THE RELATIONSHIP OF SOLUBLE STARCH STRUCTURE TO BREAD STALING

by

KATAYOON GHIASI

B.S., Tehran University, Iran, 1970

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

Approved by:

Major Professor

TABLE OF CONTENTS

INTRODUCTION

Bread staling is an economic problem whose solution should benefit both the consumer and the producer. In 1969, more than 14 billion lbs. of bread were produced in the United States. It was reported that stale returns to the bakery average 8% of the bread produced. Based on the above figure, over 110 million lbs. per year of bread can not be economically sold because of staling (1).

Bread staling is a complex physiochemical phenomena. One of the changes that occur in bread with age is that the proportion of soluble starch in the crumb is reduced (2). There is little direct information concerning the structure of water soluble starch isolated from bread. Therefore, it was felt that in order to understand more about bread staling, one should obtain more information concerning the soluble starch in bread.

It was the purpose of this study to determine the amount of soluble starch, what are its components, what is the chemical structure of the starch component and what is the effect of shortening and surfactants on the physical and chemical properties of soluble starch.

LITERATURE REVIEW

BREAD STALING

Staling is a considerable economic problem encountered in the baking industry. The higher the moisture content of the baked product in its initial fresh state, the more pronounced are the changes which occur

upon staling. Therefore, staling is a more serious problem in products such as bread than it is in cookies and crackers.

Staling has been defined as a "term which indicates decreasing consumer acceptance of bakery products caused by changes other than microbiological spoilage" (3). Bread staling includes both crust and crumb staling. The crust, which in its fresh state is relatively dry and crisp, becomes soft and leathery upon staling. The principal reaction which occurs in crust staling is the transfer of moisture from the interior of the loaf to the crust (4).

The crumb staling is a complex physiochemical phenomena. The most obvious and important attribute of crumb staling is the crumb firming (4). The theories of bread crumb staling started from the general belief that it was due to an overall loss of moisture. As early as 1852, Boussingault (5) had demonstrated that staling was not due to loss of of moisture.

In a study of changes in the starch and gluten during aging, it was shown that during storage of the isolated components there is a progressive drop in the moisture absorption capacity of pregelatinized starch and no changes in gluten on exposure to 97-98% relative humidity (6, 7). On the basis of these results, it was concluded that a transfer of moisture from the starch to the gluten in the crumb occurs during staling. However, Breaden and Willhoft believed in migration of moisture from gluten to starch, thus relating staling to stiffening of the gluten (8).

ROLE OF STARCH IN STALING

Numerous studies to determine the cause of bread staling have indicated that changes in the starch component are of major importance. To confirm the role of starch in staling, Cornford et al (9), studied the relationships between elastic modules and time and temperature on bread crumb. They concluded that crystallization of starch was the principal factor involved in crumb firmness. Similar results were found by Axford (10) and Mciver (11). Katz (12) used a X-ray diffraction technique to study bread staling and observed that fresh crumb gave a so-called V-pattern, which indicates that the starch is in an amorphous form. Upon staling, the X-ray spectrum tends to revert to the B-patterns which is typical of starch in its crystalline state. He applied the term "retrogradation" to the return of crystallinity of starch in staled bread.

This concept of how crystallinity contributes to the firming of starch gels and bread crumb is supported by experiments using cross-linked starch. Bread made from reconstituted flour in which part of the starch was replaced by chemically cross-linked starch had a firmer crumb than did control loaves containing no cross-linked starch (13, 14).

The most widely accepted explanation of the staling phenomenon to date was presented by Schoch (15). According to him, the wheat starch in dough undergoes limited swelling during baking, expelling the more soluble amylose component into the surrounding water. As swelling continues, this amylose solution becomes so concentrated that after the loaf is cooled a rigit gel develops. Therefore, in fresh bread swollen starch granules are embedded

in a firm gel of the linear amylose fraction. As the retrograded linear starch molecules are immobilized in the insoluble gel network, they do not undergo further changes on subsequent storage. Schoch, therefore, attributes the firming of the crumb structure during staling to physical changes in the branched, or amylopectin fraction, within the swollen granules. Zobel (16) reported that when heated at 95°C. under moist conditions the X-ray pattern of stale bread reverts to that of freshly-baked bread. He concluded that the amylopectin fraction of starch undergoes change during bread staling because only a retrograded amylopectin gel can be reversed by those conditions.

ROLE OF SOLUBLE STARCH IN STALING

Appreciable amount of starch in fresh bread is soluble and can be extracted from the crumb by cold water (2). The so-called soluble starch of bread consists of both starch and a pentosan fraction (17). It was reported (17) that the building units of water soluble polysaccharide in fresh bread are primarily glucose, arabinose, and xylose in the mole ratio of 5:1:1, and after 5 days the mole ratio has changed to 3,3:2:1, indicating that the amount of pentose containing polysaccharide has increased (17). During staling, the soluble starch content progressively decreases. This change in soluble starch has been used to estimate the extent of staling (12).

Schoch and French (2) 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 and that the linear fraction has no influence on staling, because it was already retrograded. It was also reported that (18, 19) the amount of amylose in the extracted

soluble starch was very small and contributed to staling primarily during the first day of storage. The amount of soluble starch from fresh bread was greater and had lower iodine affinities than the solubles from 50% starch pastes, probably due to the influence of other bread constituents (2). Little information is available in the literature concerning the structure of water soluble starch from bread.

EFFECT OF SURFACTANTS ON BREAD STALING

Today the use of surfactant is the most effective means of retarding bread staling. In spite of the extensive use of surfactant in bread production, explanation of their effect as antistaling agents is still elusive. Early workers postulated that during baking, surfactants adhere to the surface of the starch, and prevent amylose from leaching out, thus retarding staling (20-25). It was reported (25) that monoglyceride and shortenings produce softer bread by keeping the soluble starch within the granules and by decreasing the gelatinization of the starch thereby allowing an increase in the moisture available for the hydration of gluten.

Coppock (26) discussed several theories of monoglyceride action in bread making: 1) dispersion of fat through the dough; 2) retention of soluble starch in the granules; 3) retardation of gelatinization; 4) supplying more moisture to the gluten; and 5) retention of moisture initially by gluten. It was reported that (26) stale bread containing no added emulsifier will freshen upon heating at a more rapid rate than will bread of the same age containing added emulsifier. Osman and Dix (27) demonstrated that surface active agents can dramatically influence maximum viscosity temperature and gel strength of starch.

Many workers (27-30) have studied the complexing ability of amylose with surfactants and attempted to find a relationship between amylose complex formation and the surfactants effect on staling of bread. Schoch (15) postulated that when a surfactant enters the starch granules it immediately complexes with amylose forming a helix. Mikus et al. (28) observed that the surfactant complex with amylose resembled the complex of iodine and amylose. The crystalline amylose surfactant complex (31, 32), investigated by X-ray diffraction, confirmed complex formation between amylose and surfactant. In this respect, Osman et al. (29) found no apparent relationship between complex formation with the amylose and anti-firming effects on the bread crumb. DeStefanis et al. (33) demonstrated that surfactants form a complex during baking not only with amylose but also with amylopectin.

Krog (34) showed that monoglycerides were the best complexing agents with amylose. He also demonstrated that the ionic surfactants sodium and calcium stearyl 2 - lactylate (SSL and CSL) are good complexing agents. According to Knightly (35), saturated monoesters generally produce better results than do unsaturated ones.

In addition to their role in staling, some surfactants have been found to promote compatibility between soy or other proteins and wheat flour proteins (36, 37). It has been reported that SSL enhanced the quality of bread, especially when a foreign flour was introduced in the bread system (37-39). SSL imparts strength to the dough giving it tolerance to withstand production and ingredient variations. It binds with the gluten and make it more extensible and stronger and forms complexes with starch that retard gelatinization during baking and slows retrogradation after baking (40).

COMPOSITION AND STRUCTURE OF WATER-SOLUBLE PENTOSAN

The nonstarchy polysaccharides in water extracts of wheat flour are composed of arabinose, xylose and galactose. The water soluble polysaccharide complexes (crude pentosan) of flour have been separated into pentosan-rich and hexosan-rich fraction by acetylation and fractional precipitation (41). The pentosan fraction was composed of L-arabinose and D-xylose residues, and the hexosan-rich component appeared to consist of soluble starch or dextrins, and a galactan or pentose-galactan (41). In a further study Perlin (42) proposed a basic repeating unit for the soluble pentosan by using graded acid hydrolysis, methylation and periodate oxidation; the repeating unit involved a straight chain of β-D xylose residues (41-44) to which are appended single units of anhydro L - arabinofuranose through 1,2 and 1,3 linkages, making it a highly-branched structure.

The structure of the water soluble pentosans has been subsequently studied by other workers (43, 44) and the results generally support Perlin's work (41, 42). Water soluble pentosans have been fractionated on DEAE-cellulose columns by different workers (45-48). Kuendig et al. (46) reported that one of the five fractions obtained was an arabinoxylan, free of galactose and protein. This fraction corresponded to the pentosan reported by Perlin (41, 42). The other 4 fractions were more complicated and contained various amounts of protein, arabinose, xylose, and galactose and were found to be glycoproteins by Neukom et al. (49).

Neukom et al. (49) studied the structure of fraction 2 from DEAE cellulose by using the proteolytic enzyme pronase to digest this fraction. They obtained two fractions, one an alcohol-insoluble arabinoxylan containing

some residual protein but no galactose, the second being an alcohol-soluble, nondialyzable arabinogalactan containing some protein but no xylose. The results indicated that galactose was not linked directly to the xylan chain but rather occurs as an arabinogalactan which very likely was connected via a polypeptide bridge to arabinose in the arabinoxylan. It was suggested that at least three different types of carbohydrate-protein linkages could occur between carbohydrate residues and amino acid residues in the protein (50, 51). Fincher et al. (52) studied the structure of arabinogalactan-peptide by partial degradation with oxalic acid and NaOH. They observed that the linkage between polysaccharide and peptide involves galactose and hydroxyproline residues and is glycosidic in nature.

Water-soluble pentosan was fractionated on sepharose-4B columns by Fincher and Stone (53). The elution profile indicated the presence of a high molecular weight compound with an apparent molecular weight in the range 7×10^4 to 10^6 and a low molecular weight polysaccharide with molecular weight less than 7×10^4. Fractional precipitation with ammonium sulphate followed by gel filtration on sepharose-4B indicated that the precipitate was arabinoxylan and the soluble component in ammonium sulphate was a arabinogalactan fraction, the latter being associated with a hydroxy proline-rich peptide.

There is little information in the literature concerning the pentosans found in bread. Gilles et al. (17) studied the water-soluble polysaccharides derived from bread crumb and reported that the pentosan in the water-soluble polysaccharide of bread crumb possesses a highly branched

structure that was structurally similar to the pentosan in the original flour.

EFFECT OF WATER-SOLUBLE PENTOSANS ON BREAD QUALITY

Water-soluble and water-insoluble pentosans are extremely hydrophilic. Kulp (54) demonstrated the hydrophilic nature of pentosan fraction by farinograms. He reported that pentosans increased the water absorption of flour and produced doughs which were stiffer and drier than normal doughs. Pentosan added to the dough also increased dough development time and the viscosity of the dough.

Finney (55) first demonstrated the importance of the water-soluble components of flour in the bread-baking performance of flour. Pence (56) reported that soluble pentosan had little effect on the baking performance of doughs reconstituted without the soluble fraction of flour, but the handling properties of dough were distinctly improved. It was reported that the carbohydrate rather than protein components of pentosan preparation affected bread making quality (56-58). Lin and Pomeranz (59) reported that crude pentosan were more effective in improving loaf volume and crust color than purified pentosans. Hoseney et al. (58) found that the water-soluble fraction of flour was required to produce a normal loaf of bread and was not responsible for the loaf volume difference between good and poor quality wheat varieties.

D'Appolonia et al. (60) in their baking study of purified pentosans, fractionated by DEAE-cellulose chromatography, reported that fractions 1 and 2 which contained arabinose, xylose, and small amounts of protein, did not enhance bread loaf volume of starch-gluten loaves. However, fractions 3, 4, and 5

which contained the above sugars and galactose, as well as greater amount of proteins, showed a marked improving effect on loaf volume.

EFFECT OF PENTOSAN ON BREAD STALING RATE

Pentosans have been reported to decrease the retrogradation of starch gels upon aging (18), and the effect exerted by the water-insoluble pentosans was more pronounced as opposed to water-soluble pentosans. The water-soluble pentosans slowed the rate of retrogradation by affecting the amylopectin fraction, while the water-insoluble pentosans retarded the rate of retrogradation by affecting both amylose and amylopectin. Similar results were obtained when the effect of pentosans on bread staling rate was investigated. Kinetic studies showed that pentosans decrease the amount of starch component available for crystallization, thus decreasing the bread staling rate (19).

STRUCTURE OF STARCH

Starch is a natural high polymer, built up through successive condensation of glucose units by enzymic process in the plant. Most starches consist of a mixture of two types of polymers, namely amylose and amylopectin, which are present in varying amounts. The amylose component considered to be entirely made of α- (1,4) linkages and therefore lacks any fine structure (61). However, further investigation indicated the presence of very small proportion of (1,6) α-D glucosidic linkages (62-65). Amylopectin is the branched fraction and contains (1,6) linkages at the branch points, in addition to the (1,4) linkages contained in the amylose.

ENZYMES INVOLVED IN STARCH ANALYSIS

The availability of enzymes with known specificity has greatly facilitated the study of the structure of amylose and amylopectin, besides application to the analysis of unknown polysaccharides (66). Hydrolytic enzymes are the most useful class of enzymes in analysis of polysaccharide structure, and will be reviewed.

β-amylase is an exo-acting enzyme that split alternate (1,4) bonds in amylaceous polysaccharides to yield β-maltose. It can not split (1,6)-bonds and therefore acts only on the outer chains of amylopectin. Side chains are trimmed to leave (1,6)-α-D linked maltosyl or maltotriosyl groups, depending on whether the outer chains contain even or odd number of D-glucose residues (67, 68).

Phosphorylase is an exo-acting enzyme that release α-D-glucose residues from the nonreducing end as α-D-glucosyl phosphate. The specificity of the enzyme is such that, on reaching the limit of hydrolysis, four D-glucose residues are left on both the A-chains and outer B-chains (69). The dextrin prepared by the action of phosphorylase is called a Ø-limit dextrin. When phosphorylase and β-amylase are used in succession, a Ø, β-dextrin is obtained which will have all A-chains and outer B-chain with two glucose residues in length (69).

Glucoamylase is a microbial enzyme which hydrolyzes amylaceous polysaccharides and derived oligosaccharides almost completely to D-glucose in a stepwise fashion from the non-reducing chain ends (70). The enzyme has the ability to cleave both (1,4) and 1,6) -α-D-glucosidic bonds in these polysaccharides. In addition the enzyme can break (1,3) α-D-glucosidic

linkages in oligosaccharides (71). Presence of at least one impurity, alpha amylase, in glucoamylase preparation is necessary for quantitative determination of amylaceous polymers (72).

Debranching enzymes or enzymes that specifically hydrolyze the (1, 6)-α-D-glucosidic inter-chain linkages, have a great deal of importance in the analysis of polysaccharides. Two similar enzymes in this group are pullulanase and R-enzymes. Pullulanase was first reported by Bender and Wallenfels as an extracellular enzyme of Aerobacter aerogenes (73, 74). The enzyme was found to be closely similar to the already known R-enzyme from plant (75, 77, 78). R-enzyme debranches amylopectin incompletely, but act on amylopectin β-dextrin more efficiently (77). Pullulanase, however, completely debranches amylopectin and amylopectin β-limit dextrins (75). Both enzymes have little or no action on glycogen (75,76,79).

Isoamylase is a debranching enzyme which is even more specific. However it completely debranches glycogen, amylopectin and phosphorylase limit-dextrins of the polysaccharides, it has a limited action on β-limit dextrins of glycogen and amylopectin (80). This is because cytophage isoamylase will not hydrolyze (1,6) bonds bonded to α-maltosyl residues (80). Another specificity of isoamylase is that it can hydrolyze (1,6)-α-D glucosidic linkages which are at a true branch point, that is, to primary hydroxyl group of α-D-glucose residues linked through both C-1 and C-4 (66). Thus, the enzyme has no action on the linear polysaccharide pullulan.

USE OF ENZYMES IN STRUCTURAL ANALYSIS OF STARCH

In the period before it became possible to explore the fine structures of glycogen and amylopectin with enzymes, three structures were proposed by

Haworth and coworkers (81), Staudinger and Husemann (82) and Meyer and coworkers (83, 84). Their structures differed only in the way in which the unit chains were arranged. The application of debranching enzymes made it possible to distinguish between the alternatives. The regularly rebranched structure proposed by Meyer (83, 84) was the only one of the existing proposals that could explain the results with debranching enzyme experiments on glycogen and amylopectin.

The enzymic investigations were basically concerned with determining the ratio of A to B-chain (76). A-chains are those chains in amylopectin which are unbranched while B-chains are those chains to which another chain is attached by an α-(1,6) linkage. Every molecule is supposed to contain a single C-chain, unsubstituted at its reducing end (85). The enzymatic analysis gave A:B chain ratios of about unity (85) fitting the Meyer model. However, the arrangement of A- and B-chains were still not clear.

By using the debranching enzyme, isoamylase, further information on the arrangement of A- and B-chains was obtained (80). The Ø-beta dextrins of polysaccharides were prepared by exhaustive treatment with rabbit-muscle phosphorylase, followed by β-amylase (69), converting all the A-chains of amylopectin and glycogen into maltosyl residues (69). On successive treatments of this Ø, β-limit dextrin with cytophage isoamylase and β-amylase, glycogen yield 44% maltose and amylopectin yield 29% maltose. It is obvious that, if all B-chain carry A-chains, as required in the structure of Meyer and coworkers (83, 84) and if all of the chains extend to the periphery of the molecule, debranching of the Ø-beta dextrins should result in a series of maltosyl terminated oligosaccharide, and nothing

else, which would be resistant to the action of β-amylase. Therefore a new arrangement of A- and B-chain was proposed by Whelan and coworkers (85). In this structure half of the B-chains carry A-chains, and furthermore, that some B-chains do not reach the surface of the molecule.

An important characterizing feature of the branched polymers glycogen and amylopectin is their average unit-chain length. Enzymatic methods for determination of average chain length (85, 88) are superior to chemical methods. Since the unit chains of the polysaccharide are left intact and the length distribution can be examined by gel filtration (85). Examination of the unit-chain profile of debranched glycogen and amylopectin on molecular seives, (89) gave supporting evidence for the correctness of the model proposed by Whelan and coworkers (85).

MATERIALS AND METHODS

FLOUR SAMPLE

The flour sample 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 X 5.7), an ash of 0.40, a medium mixing time and good loaf volume potential.

BREAD SAMPLE

Bread was made using a straight-dough procedure with a 3 hr. fermentation, a 55 min. proof time at 86°F. and a 25-minute bake at 436°F. The baking formula based on flour weight was as follows:

Flour (14% m.b.)	% 100
Water	variable
Sugar	6.0
Salt	1.5
NFDM	4.0
Shortening	3.0
Yeast	2.0
Malt (12 α-amylase units/g)	0.75
Oxidant	20 PPM $KBrO_3$

Three more bread samples were made by the above procedure and formula with the exception that one contained no shortening, and in the other two samples the shortening was replaced by 0.5% SSL or monoglyceride.

Bread after baking was cooled at room temperature and stored in sealed containers to prevent moisture loss. After 1 and 5 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 the procedure of Schoch and French (2). A sample (25 gm., on as is basis) was placed in a centrifuge bottle. Approximately 100 ml. of distilled water was added and the mixture was stirred for 1 minute with a propeller-type stirrer to give a smooth paste. The bottle was placed in a water bath at 30°C. for 30 minutes, with stirring to keep the bread crumb suspended. The mixture was then centrifuged for 5 minutes

at 2000 RPM and the supernatant decanted. The procedure was repeated two more times and the filtrate and wash waters were combined and treated with 3-4 volumes of methanol to floculate the soluble starch, the mixture was then heated on a steam bath for one hour 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 DETERMINATION

Moisture was determined in bread samples prior to extraction according to AACC method 44-15A (90).

PROTEIN DETERMINATION

Protein content (N X 5.7) of soluble starch samples were determined by the approved AACC method 46-13 (90).

IODINE AFFINITY

To remove traces of fatty materials, the soluble starch samples were soxhlet-extracted for 24 hours with methanol prior to determining iodine affinity. Iodine affinities were determined by potentiometric titration (91) using a Beckman pH meter with a platinum electrode calibrated with a voltage reference cell. The readings were taken after stabilization of the electrodes.

DETERMINATION OF TOTAL POLYSACCHARIDE

The total amount of polysaccharide in each sample was determined by using the phenol-sulphuric acid procedure (92) using a standard curve prepared from D-glucose.

DETERMINATION OF GLUCOAMYLASE ACTIVITY

Glucoamylase solutions were diluted to approximately 0.05 units/ml. with 0.05 M. citrate buffer (pH 4.8). Enzyme activity was assayed by incubating aliquots (1 ml.) of the enzyme solution with 3 ml. of 94% soluble starch solution (commercial) for 1 hr. at 30°C. (95). The reaction was stopped by heating in a boiling water bath to inactivate the enzyme. The amount of glucose produced was measured by Nelson's colorimetric copper procedure (94) calibrated against D-glucose. One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of glucose per minute under the condition of assay.

DETERMINATION OF TOTAL STARCH

Soluble starch (1 mg./ml.) was pipetted into a test tube containing 10 I.U. glucoamylase (Grade 11, from Rhizopus genus, Sigma Chemical Co., St. Louis, Mo.) in 5.0 ml. of 0.05 M citrate buffer (pH 4.8) (93). The tube was incubated at 37°C. for 60 minutes. One ml of this solution was taken and reducing power determined by Nelson's colorimetric copper procedure (94) calibrated against D-glucose.

$$\% \text{ Total Starch T} = \frac{\text{glucose released x .9}}{\text{sample wt.}} \times 100$$

DETERMINATION OF β-AMYLOLYSIS LIMIT

Soluble starch samples were hydrolyzed by sweet potato β-amylase (crystalline, type I-B, Sigma Chemical Co., St. Louis, MO.) according to Whelan's procedure (96), with the exception that reduced glutathione and serum albumin were omitted from the digests (66). Twenty mg. of sample was

incubated with 750 units of sweet potato β-amylase in 5 ml. of 0.02-0.04 M. acetate buffer, pH 4.8, at 35°C. for 24 hr. The degree of β-amylolysis was determined by estimating the amount of maltose liberated, using Nelson's colorimetric copper method (94).

PREPARATION OF PURIFIED WATER-SOLUBLE PENTOSAN

Water soluble pentosan were extracted from soluble starch by a modification of the Medcalf et al. procedure (48). Freeze dried soluble starch (1 gm.) were completely dissolved in 50 ml. of distilled water one day before treatment with alpha-amylase. Bacillus sulitilis alpha amylase (25 mg., Type II-A, Sigma Chemical Co., St. Louis, Mo.) was dissolved in 50 ml. of 0.02 M. sodium phosphate buffer, pH 7.2, containing 0.04 M sodium chloride (97). The solutions were combined and dialyzed 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 x g. for 10 minutes. The supernatant solution was dialyzed against distilled water for 48 hr. and freeze-dried.

FRACTIONAL PRECIPITATION WITH AMMONIUM SULPHATE

Solutions of soluble starch (2 mg./ml.) were prepared in 0.1 M phosphate buffer (pH 7.0) and $(NH_4)_2SO_4$ (analytical grade) was added slowly to saturation. After stirring for 3 hrs. the solutions were allowed to stand overnight at 4°C. The precipitated polysaccharide was collected by filtration on glass fiber paper and washed thoroughly with saturated $(NH_4)_2SO_4$ in 0.1 M phosphate buffer (pH 7.0). The precipitate was

dissolved in water and dialyzed until free of $(NH_4)_2SO_4$. The combined supernatant and washings were also dialyzed exhaustively against water (53).

GEL-FILTRATION CHROMATOGRAPHY ON SEPHAROSE 4B

Samples (5-15 mg) were dissolved and loaded onto a column (60 by 2.6 cm) of sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). 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 cross linked (70 x 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 minute intervals (6 ml.), total amount of polysaccharide in each fraction was determined using the phenol-sulphuric acid procedure (92).

DEBRANCHING WITH PULLULANASE

Pullulanase was tested for amylases activity prior to debranching. Analysis for amylases activity was by preparing digests containing: 0.2 ml Nutritional Biochemical Co., amylose in DMSO (40 mg/ml), 0.7 ml 0.02 M citrate phosphate buffer, pH 5.0 and 0.1 ml pullulanase (10 mg/ml 0.02 M citrate phosphate buffer pH 5.0). The digest was inculated 48-72 hours at 30°C and reducing sugar was measured by Nelson Colorimetric Procedure (94).

Debranching of soluble starch sample was conducted at 37°C. in digest containing 2-5 mg./ml. substrate, 3-5 units/ml. pullulanase (Enterobacter aerogenes, Sigma Chemical Co., St. Louis, Mo., 150 I.U./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 24 hours incubation, the digest was heated in boiling water bath to inactivate the enzyme and insoluble material was removed by filtration (98).

GEL-FILTRATION OF DEBRANCHED SOLUBLE STARCH

5 to 10 mg. of the debranched samples were fractionated on a Bio-gel P-10 column (2.6 x 70 cm.) at room temperature (98). Elution was carried out with 0.01 M phosphate buffer, pH 7.0, containing 0.02% sodium azide to prevent bacterial growth. Six ml. fractions were collected every 30 minutes. Total carbohydrate content in each fraction was measured by the phenol-sulphuric acid method(92).

AVERAGE UNIT-CHAIN LENGTH OF AMYLOPECTIN

The fractions on Bio-gel P-10 column that was eluted after the void volume (debranched amylopectin), were combined and concentrated. The average unit-chain length ($\overline{C.L.}$) were determined (86) by dividing the total amount of polysaccharide as measured by phenol-sulphuric acid procedure (92) by the amount of reducing groups released during debranching. Reducing sugars were determined by Nelsons colorimetric procedure (94) except that all volumes were reduced by a factor of two to increase the sensitivity of the method. The method was calibrated using D-glucose as standard.

PREPARING β-AMYLASE LIMIT DEXTRINS

β-amylase limit dextrin of soluble starch were prepared by exhaustive treatment of solutions of the polysaccharides (20-30 mg/ml in 100 mM acetate buffer, pH 4.8) with β-amylase (380 units/ml.) in a dialysis bag. The digest was continuously dialyzed against 100 mM acetate buffer to remove maltose. After complete digestion, the solutions were boiled for 15 minutes to inactivate β-amylase, centrifuged, dialyzed against distilled water and freeze-dried (66, 99).

DETERMINING THE RATIO OF A-CHAINS to B-CHAINS

The ratio of A-chains to B-chains in amylopectin was determined by Isoamylase (glycogen 6-glucano hydrolase, EC-3.2.1.68) which showed to be free of α-1,4 endoglucanase activity and Pullulanase (Pullulan 6-glucanohydrolase, EC. 3.2.1.41) using β-limit dextrins as described by Marshall and Whelan (99). The amount of reducing sugars liberated in 24 hrs. by the action of (a) Isoamylase (Lytic enzyme Li, Gallard-Schlesinger, Carli Place, New York) (b) Isoamylase plus pullulanase on the dextrins were measured in digest containing substrate (2-5 mg./ml), isoamylase (0.06 units/ml.) and 20 mM. sodium acetate buffer pH 5.5 at 37°C. Pullulanase (3-5 units/ml.) was added after 12 hrs pre-incubation of the digest with isoamylase alone, to prevent the inhibition of one enzyme by the other (99). The ratio of A-chains to B-chains was calculated from the increase in reducing power following the action of debranching enzymes as shown below (66).

USING β-LIMIT DEXTRINS	REDUCING POWER MEASURE
Isoamylase	B + 1/2 A chains
Pullulanase	B + A chains
Difference	1/2 A-chain

To determine the completeness of debranching with isoamylase or pullulanase, an aliquot (0.5 ml.) of the supernatant solution was treated with 380 units of β-amylase in 0.5 ml. of 100 mM. sodium acetate buffer, pH 4.8, for 24 hours at 37°C. (98). Reducing power was measured as glucose by Nelson colorimetric copper procedure (94).

RESULTS AND DISCUSSION

EFFECT OF STALING ON QUANTITY OF SOLUBLE STARCH

The amount of solubles extracted from bread baked with shortening, no shortening, SSL and monoglyceride is presented in Table 1. The bread containing SSL and monoglyceride did not contain shortening. The data show that there is a decrease in quantity of soluble starch extracted from bread stored for five days compared to that extracted from one day old bread. This is in good agreement with the work reported by French and Schoch (2). Relatively little soluble starch was extracted from bread containing the surfactants compared to the other samples. These data support the theory of Strandine et al. (25) that the presence of an emulsifier minimizes the release of soluble starch.

The difference between the amount of soluble starch extracted from bread one and five days after baking was highest in bread baked without

Table 1. Soluble starch extracted from bread make with different treatment and stored for different times.

Treatment	Age (days)	Ave. % Soluble Extracted	Difference
Control	1	4.3	0.9
	5	3.4	
Without Shortening	1	4.2	1.1
	5	3.1	
+ SSL	1	3.1	0.2
	5	2.9	
+ Monoglyceride	1	3.8	0.4
	5	3.4	

Standard Deviations = 0.08

shortening (1.1%) and was lowest for bread baked with SSL (0.2%). The difference was 0.4 and 0.9 percent, respectively, for bread made with monoglyceride and the control bread containing shortening. These data indicate that less soluble starch become insoluble as a result of storage in bread baked with surfactant, and more become insoluble during storage of bread made with no shortening.

Early workers postulated that during baking, surfactants adhere to the surface of the starch, hindering the amylose from leaching out (20-25). The crystalline amylose surfactant complex, investigated by X-ray diffraction, confirmed complex formation between amylose and surfactants. Destefanis et al. (33) reported that surfactant forms a complex during baking not only with amylose but also with amylopectin. It has been postulated (26) that one of the functions of surfactant in bread is retension of soluble starch in the granules. The data that we obtained also indicate that addition of surfactant to the system decreases the release of soluble starch from bread and also less soluble starch become insoluble as bread ages. This phenomena is probably due to complex formation of surfactant with one or more of the starch components in the bread (32-34). It has been postulated that soluble starch acts as cementing substance between starch granules and gluten and therefore contribute to crumb firming (25), but surfactant minimize the release of soluble starch from bread. Those results may be one of the reasons for the antistaling effect of surfactants and shortening in bread. The protein content of soluble starch is presented in Table 2.

Table 2. Protein Content of Water Soluble Starch

Treatment	% Protein	
	One day	Five days
S. Starch Content	6.4	3.3
Without Shortening	4.1	4.6
+ SSL	5.9	6.7
+ Monoglyceride	5.0	5.8

IODINE AFFINITY OF SOLUBLE STARCH

Analysis by some type of iodine complex formation has bean a valuable tool in differentiating between linear and branched starch material. Iodine reacts with linear components or segments of starch and gives a blue iodine color with chain lengths in excess of forty glucose units. Shorter chains give red, brown or yellow complexes (100). Native amylose absorbs 19-20% of its weight of iodine from a dilute solution of I_2 in KI, whereas amylopectin absorbs little (0.5%) or none (101). Short amylose chains, or amylopectins with long outer chains, also absorb iodine, but only at higher iodine concentrations.

Iodine affinity of the soluble starch samples are presented in Table 3. The values are much lower than those reported for pure amylose and closer to those reported for amylopectin. These data support the conclusion of French and Schoch (2) that the soluble starch extracted from bread is mainly amylopectin. Iodin affinities were lower for the samples containing surfactants indicating a lower amount of amylose in those samples.

The complexing ability of amylose with surfactant has been studied by many authors (27-30) and it has been postulated that the surfactant enters the starch granules and forms an insoluble complex with amylose. The postulated complex is in the form of a helix (15) and resembles that formed by iodine and amylose (28). This may explain the lower iodine affinity of samples containing surfactant. The sample baked without shortening gave high iodine affinity values indicating that more amylose was leached from the starch granules and thus into the soluble fraction.

Table 3. Iodine Affinity (%) of Soluble Starches Samples

Treatment	Age (days)	Iodine Affinity
Control	1	2.60
	5	1.86
Without Shortening	1	4.60
	5	4.30
+ SSL	1	1.20
	5	0.85
+ Monoglyceride	1	1.40
	5	0.90

TOTAL CARBOHYDRATE, STARCH AND PENTOSAN CONTENT OF SOLUBLE STARCH

The total carbohydrate content of the soluble fraction extracted from bread was determined by phenol-sulphuric acid (92) and the total starch by glucoamylase digestion (93). The pentosan content was calculated by taking the difference between the total carbohydrate and starch. The quantity of starch extracted from bread decreased during storage, while the amount of pentosan increased (Table 4). These data are in agreement with Gilles et al. (17) who reported that the soluble starch in stale crumb bread contained more pentosan than found in fresh crumb.

As is shown in Table 4, samples extracted from bread made with surfactants had higher pentosan contents and lower starch contents compared to the samples baked without surfactants. There was not a significant difference in the pentosan contents from samples of bread baked with no shortening during storage. However, there was a slight decrease in starch content of those samples. The higher pentosan content of the water soluble fraction extracted from bread made with surfactants is probably because of the function of surfactants in bread.

After the samples extracted from bread with water were precipitated with methanol, dried, and redissolved in water, the solutions were cloudy. In an attempt to eliminate the problem they were dissolved in potassium hydroxide and neutralized with acid. However the solutions were still not clear. This indicated that some of the components in the extracted sample were becoming insoluble during sample preparation. Therefore, the solutions were filtered and total carbohydrate and starch on the filtrates were determined. There was a decrease in starch content from 4-9% in different

Table 4. Total Carbohydrate and Starch in Soluble Starch Samples

Treatment	Age (days)	Total CHO (%)	Total Starch (%)	Difference Between Total CHO & Starch (Other CHO's)
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
+ SSL	1	80.3	59.9	20.4
	5	78.7	56.3	22.4
+ Monoglyceride	1	78.7	63.5	15.2
	5	77.6	57.0	20.6
Standard Deviation		0.02	0.03	

samples by filtration (Table 5). These results confirm that a small amount of soluble starch has become insoluble during the isolation procedure. If during storage starch in the bread is slowly retrograding, one would expect less change in starch solubility for the five days old sample than with the one day old sample. The data in Table 5 show less change in the five day old sample.

β-AMYLOLYSIS OF SOLUBLE STARCH

β-amylolysis limits of soluble starch are given in Table 6. β-amylolysis values increased during storage of bread in all samples, except for the sample baked with no shortening. As shows by the iodine affinity values, the soluble starch of bread crumb appeared to be mainly amylopectin. Therefore, one would expect a relatively low β-amylolysis value. However, our data showed a very high conversion to maltose (71-80%). Those values are similar to what has been reported for amylose preparations (98). 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 from one day old control bread was used for gel filtration on different sepharose columns. The molecular size distribution of soluble starch sample is shown in Fig. 1. A purified pentosan was prepared by treating the soluble starch fraction with α-amylase and dialyzing, the purified pentosan was also placed on the 4B column to identify the pentosans in the soluble starch fraction (Fig. 1). The purified pentosans give one broad peak that corresponds to the higher molecular weight

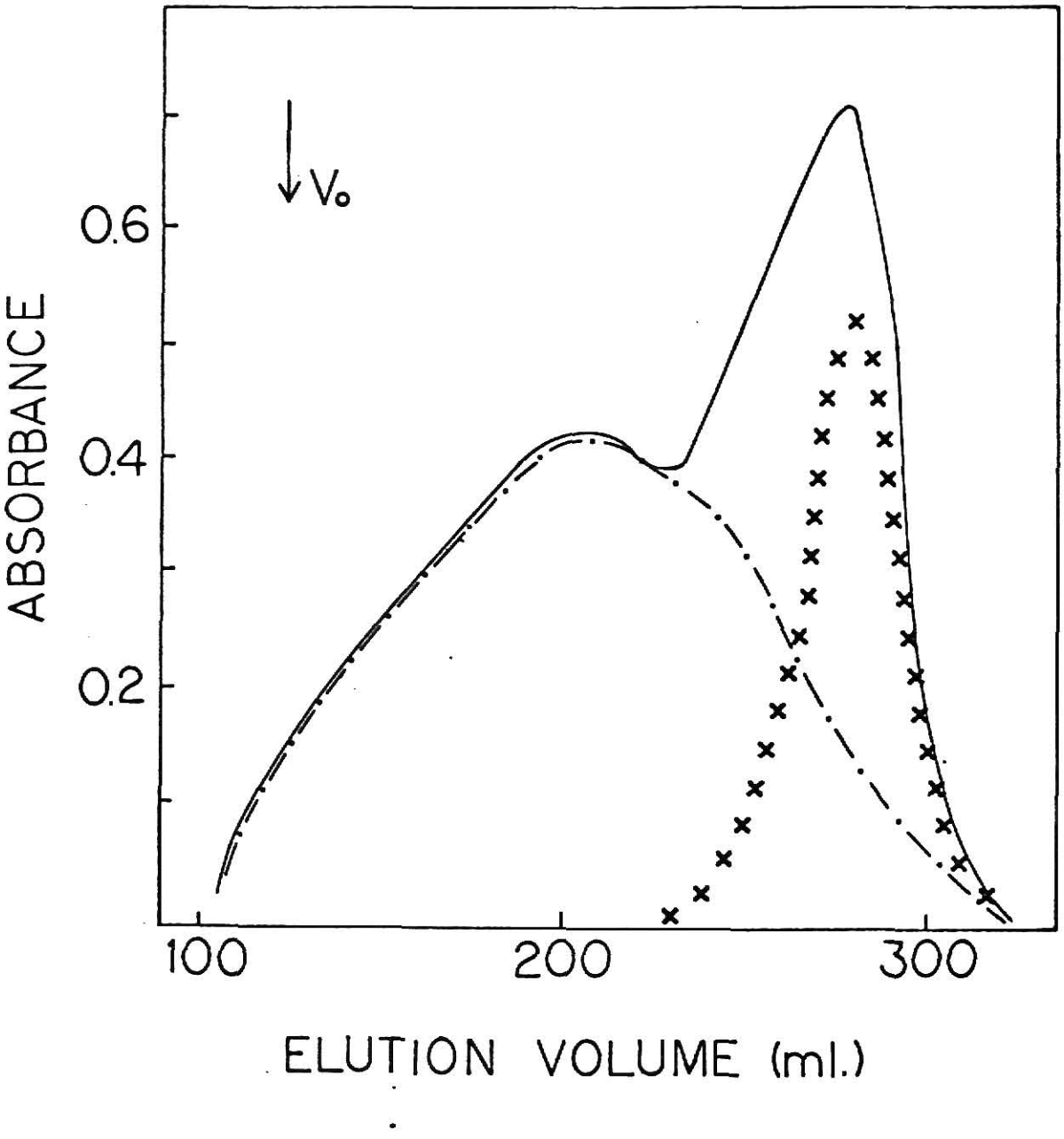
Table 5. Total Carbohydrate and Starch in Filtered Soluble Starch Samples

Treatment	Age (days)	Total CHO (%)	Total Starch (%)	Difference Between Total CHO before & after Filtration	Difference Between Total Starch before & 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
+ SSL	1	72.0	52.7	8.3	7.3
	5	72.0	51.0	6.7	5.3
+ Monoglyceride	1	75.7	55.0	3.0	8.5
	5	70.2	50.0	7.4	7.0

Table 6. 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
+ SSL	1	45.41	75.81
	5	45.00	79.90
+ Monoglyceride	1	44.75	70.48
	5	44.38	77.86

Fig. 1. Elution profiles of Soluble Starch (——), Pentosan (-∘-∘) and the Soluble Fraction in Saturated Ammonium Sulfate (+++) on Sepharose 4B Column.


↓Vo
0.6
0.4
0.2
ABSORBANCE
100
200
300
ELUTION VOLUME (ml.)

component reported by Fincher and Stone (53). The lower molecular weight component reported by Fincher and Stone was missing in this preparation. Fincher and Stone reported that the high molecular weight pentosan fraction was precipitated with ammonium sulfate. Therefore, the soluble starch fraction were treated with saturated ammonium sulfate, after centrifugation the supernatant fraction dialyzed and also applied to sepharose 4B column. It is obvious that the starch components of the soluble starch sample and in the fraction soluble in saturated ammonium sulfate are eluted in the second peak about the V_t of the column. The soluble starch on Sepharsoe 4B at pH 12 eluted at the V_t of the column while a normal wheat starch sample eluted at the V_o of the column (102). These data show that soluble starch sample is much smaller than regular starch.

MOLECULAR SIZE DISTRIBUTION ON SEPHAROSE 6B-CL

The soluble starch fraction extracted from bread and purified pentosans 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 showing that much of the starch had a relatively low molecular weight.

β-Limit dextrin was prepared from the total soluble starch fraction by exhaustive treatment with β-amylase and dialyzing to remove maltose. The β-limit dextrin was also fractionated on sepharose 6B column (Fig. 3). It should be mentioned that the β-limit dextrin was not pure because it still contained all the pentosan originally in the soluble starch sample. The elution profile shows that only a small proportion of sample was eluted at the void volume. The fraction in the void volume is certainly

Fig. 2. Elution Patterns of Soluble Starch (+++) and the Pentosan (——) on Sepharose 6B-CL Column.

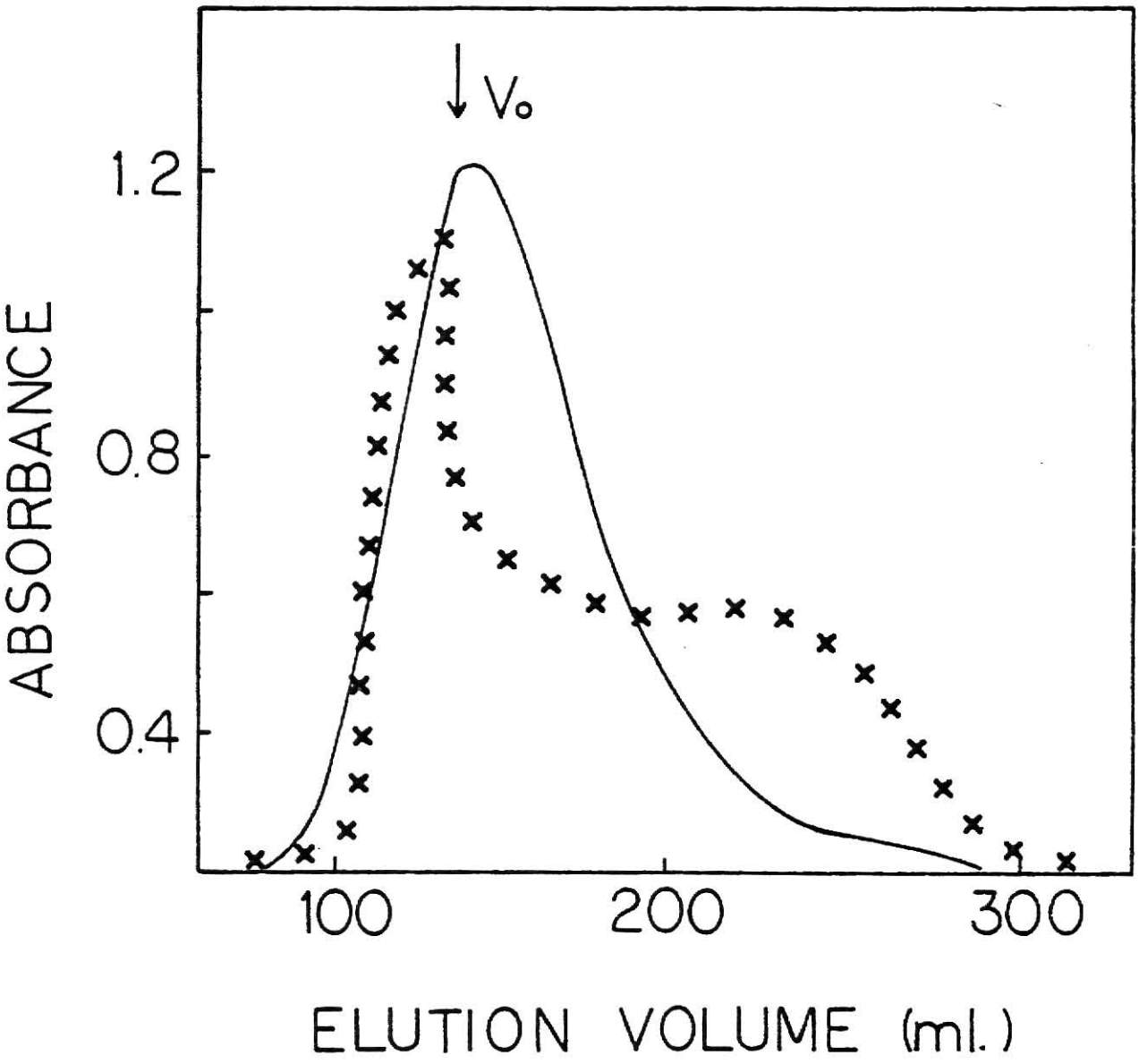
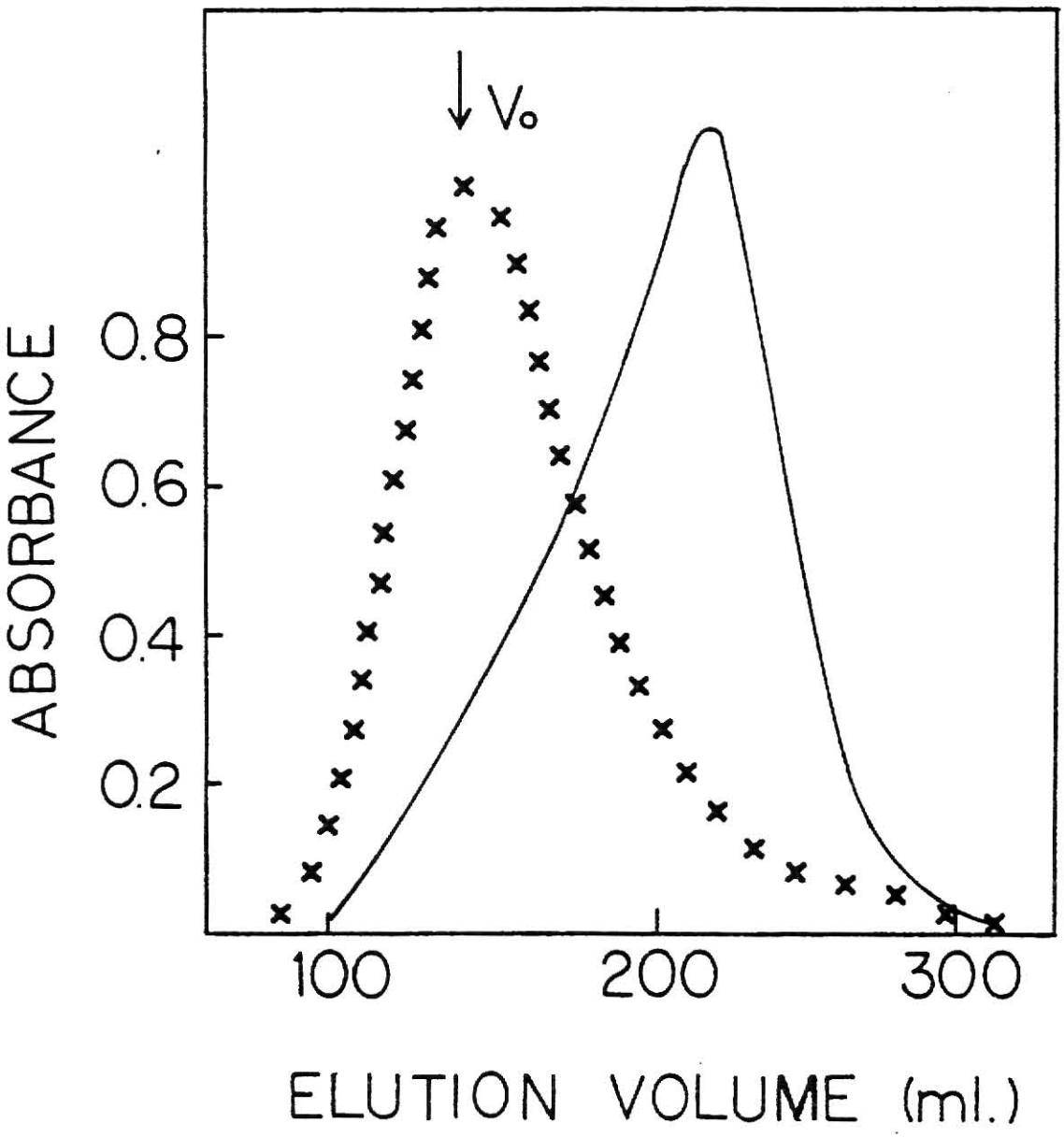

Vo
1.2
0.8
0.4
ABSORBANCE
100
200
300
ELUTION VOLUME (ml.)

Fig. 3. Elution Profiles of B-Limit Dextrin (——) and the Pentosan on Sepharose 6B-CL Column.


Vo
ABSORBANCE
0.8
0.6
0.4
0.2
100
200
300
ELUTION VOLUME (ml.)

pentosan. These data suggest that β-limit dextrin from soluble starch sample are smaller in size than found for regular β-limit dextrin (102). This result was furthermore supported by fractionation of β-limit dextrin on Biogel P-10 column (Fig. 4). Most of the sample was eluted at the void volume of the column but a significant amount was eluted later. This also indicates that the β-limit dextrin from soluble starch is small in size and there are some relatively small fragments.

GEL FILTRATION OF DEBRANCHED SOLUBLE STARCH

The soluble starch sample was debranched with Pullulanase and fractionated on sepharose 6B column (Fig. 5). Debranching would degrade the amylopectin to small fragments that should elute at the V_t of the column. Comparing Fig. 2 and 5, it is clear that almost all the starch was eluted at the V_t of the column after debranching. Thus, the starch in the soluble sample is mostly amylopectin.

The debranched sample was also fractionated on a Biogel P-10 column (Fig. 6). Two major peaks were obtained. The first peak eluted at the void volume of column, is composed of material with higher molecular weight. The first fraction was incubated with glucoamylase and showed only a slight amount of starch present. This presumably is amylose that would not debranch with pullulanase and therefore eluted in the void volume of the column along with pentosan. However pentosan makes up the major part of the first peak and amylose is present in very small amounts.

The second major peak was considered to consist of the debranched amylopectin with much lower molecular weight. The second peak has a leading

Fig. 4. Elution Profile of B-Limit Dextrin on Bio-Gel P-10 Column.

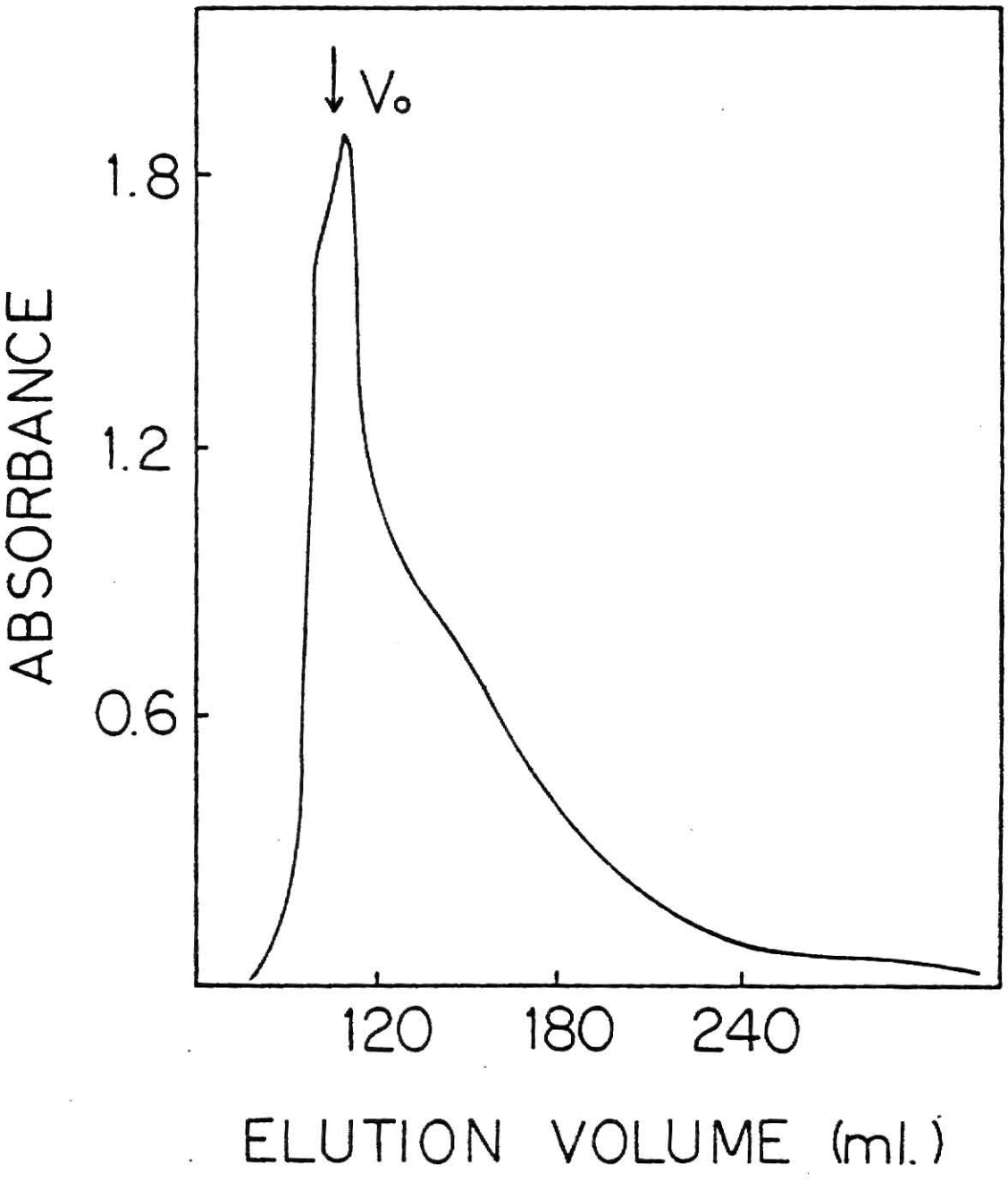

↓ Vo
1.8
1.2
0.6
ABSORBANCE
120
180
240
ELUTION VOLUME (ml.)

Fig. 5. Elution Profile of Soluble Starch Debranched by Pullulanase on a Sepharose 6B-CL Column.

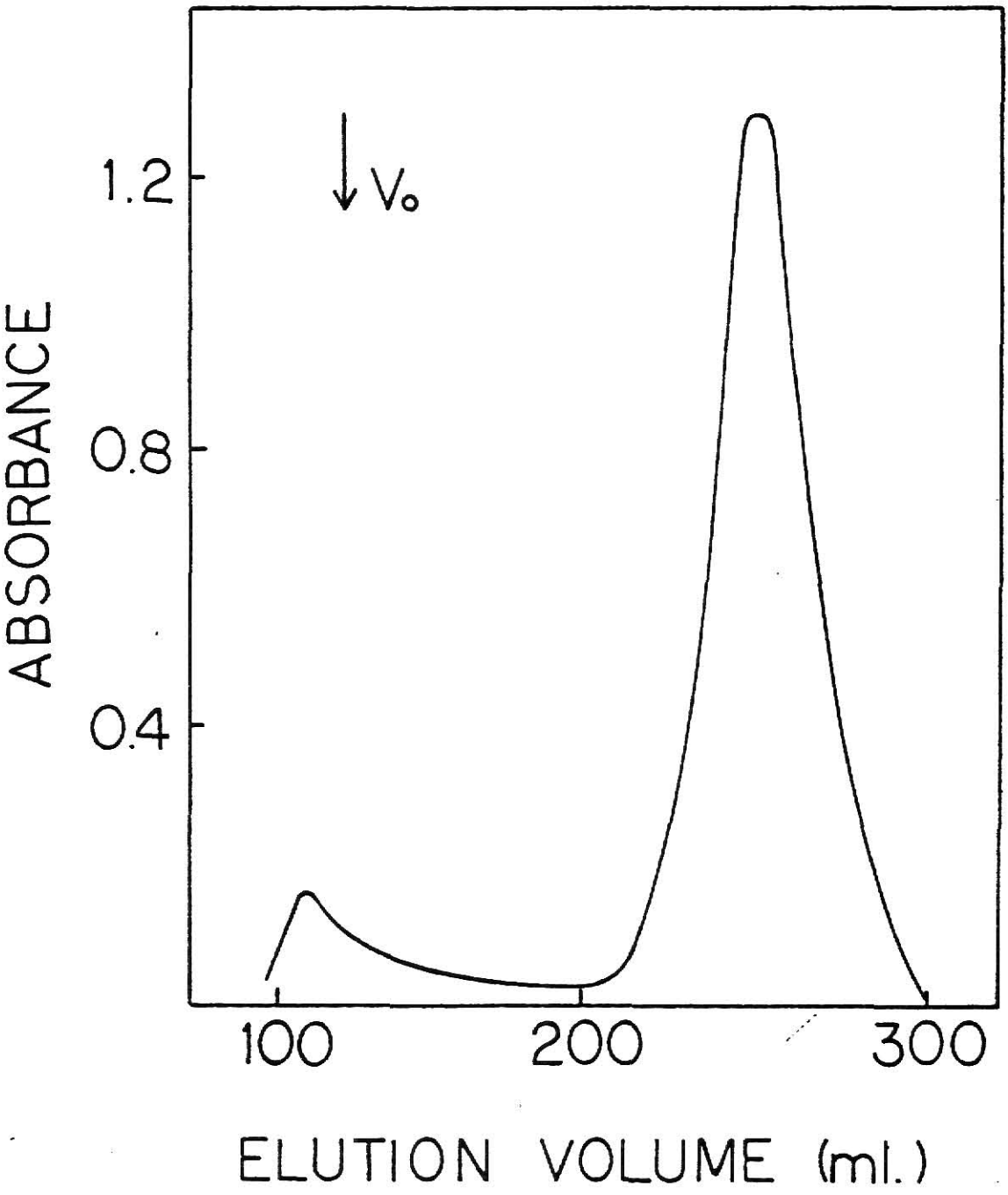
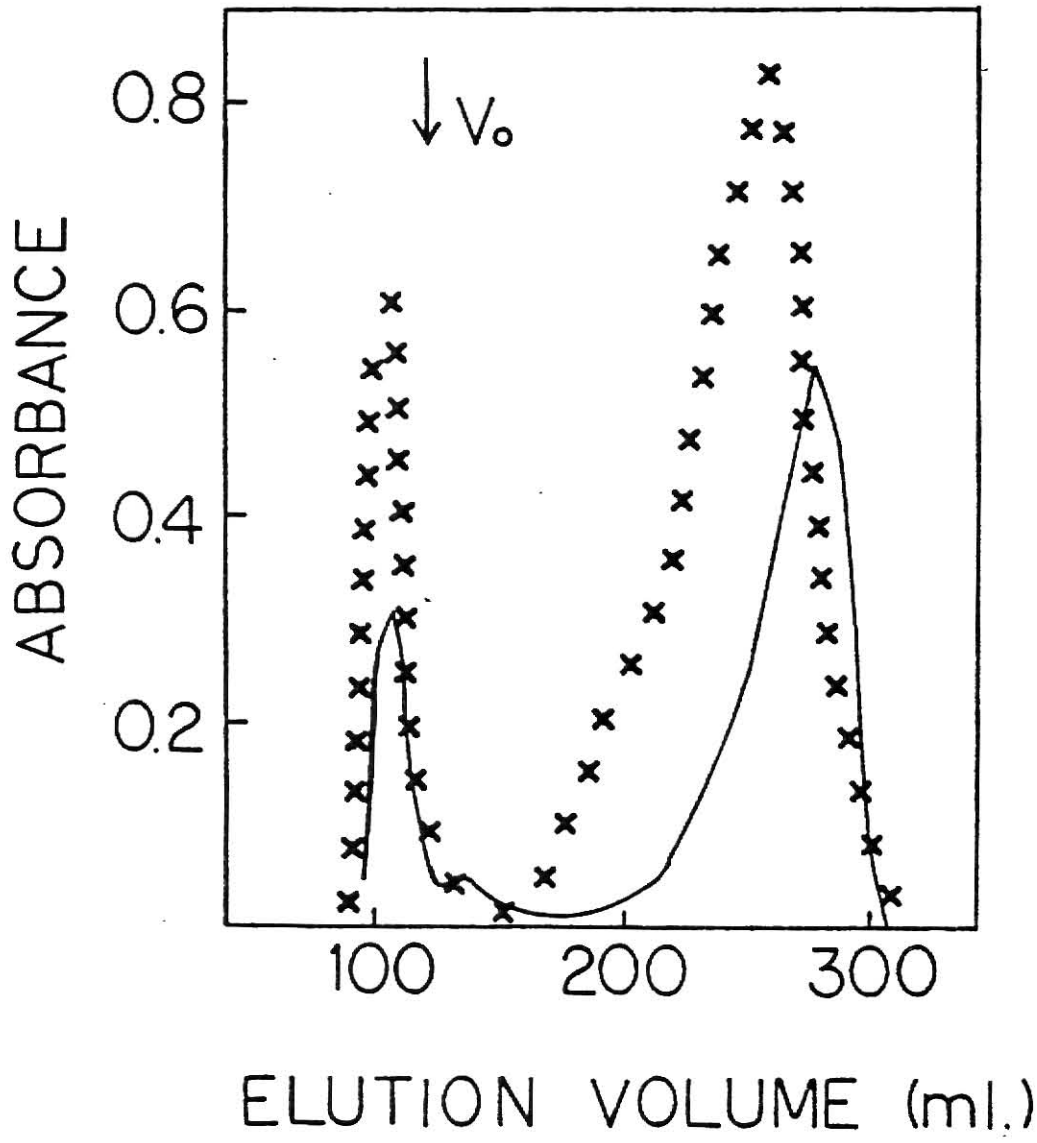

ABSORBANCE
1.2
0.8
0.4
V_o
100
200
300
ELUTION VOLUME (ml.)

Fig. 6. Bio-Gel P-10 Fractionation of the Products of Pullulanase Debranching of the Soluble Starch Extracted from 1-day Old (——) and 5 day old (+++) Control Bread.


0.8
0.6
0.4
0.2
ABSORBANCE
↓ Vo
100
200
300
ELUTION VOLUME (ml.)

shoulder which indicates that there is bimodal distribution of chain-length with the majority of chains being low molecular weights. The soluble starch fraction from bread baked with no shortening, and bread containing SSL and monoglycerides at 1 day storage and all the samples at 5 days storage were debranched with Pullulanase and placed on the Biogel P-10 column (Fig. 7-9). All the debranched samples gave similar elution profiles.

AVERAGE UNIT-CHAIN LENGTH ($\overline{C.L.}$) OF AMYLOPECTIN

The average unit-chain length ($\overline{C.L.}$) of amylopectins were determined by debranching of the samples with Pullulanase and fractionating on Bio-gel P-10 column. The second peak (Fig. 6-9) which contained the debranched fractions were combined, concentrated, and the average unit-chain length ($\overline{C.L.}$) was calculated from the total amount of polysaccharide (92) divided by the reducing groups (94) liberated during debranching (86) and is presented in Table 7.

The sample with SSL had the longest ($\overline{C.L.}$ 16) and the sample with no shortening the shortest ($\overline{C.L.}$ 11). The average unit-chain length are shorter for all samples than that reported for regular wheat amylopectin (98). This result suggests that soluble starch amylopectin chains from bread are shorter in length.

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 (99). The ratio of A-chains to B-chains was 2:1 for amylopectin of soluble starch from control bread one day after baking. Compared to a

Table 7. Average Unit-Chain Lengths ($\overline{C.L.}$) of Amylopectins from Soluble Starch Samples

Source	Age (days)	$\overline{C.L.}$
Control	1	12
	5	11
Without Shortening	1	11
	5	10
+ SSL	1	16
	5	12
+ Monoglycerides	1	13
	5	12

Fig. 7. Bio-Gel P-10 Fractionation of the Products of Pullulanase-Debranching of Soluble Starch from Bread Baked with no Shortening and Isolated after 1 Day (——) and 5 days (+++) of Baking.

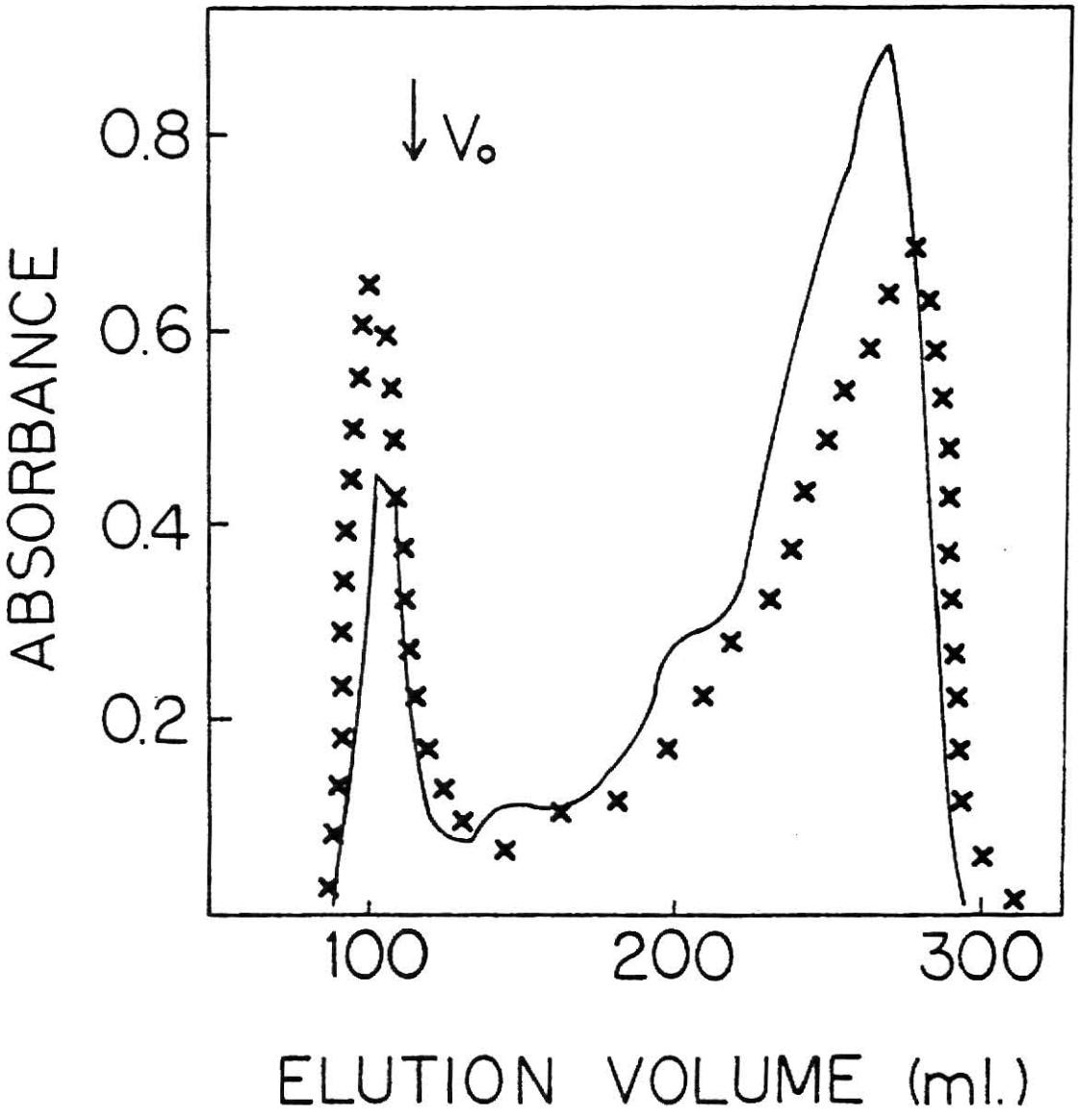

ABSORBANCE
0.8
0.6
0.4
0.2
↓ Vo
100
200
300
ELUTION VOLUME (ml.)

Fig. 8. Fractionation of Pullulanase-Debranched Soluble Starch from Bread Baked with SSL and Isolated After 1 day (——) and 5 Days (+++) of Storage on a Column of Bio-Gel P-10 Column.

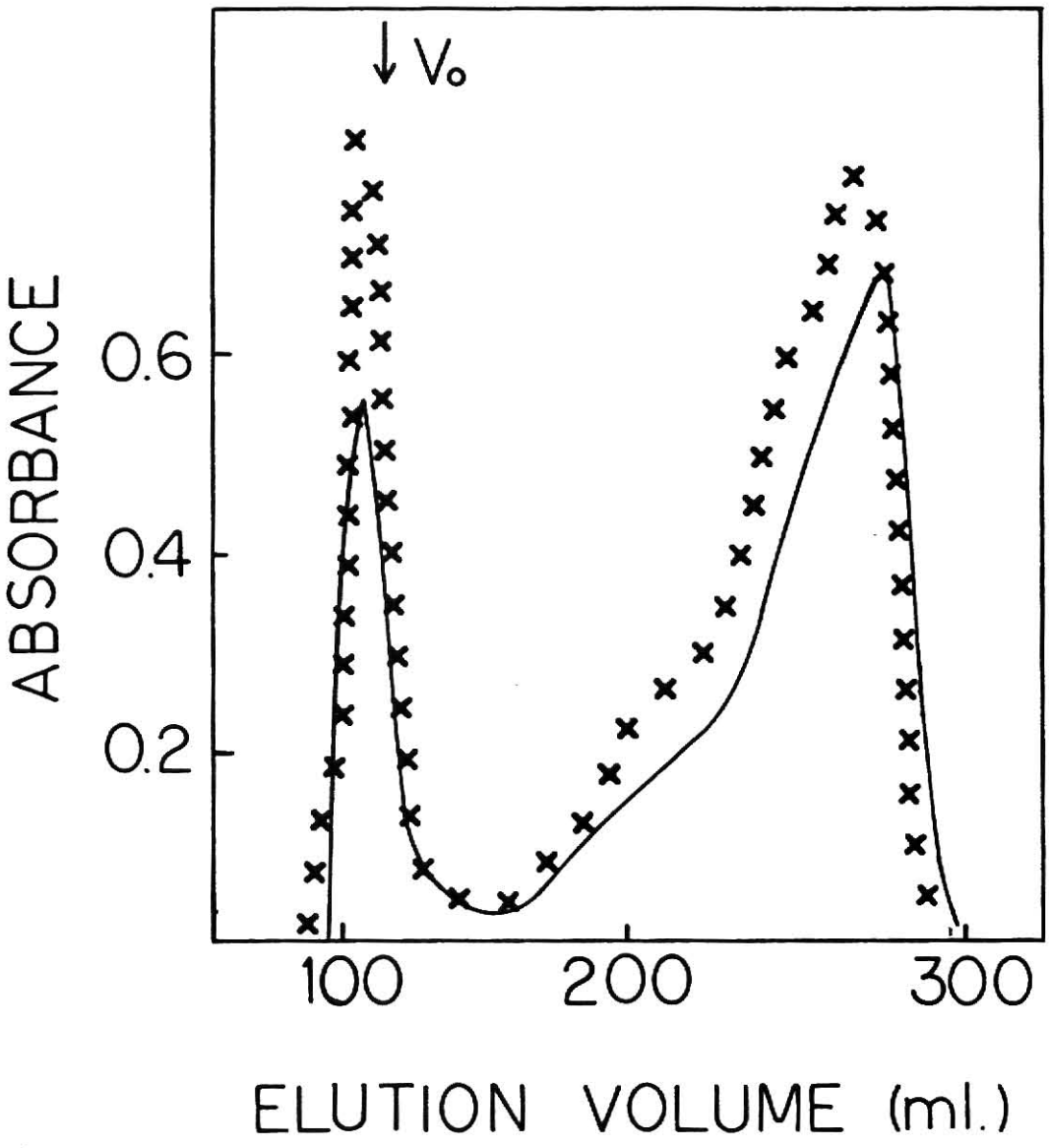
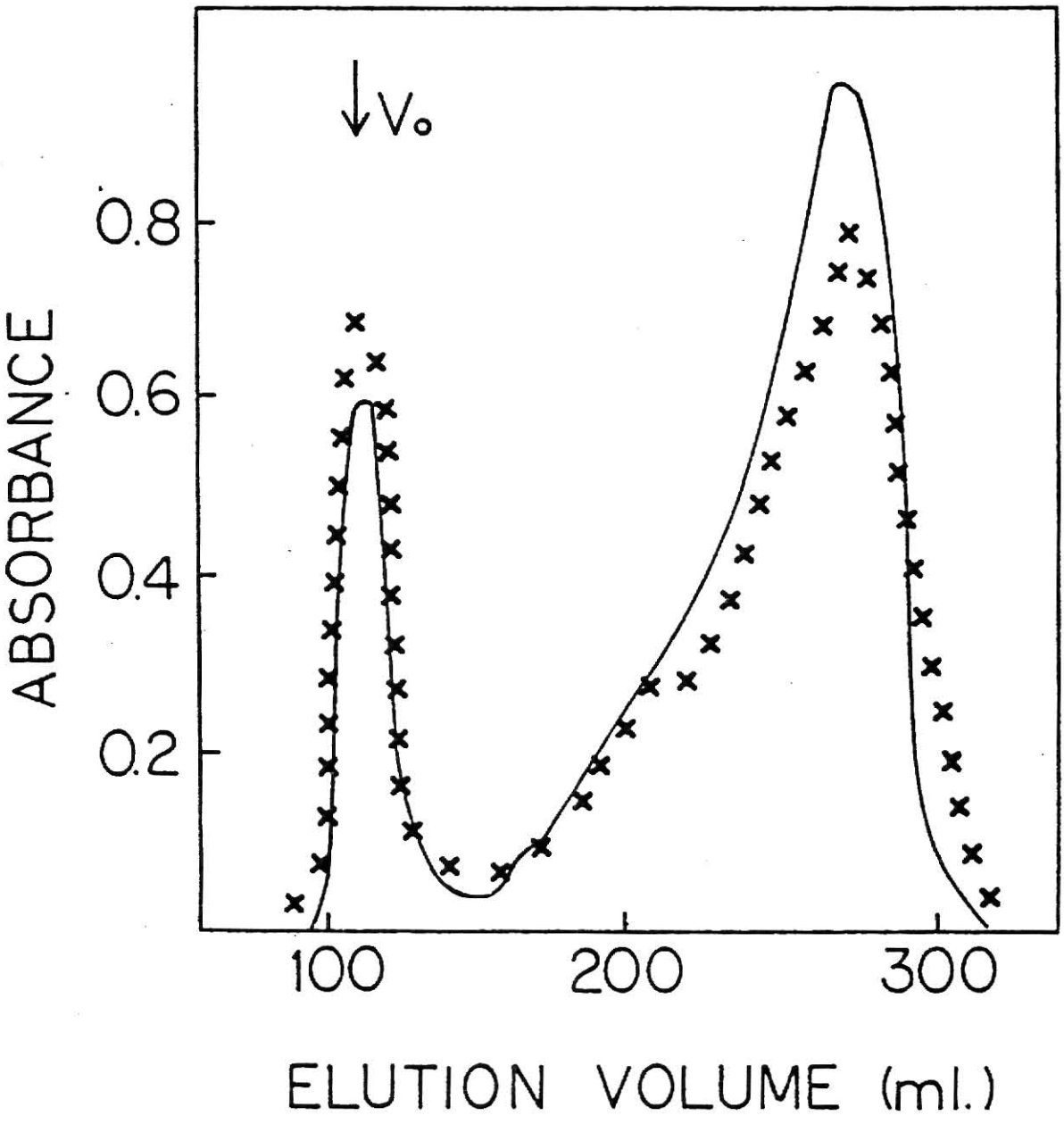

↓ Vo
ABSORBANCE
0.6
0.4
0.2
100
200
300
ELUTION VOLUME (ml.)

Fig. 9. Elution Profiles of Pullulanase-Debranched Soluble Starch from Bread Baked with Monoglycerides and Isolated After One Day (——) and 5 Days (+++) of Storage on a Bio-Gel P-10 Column.


↓Vo
0.8
0.6
0.4
0.2
ABSORBANCE
100
200
300
ELUTION VOLUME (ml.)

1.5:1 ratio of A- to B-chains which has been reported for an unknown wheat sample (99), the ratio of A- to B-chains is higher in soluble starch extracted from bread. The higher ratio of A- to B-chains in soluble starch amylopectin may be related to its high β-amylolysis values. A-chains are unbranched and B-chains are those to which another chain is attached, increasing A-chains to B-chains ratio indicates an increasing extent of multiple branching (99).

SUMMARY AND CONCLUSION

An appreciable amount of starch in fresh bread is soluble and can be extracted from the crumb by cold water. Relatively little soluble starch was extracted from bread containing the surfactants compared to the control and bread baked with no shortening. There was a decrease in quantity of soluble starch extracted from bread stored for five days compared to that extracted from one day old bread. The difference between the amount of soluble starch extracted from bread one and five days after baking was highest in bread baked without shortening and was lowest for bread baked with SSL which indicated that less soluble starch became insoluble as a result of storage in bread baked with surfactant, and more became insoluble during storage of bread baked with no shortening.

Iodine affinity values were lower for the sample containing surfactants indicating the lower amount of amylose in these samples and higher iodine affinity values of the sample baked with no shortening indicated that more amylose was leached from the starch granules in the absence of shortening. Low iodine affinity values of soluble starch samples suggested that the soluble starch extracted from bread is mainly amylopectin.

The quantity of total starch content in soluble starch sample as was measured by glucoamylase digestion, decreased during storage, while the amount of pentosan increased. Samples extracted from bread made with surfactants had higher pentosan content and lower starch content compared to the samples baked without surfactants. There was a decrease in starch content from 4-9% in different samples when the solutions were filtered. It indicated that a small amount of soluble starch has become insoluble during the isolation procedure.

Hydrolysis of soluble starch with β-amylase gave a high conversion to maltose which did not seem to be in agreement with iodine affinity values.

The soluble starch sample, the purified pentosan from that sample and soluble fraction in saturated ammonium sulfate of the total sample was applied to sepharose 4B column. The results showed that the purified pentosan is composed of only one fraction, the high molecular weight fraction which precipitate with ammonium sulfate. The low molecular weight fraction which is soluble in saturated ammonium sulfate was missing from the pentosan sample. The soluble starch sample was found to be much smaller than regular starch because it was eluted later on the column. Molecular size distribution of the soluble starch sample and the purified pentosan on sepharose 6B-CL column also indicated that the soluble starch sample are smaller in size than regular starch. Gel filtration of β-limit dextrin on sepharose 6B-CL and on Biogel P-10 column indicated that the β-limit dextrin from soluble starch sample is smaller in size and there are relatively small fragments.

When the soluble starch samples were debranched with pullulanase and then chromatographed on a column of Biogel P-10, two peaks were observed for all the samples with similar elution profiles. The first peak which was at the void volume of column, was composed of material with higher molecular weight that did not debranch with pullulanase. Pentosan made up the major part of the first peak and amylose was present in very small amounts. The second peak which contained the debranched material had a bimodal distribution of chain-length with the majority of chains being low molecular weight. There results were furthermore confirmed by the gel filtration of the debranched sample on sepharose 6B-CL. It also showed that

the amylose in soluble starch sample are very small in quantity and amylopectin is the major starch component of the soluble starch sample.

The average unit-chain length were shorter for soluble starch amylopectin compare to regular amylopectin. Soluble starch amylopectin had higher ratio of A to B chains compared to normal wheat amylopectin.

Now we can conclude that soluble starch sample are mainly amylopectin, but not a regular amylopectin. An amylopectin which gives low iodine affinity values and high β-amylolysis. Its β-limit dextrins are smaller in size. It has shorter chain length and higher ratio of A to B chains compared to regular amylopectin. This component should be called a degraded amylopectin. This degradation occurs during baking by the action of amylases on the starch component of the bread.

Flour milled from sound wheat contains 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 normally to compensate for the natural deficiency in alpha-amylase that is generally found in wheat flours (103). It has been suggested that alpha-amylases hydrolyze some of the starch molecules in the amorphous regions between the crystallites, therefore, decreasing the rate at which bread becomes firm during aging (104). Major amylase activity occurs during baking. As the temperature of dough increases during baking above 60°C, starch is gelatinized and becomes more susceptible to the enzyme. At the same temperature beta-amylase is inactivated, but wheat alpha-amylase remains active for another 1 or 2 minutes, until the temperature reaches 75°C (105).

Miller et al. (104) and Beck et al. (106) determined the formation of dextrins by extracting bread crumb with water and precipitating dextrins from the extract with alcohol. It was found that a control bread made from

unmalted flour contained 211 mg of dextrin in 10 g of bread crumb while supplementation with 131 or 796 alpha-amylase units (from wheat) per 700 g of flour led to the formation of 309 or 667 mg of dextrins, respectively. These results support our conclusion that some of the starch components are hydrolyzed by α-amylase during baking and the extracted water soluble starch are a degraded amylopectin.

ACKNOWLEDGEMENT

The author wishes to express her deep appreciation to Dr. R. Carl Hoseney for his advice and encouragement in conducting this research project as well as for his helpful suggestions in preparation of this manuscript.

The author gratefully values the assistance and suggestions of Dr. David R. Lineback during the planning and implementation of her research. Appreciation is also extended to Dr. E. Variano-Marston and Dr. Paul A. Seib, her committee members, for their assistance in reviewing this manuscript.

The author also wishes to extend special thanks to William Atwel, for his technical assistance, Richard Junge for baking bread and Aileen Berroth for typing various drafts of the manuscript.

Finnaly, the author owes the greatest gratitude to her husband, Hossain, for his encouragement, patience and support.

REFERENCES

1. Ponte, J. G. In Wheat Chemistry and Technology, 2nd ed., Edited by Y. Pomeranz, Am. Assoc. Cereal Chem., St. Paul, Minn. (1971).
2. Schoch, T. J., and French, D. Studies on bread staling. I. The role of starch. Cereal Chem., 24:231 (1947).
3. Bechtel, W. C., Meisner, D. F., and Bradly, W. B. The effect of the crust on the staling of bread, Cereal Chem. 30:160 (1953).
4. Maga, J. A. Bread staling. CRC Critical Reviews. In Food Technology. 5:443 (1975).
5. Boussingault, J. B. De La Transformation du Pain Tender en Pain Rassis, Ann. Chem. Phys. III, Series XXXVI, p. 490 (1852). Cited in: Willhoft, E.M.A. Mechanism and theory of staling of bread and baked goods and associated changes in textural properties. J. Text. Studies 4 (3): 292 (1973).
6. Senti, F. R., and Dimler, R. J. Changes in starch and gluten during aging of bread. Baker's Digest 34:28 (1960).
7. Taylor, N. W., Cluskey, J. E., and Senti, F. R. Moisture sorption of wheat starch and gluten in relation to bread staling. Cereal Chem. 36: 236 (1959).
8. Breaden, P. W. and Willhoft, E.M.A. Bread staling. III. Measurement of redistribution of moisture in bread by gravimetry. J. Sci. Food Agric. 22 (12):647 (1971).
9. Cornford, S. J., Axford, D.W.E., and Elton, G.A.H. The elastic modulus of bread crumb in linear compression in relation to staling. Cereal Chem. 41:216 (1964).

10. Axford, D.W.E., Colwell, K. H., Cornford, S. J., and Elton, G.A.H. Effect of loaf specific volume on the rate and extent of staling in bread. J. Sci. Food Agric. 19:95 (1968).

11. Mciver, R. G., Axford, D.W.E., Colwell, K. H. and Elton, G.A.H. Kinetic study of retrogradation of gelatinized starch. J. Sci. Food Agric. 19:560 (1968).

12. Katz, J. R. In: A Comprehensive Survey of Starch Chemistry, Walton, R. P., ed., Chemical Catalog Co., New York, 1928.

13. Prentice, N., Guendet, L.S. and Geddes, W. F. Studies on bread staling. V. Effect of flour fractions and various starches on the firming of bread crumbs. Cereal Chem. 3:188 (1954).

14. Bechtel, W. G. Staling studies of bread made with flour fraction. Cereal Chem. 36:368 (1959).

15. Schoch, T. J. Starch in bakery products. Baker's Digest. 39 (2):48 (1965).

16. Zobel, H. F. A review of bread staling. Baker's Digest. 47 (5):52 (1973).

17. Gilles, K. A., Geddes, W. F., and Smith, F. The carbohydrates of gramineae. XI. The constitution of the water-soluble polysaccharides derived from bread crumb. Cereal Chem. 38:229 (1961).

18. Kim, S. K., and D'Appolonia, B. L., Effect of pentosans on the retrogradation of wheat starch gel. Cereal Chem., 54:150 (1977).

19. Kim, S. K., and D'Appolonia, B. L., Bread staling studies. III. Effect of pentosan on dough, bread, and bread staling rate. Cereal Chem., 54:225 (1977).

20. Jongh, G. The formation of dough and bread studies. The ability of starch to form structures and the improving effect of glycerol monostearate. Cereal Chem. 38:140 (1961).

21. Bourne, E. J., Tiffin, A. I., and Weigel, H. Interaction of starch with sucrose stearates and other anti-staling agents. J. Sci. Food Agric., 11:101 (1960).

22. Whistler, R. L. and Hilbert, G. E. Extraction of fatty substance from starch. J. Am. Chem. Soc. 66:1721 (1944).

23. Schoch, T. J. and Williams, C. B. Absorption of fatty acids by the linear component of corn starch. Chem. Soc. 66:1232 (1944).

24. Lehrman, L. The nature of fatty acids associated with starch: The absorption of palmetic acid by potato and defatted corn and rice starches. J. Am. Chem. Soc. 64:2144 (1942).

25. Strandine, E. J., Carlin, G. T., Werner, G. A., and Hopper, R. P. Effect of monoglycerides on starch, flour and bread. A microscopic and chemical study. Cereal Chem. 28:449 (1951).

26. Cappock, J.B.M., Cookson, M. A. Laney, D. H., and Axford, D.W.E. The role of glycerides in baking. J. Sci. Food Agric. 5 (1):8 (1954).

27. Osman, F. M., and Dix, M. R. Effects of fats and nonionic surface-active agents on starch pastes. Cereal Chem. 37:464 (1960).

28. Mikus, F. F., Hixon, R. M., and Rundle, R. E. The complexes of fatty acid with amylose. J. Am. Chem. Soc. 68:1115 (1946).

29. Osman, E. M., Leith, S. J., and Fles, M. Complexes of amylose with surfactants, Cereal Chem. 38:449 (1961).

30. Lord, D. D. The action of polyoxyethylene monostearate upon starch with reference to its softening action in bread. J. Colloid Sci. 5:360 (1950).

31. Hellman, W. N., Fairchild, B. and Senti, F. R. The bread staling problem. Molecular organization of starch upon aging of concentrated starch gels at various moisture levels. Cereal Chem. 31:495 (1954).

32. Zobel, H. F. and Senti, F. R. The bread staling problem. X-Ray diffraction studies on bread containing a cross-linked starch and a heat stable amylase. Cereal Chem. 36:441 (1959).

33. Destefanis, V. A., Ponte, J. G., Chung, F. H., and Ruzza, N. A. Binding of crumb softeners and dough strengtheners during bread making. Cereal Foods World 21 (3):131 (1976).

34. Krog, N. Amylose complexing effect of food grade emulsifiers. Die Starke 23 (6):206 (1971).

35. Knightly, W. H. The role of surfactants in baked foods. In: Surface-Active Lipids in Food. p. 131. S.C.I. Monograph No. 32. Soc. of Chemical Industry, London (1968).

36. Pomeranz, Y., Shogren, M. D. and Finney, K. F. Improving bread making properties with glycolipids. II. Improving various protein-enriched products. Cereal Chem. 46:512 (1969).

37. Tsen, C. C., Hoover, W. J. The shortening-sparing effect of sodium stearyl-2-lactylate and calcium stearyl-2-lactylate in bread baking. Baker's Digest. 45 (3):38 (1971).

38. Tsen, C. C., Hoover, W. J. and Phillips, D. The use of sodium stearyl-2-lactylate and calcium stearyl-2-lactylate for producing high protein breads. Baker's Digest 45 (2):20 (1971).

39. Tsen, C. C. and Tang, R. T. K-State process for making high protein breads, I. Soy bread. Baker's Digest, 45:26 (1971).

40. Tenney, R. J., and Schmidt, D. M. Sodium stearyl-2-lactylate: Its function in yeast leavened bakery products. Baker's Digest 42 (6): 30 (1968).

41. Perlin, A. S. Isolation and composition of the soluble pentosans of wheat flours. Cereal Chem. 28:370 (1951).

42. Perlin, A. S. Structure of the soluble pentosans of wheat flours. Cereal Chem. 28:382 (1951).

43. Montgomery, R., and Smith, F. The carbohydrate of gramineae VII. The constitution of a water soluble hemicellulose from endosperm of wheat (Triticum Vulgare). J. Am. Chem. Soc. 77:3325 (1955).

44. Ewald, C. H., and Perlin, A. S. The arrangement of branching in an arabinoxylan from wheat flour. Can. J. Chem. 37:1254 (1959).

45. Wrench, P. M. The role of wheat flour pentosans in baking. III. Enzymatic degradation of pentosan fractions. J. Sci. Food Agr. 16:51 (1965).

46. Kuendig, W., Neukom, H., and Deuel, H. Unterschungen uber Getreideschleimstoffe. I. Chromatographische Fraktionierung von Wasserloeslichen Weizen Mehlpentosanen an Diaethylaminoaethyl-cellulose. Helv. Chem. Acta 44:823 (1961).

47. Lin, F. M., and Pomeranz, Y. Characterization of water-soluble wheat flour pentosans. J. Food Sci. 33:599 (1968).

48. Medcalf, D. G., D'Appolonia, B. L., and Gilles, K. A. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. Cereal Chem. 45:539 (1968).

49. Neukom, H., Providoil, L., Gremli, H., and Hui, F. A. Recent investigations of wheat flour pentosans. Cereal Chem. 44:238 (1967).

50. Gregory, J. D., Laurent, T. C., and Koden, L. Enzymatic degradation of chandromucoprotein. J. Biol. Chem. 239:3312 (1964).

51. Myhre, D. V. The functions of carbohydrates in baking. Baker's Digest, 44 (3):32 (1970).

52. Fincher, G. B., Sawyer, W. H., and Stone, B. A. Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. Biochem. J. 139:535 (1974).

53. Fincher, G. B., and Stone, B. A. A water-soluble arabinogalactan-peptide from wheat endosperm. Aust. J. Biol. Sci. 27:117 (1974).

54. Kulp, K. Pentosans of wheat endosperm. Cereal Sci. Today 13:414 (1968).

55. Finney, K. F. Fractionating and reconstituting techniques as a tool in wheat flour research. Cereal Chem. 20:381 (1943).

56. Pence, J. W., Elder, A. H., and Mecham, D. K. Preparation of wheat flour pentosans for the use in reconstituted doughs. Cereal Chem. 27:60 (1950).

57. Tracy, M. W. The role of wheat flour pentosans in baking. I. Enzymatic destruction of pentosans in vitro. J. Sci. Food Agr. 15:607 (1964).

58. Hoseney, R. C., Finney, K. F., Shogren, M. D., and Pomeranz, Y. Functional (breadmaking) and biochemical properties of wehat flour components. II. Role of water solubles. Cereal Chem. 46:117 (1969).

59. Lin, F. M., and Pomeranz, Y. Characterization of water-soluble wheat flour pentosans. J. Food Sci. 33:599 (1968).

60. D'Appolonia, B. L., Gilles, K. A., and Medcalf, D. G. Effect of water-soluble pentosans on gluten-starch loaves. Cereal Chem. 47:194 (1970).

61. Hassid, W. Z., and McCready, R. M. The molecular constitution of amylose and amylopectin of potato starch. J. American Chem. Soc., 65:1157 (1943).

62. Peat, S., Wehlan, W. J., and Pirt, S. J. The amylolytic enzymes of soya bean. J. Nature, 164:499 (1949).

63. Peat, S., Pirt, S. J., and Whelan, W. J. Enzymic synthesis and degradation of starch. Part XV. β-amylase and the constitution of amylose. J. Chem. Soc. 705 (1952).

64. Kjolberg, O., and Manners, D. J. The action of isoamylase on amylose. Biochem. J., 86:258 (1963).

65. Banks, W., and Greenwood, C. T. The fine structure of amylose: the action of pullulanase as evidence of branching. Arch. Biochem. Biophys., 117:674 (1966).

66. Marshall, J. J. Application of enzymic methods to the structural analysis of polysaccharides: Part I. Advances Carbohydrate Chem. and Biochem. 30:257 (1974).

67. Peat, S., Whelan, W. J., and Thomas, G. J. Evidence of multiple branching in waxy maize starch. J. Chem. Sco., 4546 (1952).

68. Summer, R. and French, D. Action of β-amylase on branched oligosaccharides. J. Biol. Chem., 222:469 (1956).

69. Walker, G. J., and Whelan, W. J. The mechanism of carbohydrase action: 8. Structures of the muscle phosphorylase limit dextrins of glycogen and amylopectins. Biochem. J., 76:264 (1960).

70. Pazur, J. H., and Ando, T. The action of an amyloglucosidase of aspergillus niger on starch and malto-oligosaccharides. J. Biol. Chem. 234:1966 (1959).

71. Pazur, J. H. and Kleppe, K. The hydrolysis of α-D-glucosides by amyloglucosidase from aspergillus niger. J. Biol. Chem. 237:1002 (1962).

72. Marshall, J. J., and Whelan, W. J. Incomplete conversion of glycogen and starch by crystalline amyloglucosidase and its importance in the determination of amylaceous polymers. FEBS Lett. 9:85 (1970).

73. Bender, H. and Wallenfels, K. Untersuchungen an Pullulan. II. Spezifischer Abbau durch ein Bakterielles Enzyme. Biochem. Z. 334:79 (1961).

74. Wallenfels, K., Bender, H., and Rached, J. R. Pullulanase from aerobacter aerogenes: Production in a cell-bound state. Purification and properties of the enzyme. Biochem. Biophys. Res. Commun. 22:254 (1966).

75. Abdullah, M., Catley, B. J., Lee, E.Y.C., Robyt, J., Wallenfels, K., and Whelan, W. J. The mechanism of carbohydrase action. II. Pullulanase, an enzyme specific for the hydrolysis of α (1, 6) bonds in amylaceous oligo- and polysaccharides. Cereal Chem. 43:111 (1966).

76. Whelan, W. J. Enzymic exploration of the structures of starch and glycogen. Biochem. J. 122:609 (1971).

77. Drummond, G. S., Smith, E. E., and Whelan, W. J. On the specificity of starch debranching enzymes. FEBS Lett. 9:136 (1970).

78. Hobson, P. N., Whelan, W. J. and Peat, S. The enzymic synthesis and degradation of starch. Part XIV. R-enzymes. J. Chem. Soc. 1451 (1951).

79. Mercier, C., Frantz, B. M., and Whelan, W. J. An improved purification of cell-bound pullulanase from aerohacter-aerogenes. Eur. J. Biochem., 26:1 (1972).

80. Gunja-Smith, Z., Marshall, J. J., Smith, E. E., and Whelan, W. J. A glycogen debranching enzyme from cytophage. FEBS Lett. 12:96 (1970).

81. Haworth, W. N., Hirst, E. L., and Isherwood, F. A. Polysaccharides. Part XXIII. Determination of the chain length of glycogen. J. Chem. Soc., 577 (1937).

82. Staudinger, H., and Husemann, E. Uber Hochpolymere Verbindungen. Justus Liebigs Annln. Chem. 527:195 (1937).

83. Meyer, K. H., Bernfeld, D. Recherches sur l'amidon V. L'amylopectin. Helv. Chim. Acta., 23:875 (1940).

84. Meyer, K. H. and Fuld, M. Recherches sur l'amidon XII. L'arrangement des restes de Glucose dans le Glycogen. Helv. Chim. Acta, 24:375 (1941).

85. Gunja-Amith, Z., Marshall, J. J., Mercier, C., Smith, E. E., and Whelan, W. J. A revision of Meyer-Bernfeld model of glycogen and amylopectin. FEBS Lett. 12:101 (1970).

86. Gunja-Smith, Z., Marshall, J. J., and Smith, E. E. Enzymatic determination of the unit-chain length of glycogen and related polysaccharides. FEBS Lett. 13:309 (1971).

87. Lee, E.Y.C., and Whelan, W. J. Enzymatic methods for microdetermination of glycogen and amylopectin, and their unit-chain length. Arch. Biochem. Biophys. 116:162 (1966).

88. Carter, J. H., and Lee, E.Y.C. An enzymic method for determination of the average chain lengths of glycogen and amylopectin. Anal. Biochem. 39:373 (1971).

89. Lee, E.Y.C., Mercier, C. and Whelan, W. J. A method for the investigation of the fine structure of amylopectin. Arch. Biochem. Biophys. 125:1028 (1968).

90. American Association of Cereal Chemists. Approved Methods of the AACC. The Association: St. Paul, Minn. (1976).

91. Schoch, T. J. Iodimetric determination of amylose. Potentiometric titration: Standard methods. Methods Carbohydrate Chem. IV:157 (1964).

92. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. Colorimetric method for determination of sugars and related substance. Anal. Chem. 28:350 (1956).

93. Shetty, R. M., Lineback, D. R., and Seib, P. A. Determining the degree of starch gelatinization. Cereal Chem. 51:364 (1974).

94. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375 (1944).

95. Lineback, D. R., Russell, I. J., and Rasmussen, C. Two forms of the glucoamylase of Aspergillus niger. Arch. Biochem. Biophys. 134:539 (1969).

96. Whelan, W. J. Hydrolysis with β-amylase and preparation of the β-amylase limit dextrin of amylopectin. Methods Carbohydrate Chem. IV:261 (1964).

97. Experimental Biochemistry, ed. by J. M. Clark, Jr., W. H. Freeman and Co., San Francisco, Ca. 213 (1963).

98. Lii, C. Y., and Lineback, D. R. Characterization and comparison of cereal starches. Cereal Chem. 54:138 (1977).

99. Marshall, J. J., and Whelan, W. J. Multiple branching in glycogen and amylopectin. Arch. Biochem. Biophys. 16:234 (1974).

100. Bailey, J. M., and Whelan W. J. I. Relationship between iodine stain and chain length. J. Biol. Chem. 236:969 (1961).

101. Lansky, S., Kooi, M., and Schoch, T. J. Properties of the fractions and linear subfractions from various starches. J. Amer. Chem. Soci., 71:4066 (1949).

102. Atwell, W. Dept. of Grain Science and Industry. Kansas State University. Unpublished work.

103. Pyler, E. J. Baking science and technology. Siebel Publishing Company, Vol. 1, 1973.

104. Miller, B. S., Johnson, J. A., and Palmer, D. L. A comparison of cereal, fungal, and bacterial alpha-amylases as supplements in bread making. Food Technol. 7:38 (1953).

105. Walden, C. C. The action of wheat amylases on starch under conditions of time and temperature as they exist during baking. Cereal Chem. 32: 421 (1955).

106. Beck, H., Johnson, J. A., and Miller, B. S. Studies on the soluble dextrin fraction and sugar content of bread baked with alpha-amylase from different sources. Cereal Chem. 34:211 (1957).

THE RELATIONSHIP OF SOLUBLE STARCH STRUCTURE TO BREAD STALING

by

KATAYOON GHIASI

B.S., Tehran University, Iran, 1970

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

ABSTRACT

The term "soluble starch" of bread as generally accepted consists of a starch and a pentosan fraction. During staling the soluble starch content progressively decreases. This effect has been used as a measure for estimating the extent of staling.

The composition of soluble starch extracted from control bread, bread baked with surfactants and no shortening was studied by iodine affinity, β-amylolysis and gel filtration. The results indicated that the starch fractions are smaller in size compared to starch isolated from flour and that the soluble starch component are mainly amylopectin. This amylopectin had shorter average unit-chain length and higher ratio of A to B chains compared to regular wheat starch. High β-amylolysis of soluble starch samples with the other information indicated that the starch component of the soluble starch samples was mainly amylopectin, but an amylopectin which has been degraded by α-amylase during baking of bread.