# Water-Soluble Dextrins from α-Amylase-Treated Bread and Their Relationship to Bread Firming[1]

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Cereal Chem. 71(3):223-226

**ABSTRACT**

White bread was supplemented with malted barley flour, four bacterial, and two fungal sources of α-amylase to ascertain their effect on bread firming. The malt-supplemented bread firmed at a faster rate than the standard, unsupplemented bread. The fungal amylases and one of the bacterial amylases reduced the rate of bread firming compared to that of the standard. The remaining three bacterial amylases reduced the rate of bread crumb significantly more than did the other treatments. High-performance anion-exchange chromatography was used to analyze the water-soluble dextrins extracted from the aged, supplemented, and unsupplemented bread crumb. Certain peak areas obtained from the chromatograms were shown to be highly correlated with a reduced rate of bread crumb firming. Other peaks were highly correlated with an increased rate of bread crumb firming. When comparing only the bacterial enzymes, the three bacterial α-amylases that produced the lowest rate of bread crumb firming produced peaks that were significantly and highly correlated to a reduced rate of bread crumb firming. The fourth bacterial amylase supplemented bread, which firmed at the same rate as the two fungal amylase supplemented breads, contained more carbohydrate in those peaks and significantly and positively correlated to an increased rate of bread crumb firming.

For years, it was generally believed that bread firming was caused by the retrogradation or recrystallization of starch, particularly the amylopectin (Schoch and French 1947, Kim and D'Appolonia 1977). Recently, a new mechanism was suggested for the firming process (Martin and Hoseney 1991). The firming mechanism postulates that interactions occur between the swollen starch granules and the continuous protein network in bread. These interactions, or cross-links, originate during the bake process. Aging permits more linkages to form and strengthens linkages already present in the baked bread.

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It has been well documented that bacterial α-amylase exerts a retarding effect on the firming rate of bread (Conn et al 1950, Zobel and Senti 1959, Dragsdorf and Varriano-Marston 1980). Martin and Hoseney (1991) reported that low molecular weight dextrins were produced during baking of bacterial α-amylase-treated dough. Those dextrins slowed bread firming by interfering with the interaction between the starch and the continuous protein matrix.

During baking, there are windows of accelerated enzyme activities that define the temperature range over which starch is readily degraded (Martin 1989). The optimal temperatures range from starch gelatinization to the enzymes' denaturation. Bacterial amylases have a wider window of activity than do the malt or fungal amylases. The temperature at which the bacterial amylases denature (95°C) exceeds that of either the malt (85°C) or fungal

(75° C) denaturation temperature (Audidier 1968).

The objective of this study was to use high-performance anion-exchange chromatography (HPAEC) to separate the water-soluble dextrins extracted from bread (α-amylase-supplemented and unsupplemented) that had been aged for five days. We wanted to determine the relationship between soluble dextrins and the rate of bread firming.

## MATERIALS AND METHODS

Bread was prepared and baked according to AACC method 10-10B (AACC 1983). The flour used was donated by Cargill (Wichita, KS) and had a protein content of 11.7%. Fermipan instant yeast (0.72% flour basis) was donated by Gist Brocades (King of Prussia, PA). Fermentation time used was 180 min.

The seven α-amylase enzymes studied were commercial preparations. Malted barley flour, was procured from the Department of Grain Science and Industry at Kansas State University, Manhattan, KS. The two fungal α-amylases tested were from *Aspergillus oryzae*: one was product A0273 from Sigma Chemical Co. (St. Louis, MO), and one was from Amano International Enzyme Co. (Troy, VA). Four bacterial α-amylase sources (*Bacillus* strain) were: Canalpha from BioCon (Lexington, KY), Dexlo-P from Gist Brocades Food Ingredients, Enzeco from EDC Development (New York), and Hi Tempase from BioCon.

The amounts of enzymes added to the doughs are listed in Table I. In the case of the malted barley flour, an equal weight of flour was removed.

The enzymatic activity of the amylase preparations were determined using the AmylaZyme AK Alpha Amylase Enzyme Activity test (Megazyme Pty. Ltd., Australia). The malted barley flour and fungal enzymes were inverted (shaken) for an additional 10 min. The enzyme activity was calculated according to the test procedure.

Bread was stored in polyethylene bags at ambient temperature for five days. Firmness of the bread crumb was measured on days 1, 3, and 5 after baking using a TA.XT2 Texture Analyzer equipped with a 25-kg compression load cell and 36-mm (dia) aluminum plunger (Texture Technologies, Scarsdale, NY) (AACC method 74-09). AACC method 74-09 was modified by changing the crosshead speed from 50 mm/min to 48 mm/min (0.8 mm/sec). The compression distance did not change.

Results of each compression were stored using the XT-RA Texture Analyzer compatible software program (Stable Micro Systems, United Kingdom). Compression force values at 25% compression, 6.25 mm, (AACC method 74-09) were taken on days 1, 3, and 5. The firmness values, in grams, were plotted as a function of time (days). The firming rate reported was calculated as the change in firmness over time (grams per day).

Extraction of water-soluble dextrins from the bread crumb was according to Martin (1989). The water-soluble dextrins were analyzed using a Dionex BioLC Model 4000i HPAEC with pulsed amperometric detection (PAD) (Dionex Corp, Sunyvale, CA). A Dionex Carbopac PA1 column (4 × 250 mm) was used with a CarboPac PA guard column (3 × 25 mm). Results were recorded on a Chromatopac CR601 digital integrator (Shimadzu, Kyoto, Japan). The sample loop size was 20 μl. Pulse potentials (volts) and durations were: E1 0.05 (t1 0.0), E2 0.05 (t2 0.50), E3 0.60 (t3 0.51), E4 0.60 (t4 0.59), E5 −0.60 (t5 0.60), E6 −0.60 (t6 0.65).

The gradient program for the HPAEC analysis consisted of eluent A (sodium hydroxide, 150 m*M*) prepared with carbonate free water, and eluent B (sodium hydroxide, 150 m*M*) containing sodium acetate (500 m*M*). Both eluents were prepared with 18 megaohm resistence deionized water. The eluent program at time zero was 80% solution A and 20% solution B. After 10 min, the eluent was 60% solution A and 40% solution B. Separations were accomplished at ambient temperatures with a flow rate of 1 ml/min. Three subsamples were taken from each extract, and there were four extracts of each treatment.

Elution times were used to identify peaks on the chromatogram. Small adjustments in time were made to line up the sample peaks. The area under each curve was an estimate of the amount of

**TABLE I**
**α-Amylase Supplementation of Dough by Type and Name**

| Enzyme | Amount (g) per 100 g of flour |
|---|---|
| Cereal | |
| Malt | 1.9774 |
| Fungal | |
| Amano | 0.0148 |
| Sigma | 0.0197 |
| Bacterial | |
| Canalpha | 0.0014 |
| Dexlo-P | 0.0001 |
| Enzeco | 0.0059[a] |
| Hi Tempase | 0.0044[a] |

[a] Liquid preparations weighed out in grams.

**TABLE II**
**Enzyme Activity of Cereal, Fungal, and Bacterial α-Amylase Determined Using MagaZyme AmylaZyme Test**

| Enzyme | Activity Units[a] per g/ ml of Enzyme | Units of Amylase Activity per 100 g of Flour |
|---|---|---|
| Cereal | | |
| Malted barley flour | 100.70 | 199.12 |
| Fungal | | |
| Amano | 680,815.00 | 10,096.82 |
| Sigma | 30,932.50 | 611.82 |
| Bacterial | | |
| Canalpha | 28,152.39 | 41.75 |
| Dexlo-P | 40,143.01 | 5.47 |
| Enzeco | 7,791.38 | 46.22 |
| Hi Tempase | 3,913.78 | 17.41 |

[a] Where activity units are milli-Somogyi units.

**TABLE III**
**Rate of Change in Firmness, Total Carbohydrate, and Total Number of Peaks Detected in HPAEC[a] Analysis of Water-Soluble Dextrin Extracts from α-Amylase-Treated and Standard Untreated Breads**

| Treatment | Rate of Change in Firmness (g/day)[b] | Average Total Carbohydrate (area)[c] | Total Number of Peaks |
|---|---|---|---|
| Standard | 159 b | 50,614 e | 28 |
| Malt | 197 a | 59,968 d | 37 |
| Amano | 127 c | 49,907 e | 35 |
| Sigma | 115 c | 58,812 d | 36 |
| Canalpha | 22 d | 113,535 b | 41 |
| Dexlo-P | 34 d | 119,608 a | 37 |
| Enzeco | 34 d | 107,752 bc | 35 |
| Hi Tempase | 109 c | 104,370 c | 35 |

[a] High-performance anion exchange chromatography.
[b] Means with same letter are not significantly different.
[c] Area under the curve represents average of twelve samples in treatment group. Results reported are $10^{-2}$ smaller than actual data.

**TABLE IV**
**Total Area[a] Detected by HPAEC[b] Analysis of Water-Soluble Dextrin Extracts from α-Amylase Treated and Standard Untreated Breads**

| Treatment | Total Area |
|---|---|
| Standard | 50,614 e |
| Malt | 59,968 d |
| Amano | 49,907 e |
| Sigma | 58,812 d |
| Canalpha | 113,535 b |
| Dexlo-P | 119,608 a |
| Enzeco | 107,752 bc |
| Hi Tempase | 104,370 c |

[a] Analysis using PC SAS software. Means with same letter are not significantly different.
[b] High-performance anion-exchange chromatography.

dextrin. The data were multiplied by a factor of $10^{-2}$ and reported as such.

Data were analyzed using PC SAS (SAS 1980). Area under the peaks were correlated with the firming rate of the corresponding bread crumb. The firming rates were determined by averaging the results of measurements on three slices of bread per loaf.

The total integrator area (sum of area under all the peaks) from each HPAEC chromatogram was used as a quantitative value to represent the total carbohydrate content of each bread extract. Areas were determined by averaging the results of measurements on three subsamples per loaf.

Reference sugar solutions of glucose DP 1-7, fructose and lactose (0.01 mg/ml) were analyzed for their retention times. Retention times were recorded for each solution using the Shimadzu integrator as previously described.

## RESULTS AND DISCUSSION

### Enzyme Activity

The amount of enzyme added to each dough was determined by subjective criteria. A level of enzyme was added to the bread dough sufficient to achieve a softening effect, yet yield a crumb that regained its elasticity after compression. The enzymatic activities of the α-amylases added to the bread doughs were determined (Table II).

### Rate of Crumb Firming

The malted barley treatments increased bread firming rate when compared to that of standard untreated bread (Table III). Martin (1989) reported similar results. Amano and Sigma fungal amylase treatments lowered bread firming rate. Hi Tempase bacterial amylase treatment had a firming rate equal to those of the fungal enzymes. Three other bacterial amylases (Canalpha, Dexlo-P, and Enzeco) were equally effective in reducing bread firming and were the most effective of the enzymes used.

### Total Carbohydrate from HPAEC Analysis

Water-soluble extracts of the bacterial-supplemented breads contained significantly more total carbohydrate than did either the fungal or malt extracts (Table III). The fact that the largest amount of total carbohydrate was detected in the soluble extracts from the bacterial-treated bread may be because of their wider window of activity. The bacterial's thermostability allows hydrolysis to take place over a longer time period during baking, resulting in a higher total carbohydrate.

It does not appear that the level of soluble carbohydrate is directly related to a reduced rate of bread crumb firming. For example, the total soluble carbohydrate from the Sigma fungal amylase and malted barley flour supplemented breads were the same, but their firming rates were different (Table III). In addition, the two fungal amylases (Sigma and Amano) and the bacterial Hi Tempase had significantly different amounts of total carbohydrate, but their rate of firming was the same (Table IV).

### Total Number of Peaks

The total number of peaks detected by HPEAC analysis of the water soluble extracts is given in Table III. Little relationship appears to exist between the number of peaks and the enzyme's ability to retard bread firming.

TABLE V
**Average Elution Times for Reference Sugars Using HPAEC[a] Analysis**

| Sugar | Average Elution Time, min |
|---|---|
| Fructose | 2.32 ± 0.000 |
| Lactose | 2.35 ± 0.000 |
| Glucose (G1) | 2.17 ± 0.005 |
| Maltose (G2) | 3.40 ± 0.002 |
| Maltotriose (G3) | 5.10 ± 0.008 |
| Maltotetraose (G4) | 6.90 ± 0.002 |
| Maltopentaose (G5) | 8.18 ± 0.008 |
| Maltohexaose (G6) | 9.23 ± 0.007 |
| Maltoheptaose (G7) | 10.14 ± 0.023 |

[a] High-performance anion-exchange chromatography.

### HPAEC-PAD Analysis of Reference Sugars

The elution times for nine reference sugars are given in Table V. Many of the dextrins in the extract of bread had longer elution times than did maltoheptaose, the largest reference sugar tested.

### Peak Areas of Water-Soluble Dextrin Extracts

Dextrins eluted during HPAEC analysis are referred to by peak number. The lower the number, the shorter the elution time, and presumably the smaller the dextrins.

Correlations were run between the area under the curve of each individual dextrin peak and the firming rate (Table VI). Results showing a significant negative correlation, such as peak 10 (Table VI), suggest an association between the peak area and a reduced rate of bread firming (softening effect by the enzyme). Thus, peaks with a significant positive correlation, are associated with an increase in the rate of bread crumb firming. Three peaks (8, 14, and 23) had significant positive correlations to the rate of bread firming (Table VI).

Assuming that all peaks with a significantly negative correlation were equally effective, the 19 values were summed for each treatment (Table VII). Making the same assumption, the three significantly positively correlated peaks were also summed. A ratio of the two sets of summed values was correlated with the staling rate ($r = 0.866$).

The correlation appears to explain the effect of the various peaks on the firming rates. However, from Table VII, it is obvious that this does not explain the faster staling rate of the Hi Tempase compared to the other three bacterial enzymes.

### Bacterial Group Correlation Results

Correlations were run for just the four bacterial enzymes. The area of each peak was correlated with its corresponding bread firming rate (Table VIII). We found fewer peaks that were sig-

TABLE VI
**Significant Correlation Coefficients ($P \leq 0.05$) for the Relationship Between Peak Area and the Mean Rate of Firming of the α-Amylase-Treated and Standard Untreated Breads**

| Peak | Correlation Coefficient | Peak | Correlation Coefficient |
|---|---|---|---|
| 6 | −0.40 | 32 | −0.59 |
| 8 | 0.49 | 34 | −0.65 |
| 10 | −0.47 | 36 | −0.75 |
| 14 | 0.67 | 38 | −0.90 |
| 16 | −0.84 | 40 | −0.89 |
| 19 | −0.73 | 42 | −0.88 |
| 20 | −0.49 | 43 | −0.70 |
| 22 | −0.86 | 45 | −0.77 |
| 23 | 0.51 | 47 | −0.79 |
| 29 | −0.50 | 48 | −0.91 |
| 31 | −0.67 | 50 | −0.72 |

TABLE VII
**Summation of the Areas under the Peak for Positive and Negative Significantly Correlated Peaks and Their Ratios**

| Treatment | Summation of Peak Area Numbers | | Ratio (+)/(−) |
|---|---|---|---|
| | Positive[a] | Negative[b] | |
| Standard | 828 | 17,336 | 0.048 |
| Malt | 1,331 | 23,746 | 0.056 |
| Amano | 600 | 19,101 | 0.031 |
| Sigma | 1,111 | 22,856 | 0.049 |
| Canalpha | 499 | 73,353 | 0.007 |
| Dexlo-P | 812 | 78,153 | 0.010 |
| Enzeco | 806 | 67,987 | 0.012 |
| Hi Tempase | 618 | 64,8226 | 0.010 |

[a] 8, 14, 23.
[b] 6, 10, 16, 19, 20, 22, 29, 31, 32, 34, 36, 38, 40, 42, 43, 45, 48, and 50.

TABLE VIII
Correlation Coefficients ($P \leq 0.05$) for the Relationship Between Peak Area and Rate of Firming of the α-Amylase-Treated Breads

| Peak | Correlation Coefficient | Peak | Correlation Coefficient |
|---|---|---|---|
| 1 | 0.71 | 29 | 0.79 |
| 16 | −0.77 | 31 | −0.65 |
| 17 | 0.90 | 32 | 0.71 |
| 19 | 0.67 | 34 | 0.64 |
| 20 | 0.79 | 38 | −0.84 |
| 22 | −0.80 | 40 | −0.85 |
| 24 | −0.85 | 42 | −0.82 |
| | | 50 | −0.63 |

nificantly correlated to reduced rates of firming than we had found previously (Table VI). Of the eight peaks (16, 22, 24, 31, 38, 40, 42, and 50) significantly negatively correlated with firming in this set, seven were also negatively correlated in the first set. Peak 24 was not significantly correlated in the first set. Interestingly, none of the three peaks that were positively correlated in the first set were positively correlated with the second set (bacterial enzymes). Instead, a new set of peaks (1, 17, 19, 20, 29, 32, and 34) were found to be positively correlated to firming. Of the seven peaks, five had been significantly negatively correlated in the first set of data.

### Mean Areas of Positive and Negative Correlated Peaks

Assuming that all the negatively correlated peaks were equally effective in retarding firming, those peaks that gave significant negative correlations with bread firming were summed for each enzyme. This was also done for those peaks giving significant positive correlations to bread firming rates (Table IX).

The results indicate that Hi Tempase had the largest total peak area of the positively correlated peaks. This was interesting, because the Hi Tempase firmed at a faster rate (Table III) than did the other three bacterial enzymes. Conversely, the three bacterial enzymes (Canalpha, Dexlo-P, and Enzeco) had the largest total peak areas for those peaks that were negatively correlated to the rate of bread firming. This corresponded to their rate of firming (Table III).

The identity of the dextrins making up the peaks with significant positive and negative correlations to the rate of bread firming is not known. A comparison between the elution time of the reference sugars (Table V) and the elution time of the peaks making up the those peaks positively and negatively correlated to the rate of bread firming shows that most of the peaks of interest eluate much later than do the standards. The elution time of 8.2 min for peak 19 suggests that it may be a G5 linear dextrin.

## SUMMARY

Malt α-amylase-treated bread did not reduce the rate of bread crumb firming when compared to that of standard untreated bread. Two fungal amylases (Amano and Sigma) and one bacterial α-amylase (Hi Tempase) significantly reduced the rate of bread crumb firming compared to that of the standard bread. Three bacterial amylases (Canalpha, Dexlo-P, and Enzeco) significantly reduced the rate of bread crumb firming when compared to that of the other treatments.

Extraction of the water-soluble dextrins from the bacterial α-amylase treated bread and analysis of the extracts using HPAEC revealed that many of the peaks in the chromatogram profiles were associated with a reduced rate of firming. Both positive and negative correlations existed between the rate of bread firming and the mean peak areas produced by HPAEC analysis of the bacterial-treated bread water-soluble extracts.

Whether these peaks are composed of linear or branched dextrins, or a mixture of both, is not determinable from this data. The elution time of many of the dextrins making up the peaks associated with firming exceeded the elution time of the G7 reference sugar reported in this article. That suggests that many of these dextrins are larger than G7.

The results of this study also indicate that not all bacterial amylases produce the same amount of the same dextrins. The extent to which different bacterial α-amylases reduced the rate of bread crumb firming differed significantly. In general, this report extends the study of Martin and Hoseney (1991) and confirms that the dextrins produced by amylases are important in controlling the rate of bread firming.

TABLE IX
Total Mean Peak Area, by Treatment, for Those Peaks with Significant Positive and Negative Correlations to Firming Rate

| Enzyme | Summation of Peak Area Numbers | |
|---|---|---|
| | Positive[a] | Negative[b] |
| Canalpha | 14,652 | 27,026 |
| Dexlo-P | 15,822 | 28,626 |
| Enzeco | 12,660 | 25,171 |
| Hi Tempase | 20,256 | 17,272 |

[a] 1, 17, 19, 20, 29, 32, and 34
[b] 16, 22, 24, 31, 38, 40, 42, and 50

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[Received August 30, 1993. Accepted January 24, 1994.]