from the RS content of the products used in the baking recipe.

RS and DF contents of the bread crumb after storage for one and seven days are shown in Table III.

Wheat flour breads versus breads with 20% CS. RS levels in wheat flour breads, with or without substitution with 20% CS, were negligible in the fresh bread. This implies that the bread-making process, with certain levels of α-amylase and heat-moisture treatment (and gelatinization) during baking, enhanced the digestibility of the starch in flour or CS (Table I).

However, after seven days the RS content had significantly increased. It is generally accepted that amylose retrogrades very quickly after gelatinization, thus such retrogradation could not account for the difference in RS levels of the breads stored for one or seven days. Further evidence stems from the DF analytical data that shows DF content of wheat flour and CS breads did not increase with storage time. This indicated that the observed increase in RS content could not be ascribed to the formation of resistant retrograded amylose. It seems probable that retrogradation of amylopectin, or changes in interactions of starch with other ingredients during storage, increased resistance of starch to hydrolysis. Because retrograded amylopectin melts between 40 and 60°C (Russell 1983, 1987; Eliasson 1985), it appeared possible that at least part of it was included in the RS assay (amylolytic treatment at 37°C) and not in the DF analysis (Termamyl treatment at 100°C).

These results are at variance with those reported earlier by Berry (1986) and Siljeström et al (1988). However, they are in agreement with the results of Schultz and Landis (1932) and Jackel et al (1952, 1953), who also found a decrease in susceptibility of bread starch to amylases (flour and malt amylase, β-amylase, and pancreatic α-amylase) with storage time. Berry (1986) found that RS in wheat bread was present immediately after baking, and that no increase occurred during storage (nine days at 21°C). However, in Berry's work, RS was determined with pancreatic α-amylase and pullulanase for 16 hr at 42°C. Siljeström et al (1988) also reported no difference in RS content in breads stored for one or six days. However, they determined RS by using more severe conditions. RS was considered the amount of starch in the DF residue that was obtained after hydrolysis with Termamyl, pepsin, and pancreatin (Asp et al 1983) and was not susceptible to amyloglucosidase.

It is of further relevance that replacing 20% of the flour with CS did not influence RS content of the breads.

Wheat flour breads versus breads with 20% HA or ERHA. Again, the RS content of the breads were lower than could be predicted from the content of their starting materials (Table I). However, substituting HA or ERHA for flour increased the RS content of the bread crumb significantly, up to about 8% when stored for one day and up to ~10% after storage for seven days. RS content of breads with ERHA were not significantly higher than RS content of breads with HA. It seems likely that the increased levels of RS could be ascribed both to increased levels of retrograded amylose and to the lower degree of gelatinization of HA starch during the breadmaking process. Gelatinization of HA occurs at much higher temperatures than that of regular starch, as already mentioned. Therefore, HA gelatinized only to

a limited degree during baking. The gelatinized fractions then could retrograde and form resistant structures. Because HA contains 75% amylose, such resistant structures were mainly retrograded amylose. In the case of ERHA, the (retrograded) crystalline fractions already present in ERHA may have recrystallized during and after the baking process. This also explains the increase in DF content (Table III) when 20% of the flour was replaced with HA or ERHA. The increase in DF content could only be explained by an increase in very resistant starch levels (retrograded amylose and very resistant high-amylose corn starch granules or granule remnants).

Sensory Analysis and Volumes of Breads with Varying Levels of RS

Figure 1 shows cross sections of the different breads. No significant sensory differences were detected between any of the breads for quality characteristics (crumb color, crumb structure, aroma intensity, taste intensity and mouthfeel). No significant aroma and taste preference differences were detected, except for the breads with 20% CS; the latter received significantly lower scores than did the other samples. Therefore, the breads with high RS levels (breads with 20% HA or ERHA) were comparable to the control wheat bread.

Loaf volumes and specific volumes are listed in Table IV. Breads baked with ERHA were significantly smaller in specific volume than were the other breads. Breads with HA were significantly smaller than the control loaves.

Bread Ageing

Staling of the breads was evaluated both by measurement of crumb firmness and by DSC, which was used to measure the melting heat of retrograded amylopectin. It is generally accepted that amylopectin retrogradation is one of the predominant phenomena associated with bread staling.

Table V shows the analysis of variance for the effect of formulation and storage time on crumb firmness. The firmness of all types of breads increased with storage time (Fig. 2). Differences in firmness between the breads became more obvious when they were stored for longer periods (seven days). Although loaves baked with ERHA had the lowest specific volumes, their crumb firmness was lower than that of the other bread types. Substituting 20% CS for flour increased crumb firmness. Substituting 20% HA for flour, on the other hand, decreased crumb firmness of the
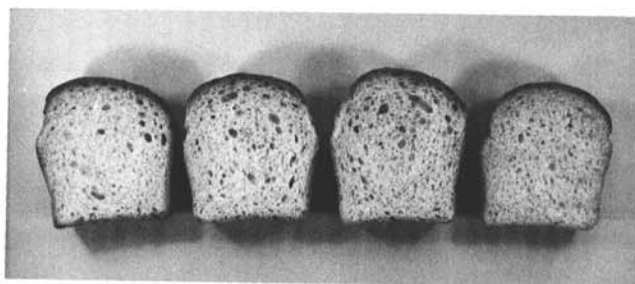

Fig. 1. Cross sections of the different breads. From left to right: regular wheat flour bread (control) and breads with 20% corn starch, high-amylose corn starch, or extruded retrograded high-amylose corn starch.

TABLE III
Resistant Starch (RS, % of dry matter) and Dietary Fiber Content
(DF, % of dry matter) of Crumb from Bread Baked from Wheat Flour
and Wheat Flour Partly Substituted by Different Starches[a]
and Stored for One or Seven Days

Bread Crumb	RS		DF	
	1 Day	7 Days	1 Day	7 Days
Wheat flour	0.0	4.0	2.4	2.4
CS	0.4	4.4	2.4	2.5
HA	7.7	10.2	6.9	9.1
ERHA	8.4	11.0	6.1	6.0

[a] CS = corn starch; HA = high-amylose corn starch; ERHA = extruded retrograded high-amylose corn starch.

TABLE IV
Volumes (ml) and Specific Volumes (ml/g) of the Loaves Baked
with and without Substitution of 24% of the Flour
by 4% Gluten and 20% of Different Starches

Bread[a]	Volume, ml	Specific Volume, ml/g
Wheat flour	663.9 (8.5)[b]	4.73 (0.07)
CS	654.3 (10.2)	4.59 (0.08)
HA	655.9 (17.0)	4.49 (0.13)
ERHA	621.5 (10.8)	4.12 (0.09)

[a] CS = corn starch; HA = high-amylose corn starch; ERHA = extruded retrograded high-amylose corn starch.
[b] Standard deviation in parentheses.

breads stored for seven days. Therefore, as indicated in Figure 2, the higher RS content in bread loaves with HA and ERHA did not imply increased crumb firmness.

In DSC analysis, an endotherm appeared between 40 and 60°C. This endotherm has been attributed to retrograded amylopectin and is also referred to as the "staling endotherm" (Russell 1983, 1987; Eliasson 1985). Table VI shows the analysis of variance for the effects of bread recipes and storage time on the staling endotherm (enthalpy values). All bread types showed an increase in enthalpy with storage time (Table VII). After one or seven days of storage, breads with CS showed a more intense staling endotherm than did the other bread types. Breads with HA or ERHA contained more retrograded amylopectin after one day of storage, compared with that of the control, but the increase during storage was less pronounced. Thus, after seven days of storage, breads with HA or ERHA showed the smallest staling endotherm. These findings are in line with the results of the compressibility measurements, except for the breads containing ERHA, which were the softest (Fig. 2) but did not have the lowest staling endotherm enthalpy values. The enthalpy values were slightly higher than those of the breads with HA. We cannot explain why, in spite of decreased specific volumes, we observed an improvement in crumb softness as a result of the addition of ERHA. The fact that more water was used in this recipe is not a key factor because baking experiments with lower levels of water did not greatly increase in crumb firmness (data not reported).

CONCLUSIONS

Although wheat flour and different starch sources contain appreciable amounts of RS and DF, processing into bread enhances the digestibility of the starch. Whereas regular, fresh, wheat bread contains virtually no RS, it is possible to produce breads that are significantly RS-enriched. The highest levels of RS were obtained when HA or ERHA were incorporated into

TABLE V
Analysis of Variance of the Effects for Bread Formulations and Storage Times on Crumb Firmness

Source of Variance	Degrees of Freedom	Mean Squares	F^a
Bread type (B)	3	10.521	35.65**
Storage time (S)	2	143.744	487.01**
B × S	6	2.533	8.58**
Error	12	0.292	...

[a] ** = significant at $P < 0.001$; F is the explained variance × (unexplained variance)$^{-1}$ = mean square × error^{-1}.
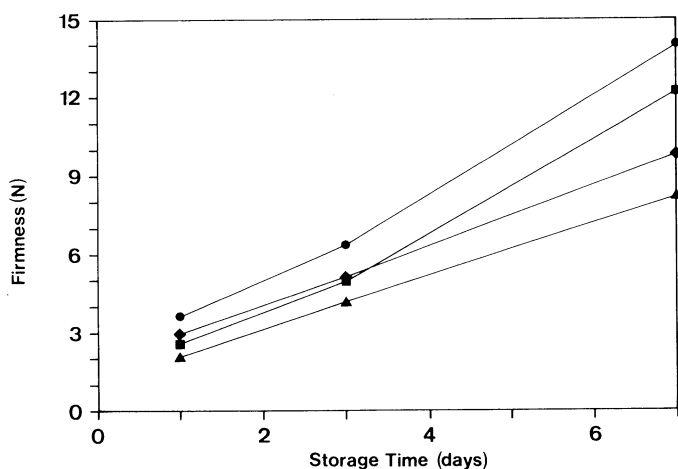

Fig. 2. Crumb firmness (resistance against 25% compression) of the different breads as a function of storage time of the breads. ■ = Wheat flour (control); ● = 20% corn starch; ◆ = high-amylose corn starch; ▲ = extruded retrograded high-amylose corn starch.

TABLE VI
Analysis of Variance for the Effects of Bread Formulations and Storage Times on the Staling Endotherm

Source of Variance	Degrees of Freedom	Mean Squares	F^a
Bread type (B)	3	0.137	3.95*
Storage time (S)	1	0.602	17.40**
B × S	3	0.085	2.46 ns
Error	16	0.035	...

[a] * and ** = significant at $P < 0.05$ and $P < 0.001$, respectively; ns = not significant; F is the explained variance × (unexplained variance)$^{-1}$ = mean square × error^{-1}.

TABLE VII
Thermo-Analytical Characteristics[a] of Bread Crumb Containing Different Starches and Stored for 1 or 7 Days

Bread Crumb[b]	1 Day		7 Days	
	T_p	ΔH	T_p	ΔH
Wheat flour	48.9	1.0	47.9	1.6
CS	48.3	1.3	47.9	1.7
HA	48.7	1.1	48.1	1.2
ERHA	50.0	1.2	48.3	1.4

[a] Peak Temperature (T_p, °C) and Enthalpy (ΔH, mJ/mg).
[b] CS = corn starch; HA = high-amylose corn starch; ERHA = extruded retrograded high-amylose corn starch.

the recipe. Such incorporations resulted in significant quantities of very resistant starch that withstood amylolysis at 100°C.

During ageing of the breads, additional quantities of RS were formed. DSC analysis showed that amylopectin retrograded. However, whether or not such material is enzyme resistant under physiological conditions is unclear at this time.

Measures taken to increase RS levels from a virtual absence to about 10% need not necessarily impair bread quality, as exemplified by the excellent softness, acceptable bread volumes, and by the appreciation in the sensory analysis. In fact, the bread-making process has more impact on the enzyme resistance of the raw materials than vice versa.

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