Characterization of Soluble Starch from Bread Crumb[1]

K. GHIASI,[2] R. C. HOSENEY,[2] and D. R. LINEBACK[3]

ABSTRACT

The soluble starch fraction extracted from bread crumb with water decreased in quantity as the bread was aged. Bread baked with surfactant but without shortening had a smaller quantity of soluble starch and the quantity did not change so much with age as did that of control bread baked with or without shortening. The soluble starch gave generally low iodine affinity values, indicating that the fraction was mostly amylopectin. However, β-amylolysis values were much higher than normal for amylopectin. Gel filtration on sepharose 4B and 6B-CL columns showed that the soluble starch was much lower in molecular weight than was normal amylopectin. Debranching the soluble starch with pullulanase followed by fractionation on Bio-Gel P-10 proved that the soluble starch was essentially all branched. The branched fraction had a shorter average chain length and a higher A-chain to B-chain ratio than did normal wheat amylopectin. Thus, the soluble starch extracted from bread crumb is an amylopectin that has been degraded by amylases during baking.

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Studies to determine the cause of bread staling have indicated that changes in the starch components are of major importance. One change in bread during storage is reduction in the amount of soluble starch (Schoch and French 1947). This change in soluble starch content has been used to estimate the extent of staling (Katz 1928). On the basis of iodine affinity values, Schoch and French (1947) reported that the water-soluble starch extracted from fresh bread was mainly amylopectin, suggesting that staling of bread is a spontaneous aggregation of the branched fraction. Kim and D'Appolonia (1977) reported that the amount of amylose in the extracted soluble starch was small and contributed to staling primarily during the first day of storage. The amount of soluble starch from fresh bread was greater and the starch had lower iodine affinities than did the soluble starch from a 50% starch paste (Schoch and French 1947). The difference was presumably the influence of other bread constituents.

Little direct information concerns the structure of water-soluble starch isolated from bread. Therefore, to understand more about bread staling, more information is needed about the soluble starch in bread. The purpose of this study was to determine the amount of soluble starch, its components, and its chemical structure and to investigate the effect of shortening and surfactants on the physical and chemical properties of soluble starch.

[1]Contribution 79-116-J, Department of Grain Science & Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan 66506.
[2]Graduate research assistant and professor, respectively.
[3]Head, Department of Food Science, Pennsylvania State University, University Park 16802.

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MATERIALS AND METHODS

Flour

The flour used was a composite of several hard red winter wheat varieties grown at several locations in the southern Great Plains. The flour had a protein content of 12.3% (N × 5.7), ash content of 0.40, a medium mixing time, and good loaf volume potential.

Bread

Bread was made using a straight-dough procedure with 3-hr fermentation, 55-min proof time at 86° F, and 25-min baking at 436° F. The baking formula, based on flour weight, was as follows: flour (14% mb), 100; water, variable; sugar, 6.0; salt, 1.5; nonfat dried milk, 4.0; shortening, 3.0; yeast, 2.0; malt (12 α-amylase units per gram), 0.75; oxidant, 20 ppm $KBrO_3$.

Three other breads were made by this procedure and formula except that one contained no shortening and the other two contained either 0.5% sodium stearoyl 2-lactylate (SSL) or monoglyceride in place of shortening.

Baked bread was cooled at room temperature and stored in sealed containers to prevent moisture loss. After one and five days of storage, bread crumb was removed and the soluble starch extracted.

Isolation of Soluble Starch

The soluble starch was isolated from the bread crumb according to Schoch and French's procedure (1947). A 25-g sample (as-is basis) was placed in a 250-ml centrifuge bottle; 100 ml of distilled water was added; and the mixture was stirred for 1 min with a propeller-type stirrer to give a smooth paste. The sample was stirred at 30° C for 30 min to keep the bread crumb suspended and then centrifuged for 5 min at 1,000 × g. The supernatant solution was decanted and the extraction procedure repeated two more times. The combined supernatant solutions and wash waters were treated with three to four volumes of methanol to flocculate the soluble starch; then the mixture was heated on a steam bath for 1 hr and allowed to settle overnight. The precipitate was washed with methanol, dissolved in water, and freeze-dried. The percentage of solubles was calculated on a dry bread basis.

Moisture and Protein Determinations

Moisture was determined in bread samples before extraction according to AACC method 44-15A. Protein content (N × 5.7) of soluble starch samples was determined by the approved AACC method 46-13.

Iodine Affinity

To remove traces of fatty materials, the soluble starch samples were Soxhlet-extracted for 24 hr with methanol before determination of iodine affinity. Iodine affinities were determined by potentiometric titration (Schoch 1964) using a Beckman pH meter with a platinum electrode calibrated with a voltage reference cell. The readings were taken after stabilization of the electrodes.

TABLE I
Soluble Starch Extracted from Bread Made with Different Treatments and Stored for Different Times

Treatment	Age (days)	Soluble Starch Extracted[a] (%)	Difference (%)
Control	1	4.3	0.9
	5	3.4	
Without shortening	1	4.2	1.1
	5	3.1	
With SSL	1	3.1	0.2
	5	2.9	
With monoglyceride	1	3.8	0.4
	5	3.4	

[a]Standard deviation = 0.08

Determination of Total Polysaccharide

The total amount of polysaccaride in each sample was determined by the phenol-sulfuric acid procedure (Dubois et al 1956) using a standard curve prepared from D-glucose.

Determination of Total Starch

Soluble starch (1 mg/ml) was pipetted into a test tube containing 10 IU of glucoamylase (Grade 11, from *Rhizopus* species, Sigma Chemical Co., St. Louis, MO) in 5.0 ml of 0.05 *M* citrate buffer, pH 4.8 (Shetty et al 1974). The tubes were incubated at 37° C for 60 min. The glucose in 1 ml of this solution was determined by measuring the reducing power, using Nelson's colorimetric copper procedure (1944). The percent of starch was calculated as follows:

$$\% \text{ total starch} = \frac{\text{glucose released} \times 0.9}{\text{sample wt}} \times 100$$

Determination of β-Amylolysis Limit

Soluble starch samples were treated with β-amylase (crystalline, type I-B, Sigma Chemical Co., St. Louis, MO) according to Whelan's procedure (1964) except that reduced glutathione and serum albumin were omitted from the digests (Marshall 1974). The sample (20 mg) was incubated with 750 units of β-amylase in 5 ml of 0.02–0.04 *M* acetate buffer, pH 4.8, at 35° C for 24 hr. The maltose liberated was determined using Nelson's colorimetric copper method (1944). The degree of β-amylolysis was calculated as follows:

$$\% \beta\text{-amylolysis} = \frac{\text{maltose} \times 0.95}{\text{starch wt}} \times 100$$

Purified Water-Soluble Pentosans

Water-soluble pentosans were isolated from soluble starch by a modification of the procedure described by Medcalf et al (1968). Freeze-dried soluble starch (1 g) was dissolved in 50 ml of distilled water, allowed to set one day, and treated with *Bacillus subtilis* α-amylase (25 mg, Type II-A, Sigma Chemical Co., St. Louis, MO). The enzyme was dissolved in 50 ml of 0.02 *M* sodium phosphate buffer, pH 7.2, containing 0.04 *M* sodium chloride. The enzyme and sample solutions were combined and incubated during dialysis against diluted (1:1) phosphate buffer solution for 48 hr at room temperature. The enzyme was then denatured by heating at 90° C for 1 hr. The coagulated protein was removed by centrifugation at 20,000 × *g* for 10 min. The supernatant solution was dialyzed against distilled water for 48 hr and freeze-dried.

Fractional Precipitation with Ammonium Sulfate

Solutions of soluble starch (2 mg/ml) were dissolved in 0.1 *M* phosphate buffer (pH 7.0) and sufficient ammonium sulfate was slowly added to give saturation. After being stirred for 3 hr, the solutions were allowed to stand overnight at 4° C. The precipitated polysaccharide was collected by filtration on glass paper and washed thoroughly with saturated ammonium sulfate in 0.1 *M* phosphate buffer (pH 7.0). The precipitate was dissolved in water and dialyzed free of ammonium sulfate. The combined supernatant solution and washings were also dialyzed exhaustively against water to recover $(NH_4)_2SO_4$ solubles (Fincher and Stone 1974).

TABLE II
Iodine Affinity Based on the Starch in Soluble Starch Samples

Treatment	Age (days)	Iodine Affinity (%)
Control	1	2.60
	5	1.86
Without shortening	1	4.60
	5	4.30
With SSL	1	1.20
	5	0.85
With monoglyceride	1	1.40
	5	0.90

Gel-Filtration Chromatography on Sepharose 4B

Samples (5–15 mg) were loaded onto a column (60 × 2.6 cm) of sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) The samples were eluted at a flow rate of approximately 10 ml/hr with a solution of 0.01*M* phosphate buffer (pH 8.3) containing 0.02% sodium azide; 4–5 ml fractions were collected.

Gel-Filtration Chromatography on Sepharose 6B-CL

Samples (5–15 mg) were chromatographed on a column of sepharose 6B-CL (cross-linked, 70 × 2.6 cm). The carbohydrate was eluted with 0.01*M* phosphate buffer, pH 12.0, containing 0.02% sodium azide. Fractions were automatically collected at 30-min intervals (~6 ml). The total amount of polysaccharide in each fraction was determined using the phenol-sulfuric acid procedure (Dubois et al 1956).

Debranching with Pullulanase

Pullulanase was tested for amylase activity before debranching. Digests were prepared; each contained 0.2 ml of amylose (Nutritional Biochemical Co.) in dimethylsulfoxide (40 mg/ml); 0.7 ml of 0.02*M* citrate phosphate buffer pH 5.0; and 0.1 ml of pullulanase (10 mg/ml in 0.02*M* citrate phosphate buffer, pH 5.0). The digest was incubated 48–72 hr at 30° C, and reducing sugar was measured by Nelson's colorimetric procedure (1944).

Debranching of the soluble starch sample was conducted at 37° C in digest containing 2–5 mg/ml of substrate and 3–5 units per milliliter of pullulanase (*Enterobacter aerogenes*, Sigma Chemical Co., St. Louis, MO, 150 IU/ml) in 0.02*M* sodium acetate buffer, pH 5.5. A drop of toluene was added to each digest to inhibit bacterial growth. After a 24-hr incubation, the digest was heated in a boiling water bath to inactivate the enzyme, and insoluble material was removed by filtration (Lii and Lineback 1977).

Gel-Filtration of Debranched Soluble Starch

Debranched samples (5–10 mg) were fractionated on a Bio-Gel P-10 column (2.6 × 70 cm) at room temperature (Lii and Lineback, 1977). Elution was accomplished with 0.01*M* phosphate buffer, pH 7.0, containing 0.02% sodium azide. Fractions (~6 ml) were collected automatically every 30 min. Total carbohydrate content in each fraction was measured by the phenol-sulfuric acid method (Dubois et al 1956).

Average Unit-Chain Length of Amylopectin

The carbohydrate (debranched amylopoectin) eluting from Bio-Gel P-10 after the void volume peak was concentrated, and the average unit-chain length (CL was determined (Gunja-Smith et al 1971) by dividing the total amount of polysaccharide by the amount of reducing groups released during debranching. Reducing groups were determined by Nelson's colorimetric procedure (1944) except that all volumes were reduced by a factor of two to increase the sensitivity of the method. D-Glucose was used as the standard.

TABLE III
Total Carbohydrate (CHO) and Starch in Soluble Starch Samples

Treatment	Age (days)	Total CHO (%)	Total Starch (%)	Difference between Total CHO and Starch (Pentosans) (%)
Control	1	78.4	66.2	12.2
	5	76.6	61.9	14.7
Without shortening	1	80.3	66.2	14.1
	5	78.4	63.5	14.9
With SSL	1	80.3	59.9	20.4
	5	78.7	56.3	22.4
With monoglyceride	1	78.7	63.5	15.2
	5	77.6	57.0	20.6
Standard deviation	...	0.02	0.03	...

β-Amylase Limit Dextrins

β-Amylase limit dextrins of soluble starch were prepared by exhaustively treating solutions of the polysaccharides (20–30 mg/ml in 100m*M* acetate buffer, pH 4.8) with β-amylase (380 units per milliliter) in a dialysis bag. The digest was continuously dialyzed against 100m*M* acetate buffer, pH 4.8, to remove maltose. After complete digestion, the solutions were boiled for 15 min to inactivate β-amylase, centrifuged, dialyzed against distilled water, and freeze-dried (Marshall 1974, Marshall and Whelan 1974).

Ratio of A-Chains to B-Chains

The ratio of A-chains to B-chains in amylopectin was determined as described by Marshall and Whelan (1974) using isoamylase (glycogen 6-glucanhydrolase, EC 3.2.1.68) and pullulanse (pullulan 6-glucanohydrolase, EC 3.2.1.41).The amount of reducing sugars liberated in 24 hr by the action on the dextrins of 1) isoamylase (lytic enzyme, Ll from cytophaga, Gallard-Schlesinger, Carli Place, NY) or 2) isoamylase plus pullulanase was measured in digest containing substrate (2–5 mg/ml), isoamylase (0.06 unit per milliliter), and 20m*M* sodium acetate buffer, pH 5.5, at 37° C. To prevent inhibition of one enzyme by the other (Marshall and Whelan 1974), pullulanase (3–5 units per milliliter) was added after a 12-hr preincubation of the digest with isoamylase alone. The ratio of A-chains to B-chains was calculated from the increase in reducing power following the action of debranching enzymes (Marshall 1974).

To determine the completeness of debranching with isoamylase or pullulanase, 0.5 ml of the supernatant solution was treated with 380 units of β-amylase in 0.5 ml of 100m*M* sodium acetate buffer, pH 4.8, for 24 hr at 37° C (Lii and Lineback 1977). Reducing power was measured as glucose by Nelson's colorimetric copper procedure (1944).

TABLE IV
Total Carbohydrate (CHO) and Starch in Filtered Soluble Starch Samples

Treatment	Age (days)	Total CHO (%)	Total Starch (%)	Difference Between Total: CHO Before and After Filtration	Difference Between Total: Starch Before and After Filtration
Control	1	73.7	57.0	4.7	9.2
	5	73.2	55.4	3.4	6.5
Without shortening	1	73.2	59.9	7.1	6.3
	5	76.54	58.9	1.9	4.6
With SSL	1	72.0	52.7	8.3	7.3
	5	72.0	51.0	6.7	5.3
With monoglyceride	1	75.7	55.0	3.0	8.5
	5	70.2	50.0	7.4	7.0

TABLE V
β-Amylolysis of Soluble Starch Samples

Treatment	Age (days)	% Conversion to Maltose: Based on Whole Sample	% Conversion to Maltose: As % of Soluble Starch Content
Control	1	50.32	76.00
	5	50.00	80.70
Without shortening	1	51.45	77.72
	5	45.43	71.50
With SSL	1	45.41	75.81
	5	45.00	79.90
With monoglyceride	1	44.75	70.48
	5	44.38	77.86

RESULTS AND DISCUSSION

Effect of Staling on Quantity of Soluble Starch

The amounts of soluble starch extracted from bread baked with shortening, no shortening, SSL, or monglyceride are listed in Table I. The breads containing SSL or monoglyceride did not contain shortening. The data show a decrease in the quantity of soluble starch extracted from five-day-old compared with that from one-day-old bread. The results are in good agreement with the work of Schoch and French (1947). The difference between the amounts of soluble starch extracted from bread one and five days after baking was highest in bread without shortening (1.1%) and lowest in bread baked with SSL (0.2%). The difference was 0.4 and 0.9%, respectively, for bread made with monoglyceride and the control bread containing shortening. These data indicate that addition of surfactant to the system decreases the release of soluble starch from bread and results in less soluble starch becoming insoluble as bread ages.

Iodine Affinity of Soluble Starch

Iodine affinities of the soluble starch samples are presented in Table II. The values were lower for the samples containing surfactant, indicating a lower amount of amylose in those samples. Iodine affinity for the sample baked with no shortening indicated that more amylose was leached from the starch granules in the absence of shortening. The generally low iodine affinity values suggested that the soluble starch extracted from bread was mainly amylopectin.

Total Carbohydrate, Starch, and Pentosan Content of Soluble Starch

The quantity of starch extractable from bread decreased during storage, and the quantity of pentosans (difference between total carbohydrate and starch) increased (Table III). These data agree with those reported by Gilles et al (1961), which showed that the soluble starch in stale bread contained more pentosan than did the soluble starch in fresh crumb.

As is shown in Table III, samples extracted from bread made with surfactant had higher pentosan content and therefore lower starch content than did the samples baked without surfactant. Differences were not significant between the pentosan content of bread baked with no shortening and that of the control.

After the water-extracted samples were precipitated with methanol, dried, and redissolved in water, the solutions were cloudy. In an attempt to eliminate the problem, the samples were dissolved in potassium hydroxide and neutralized with acid, but the solutions were still not clear. This indicated that some components in the extracted samples were becoming insoluble during sample preparation. Therefore, the solutions were filtered and the filtrates analyzed for total carbohydrate and starch. The decrease in starch content ranged from 5 to 9% as a result of filtration (Table IV). Thus a small amount of soluble starch became insoluble during the isolation procedure. If starch in bread were slowly retrograding during storage, less change in starch solubility would be expected for the five than for the one-day-old sample. The data in Table IV show less change for the five-day-old sample.

β-Amylolysis of Soluble Starch

β-Amylolysis limits of soluble starch are given in Table V. β-Amylolysis values increased during storage in all samples except the sample baked with no shortening. The iodine affinity values showed the soluble starch of bread crumb to be mainly amylopectin. A relatively low β-amylolysis value would therefore be expected, but our data showed a very high conversion to maltose, 71–81%, similar to that reported for amylose preparations (Lii and Lineback 1977). These data suggest that the samples are either amylose with unusual iodine affinity values or amylopectin with very high β-amylolysis limits.

Molecular Size Distribution On Sepharose 4B

The soluble starch sample from the one-day-old control bread was fractionated on a sepharose 4B column (Fig. 1). A purified pentosan was prepared by treating the soluble starch fraction with α-amylase and dialyzing. The purified pentosan gave one broad peak on sepharose 4B that corresponded to the higher molecular weight wheat flour component reported by Fincher and Stone (1974) and also eluted at the same volume as one of the two components in the soluble starch sample. The lower molecular weight component reported by Fincher and Stone was not present in this preparation. Fincher and Stone reported that the high molecular weight pentosan fraction was precipitated with saturated ammonium sulfate. Therefore, the soluble starch fraction was treated with saturated ammonium sulfate. After centrifugation, the supernatant fraction was dialyzed and placed on a sepharose 4B

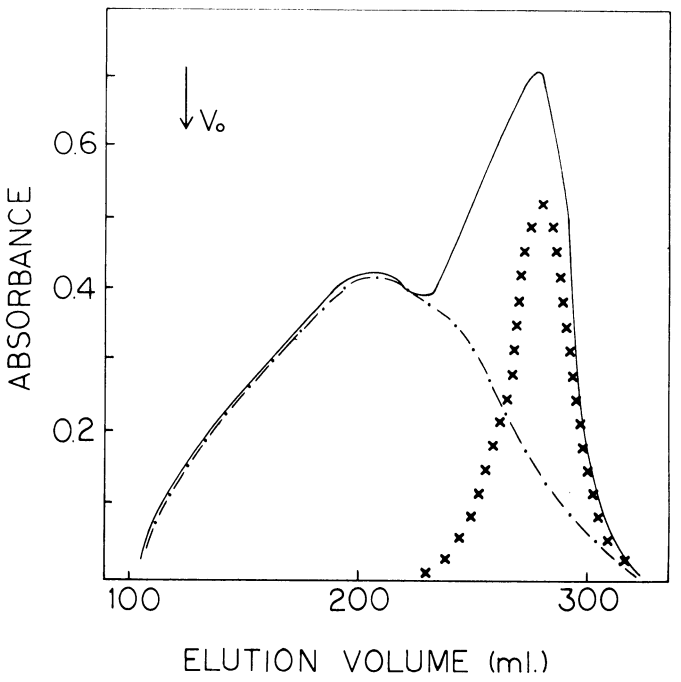

Fig. 1. Elution profiles of soluble starch (——), pentosan (-·-·-·), and the soluble fraction in saturated ammonium sulfate (xxx) on a column of sepharose 4B.

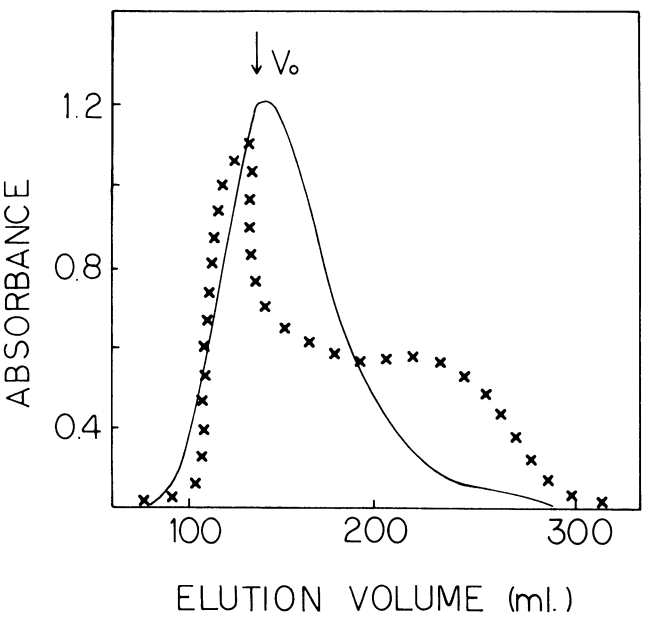

Fig. 2. Elution patterns of soluble starch (xxx) and pentosan (——) on sepharose 6B-CL column.

column. The starch component of the soluble starch sample and the fraction soluble in saturated ammonium sulfate eluted in the second peak, at about the V_t of the column. Because the soluble starch eluted at the V_t of the column and because normal wheat starch elutes at the V_o of the column,[4] the soluble starch sample was lower in molecular weight than is regular starch.

Molecular Size Distribution on Sepharose 6B-CL

The soluble starch fraction extracted from bread and the purified pentosan fraction prepared from that soluble starch were fractionated on sepharose 6B-CL (Fig. 2). The purified pentosan eluted as one peak, mostly in the void volume. The soluble starch fraction had material eluting at the void volume and a sizable quantity of carbohydrate that eluted later, indicating that much of the starch had a relatively low molecular weight.

Gel-Filtration of Debranched Soluble Starch

The soluble starch sample was debranched with pullulanase and fractionated on a column of sepharose 6B (Fig. 3). Debranching should degrade the amylopectin to small fragments that should elute at the V_t of the column. Comparison of Figs. 2 and 3 shows clearly that almost all the starch was eluted at the V_t of the column after debranching. Thus the starch in the soluble sample was mostly amylopectin.

The debranched sample was also fractionated on a Bio-Gel P-10 column (Fig. 4). Two major peaks were obtained. The first peak, eluted at the void volume of the column, was composed of material with higher molecular weight. The first fraction was incubated with glucoamylase and showed only a small amount of starch (presumably amylose that would not debranch with pullulanase and therefore eluted in the void volume of the column along with pentosan). However, pentosan made up the major part of the first peak and amylose was present in very small amounts.

The second major peak was the debranched amylopectin with much lower molecular weight. The seond peak had a leading shoulder that showed the bimodal distribution of chain-lengths, with most chains being of low molecular weight. The soluble starch fractions from bread baked with no shortening and bread containing SSL or monoglycerides at one day of storage and all the samples at five days of storage were debranched with pullulanase and placed on the Bio-Gel P-10 column. All the debranched samples gave similar elution profiles.

[4] W. Atwell, R. C. Hoseney, and D. R. Lineback. Debranching of wheat amylopectin. Unpublished data.

Average Unit-Chain Length ($\overline{CL}$) of Amylopectin

The $\overline{CL}$ of amylopectins from the soluble starch samples were determined after debranching with pullulanase and fractionating on a column Bio-Gel P-10. The second peaks (Fig. 4), which contained the debranched fractions, were concentrated, and the $\overline{CL}$ was calculated as the total amount of polysaccharide divided by the reducing groups liberated during debranching (Table VI).

The sample baked with SSL had the longest $\overline{CL}$ and the sample without shortening the shortest. The $\overline{CL}$ lengths were shorter for all samples than that reported for regular wheat amylopectin (Lii and Lineback 1977).

TABLE VI
Average Unit-Chain Lengths ($\overline{CL}$) of Amylopectin from Soluble Starch Samples

Source	Age (days)	$\overline{CL}$
Control	1	12
	5	11
Without shortening	1	11
	5	10
With SSL	1	16
	5	12
With monoglyceride	1	13
	5	12

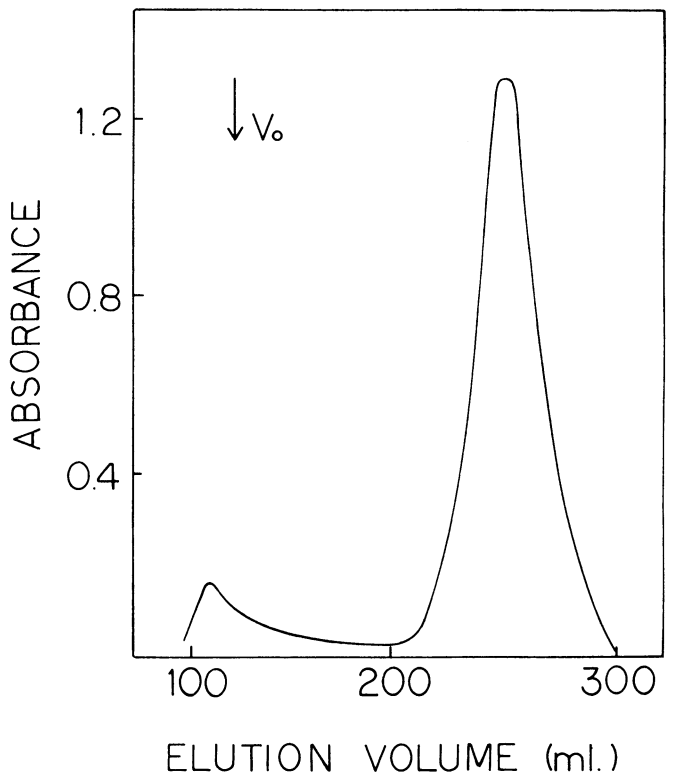

Fig. 3. Elution profile of soluble starch debranched by pullulanase on a column of sepharose 6B-CL.

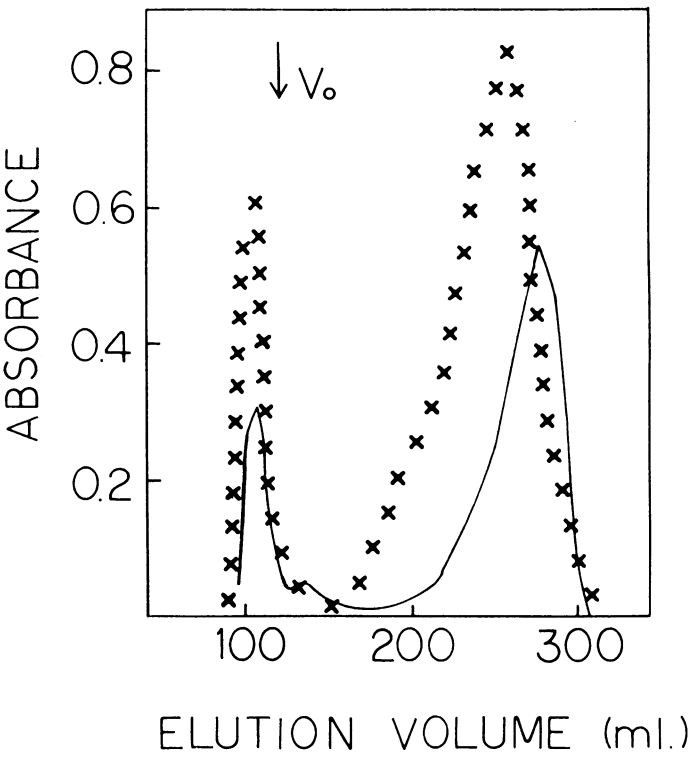

Fig. 4. Bio-Gel P-10 fractionation of the products of pullulanase debranching of the soluble starch extracted from one-day-old (——) and five-day-old (xxx) control bread.

Ratio of A-Chains to B-Chains

The ratio of A-chains to B-chains was calculated from the increase in reducing power following the action of debranching enzymes on β-limit dextrin (Marshall and Whelan 1974). The ratio of A-chains to B-chains was 2:1 for amylopectin from soluble starch isolated from control bread one day after baking, compared with 1.5:1 for an unknown wheat sample (Marshall and Whelan 1974). The ratio of A-chains to B-chains was higher in soluble starch extracted from bread. The higher ratio of A-chains to B-chains in soluble starch amylopectin may explain its high β-amylolysis values. A-chains are unbranched and thus are degraded by β-amylase.

Soluble Starch from Bread

The soluble starch samples extracted from bread are mainly amylopectin but not a normal amylopectin. The soluble amylopectin has low iodine affinity values and high β-amylolysis limits. It has a lower molecular weight, shorter average chain length, and higher ratio of A-chain to B-chains than does regular amylopectin. This component is a degraded amylopectin. The degradation occurs by the action of amylases during baking.

Flour milled from sound wheat has a relatively high content of β-amylase but little α-amylase. The purpose of diastatic supplementation, which is widely used in the baking industry, is to compensate for sound wheat flour's natural deficiency in α-amylase (Pyler 1973). α-Amylase hydrolyzes starch and thus decreases the rate at which bread becomes firm (Miller et al 1953). Major amylase activity occurs during baking. As the temperature of dough increases during baking, starch is gelatinized and becomes more susceptible to the enzyme until the enzyme is inactivated. β-Amylase is inactivated, but malt α-amylase remains active for a period of time (Walden 1955).

Miller et al (1953) and Beck et al (1957) determined the formation of dextrins by extracting bread crumb with water and precipitating the dextrins from the extract with alcohol. They found that a control bread made from unmalted flour contained 211 mg of dextrins in 10 g of bread crumb and that doughs supplemented with 131 or 796 units of wheat α-amylase per 700 g of flour gave 309 and 667 mg of dextrins per 10 g of bread crumb, respectively. Those results support our conclusion that some of the starch components are hydrolyzed by α-amylase during baking and that the extracted water soluble starch from bread is a degraded amylopectin.

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