This document is supplied on the condition that it will be used solely for research, further reproduction is prohibited by copyright law

4. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (9th ed.). The Association: Washington, D.C. (1960).
5. SPACKMAN, D. H. Accelerated system for automated analysis of amino acids. Fed. Proc. 22: 244 (1963).
6. FINLEY, J. W., JOHNSTON, P. H., and FRIEDMAN, M. A potential improved tryptophan analysis of food proteins in the presence of carbohydrates, p. 453. In: Protein nutritional quality of foods and feeds, Part I, ed. by M. Friedman. Marcel Dekker: New York (1975).
7. MOORE, S. On the determination of cystine as cysteic acid. J. Biol. Chem. 238: 235 (1963).
8. KOHLER, G. O., and PALTER, R. Studies on methods of amino acid analysis of wheat products. Cereal Chem. 44: 512 (1967).

[Received January 2, 1976. Accepted May 18, 1976]

CHARACTERIZATION AND COMPARISON OF CEREAL STARCHES[1]

C.-Y. LII[2] and D. R. LINEBACK

ABSTRACT Cereal Chem. 54(1): 138-149

Starches were isolated from hard wheat (two varieties), soft wheat, club wheat, durum wheat, triticale, rye, and *Triticum dicoccum* (Emmer). All starches lost birefringence within the range 50.0° to 64.5°C. Scanning electron microscopy revealed similar granule sizes and shapes for all starches, except rye. Iodine affinities of the starches ranged from 4.36 to 4.91%. Brabender amylograms obtained for each starch were characteristic of normal cereal starches. When amylopectin fractions isolated from each starch were debranched with isoamylase and subjected to gel filtration through Bio-Gel P-10, two chain populations of carbohydrate with $\overline{d.p.}$ of approximately 11-25 and 52-60 were obtained from each amylopectin. Average unit-chain lengths of the amylopectins ranged from 15 for triticale to 26 for rye. The ratio of A-chains to B-chains in each amylopectin, determined enzymatically using ϕ, β-dextrins, and β-limit dextrins, ranged from 1.21 to 2.11. The amylose fractions isolated from each starch had β-amylolysis limits ranging from 76.6 to 81.6%. The wheat starches investigated appeared to have definite, but minor, differences in structure.

The role of starch in forming baked products has not been completely elucidated. Harris (1), Sandstedt (2), and Hoseney *et al.* (3) reported that rice, corn, waxy maize, and potato starches do not have the breadmaking quality that wheat starch has, but Hoseney *et al.* (3) found that rye and barley starches very nearly do. Medcalf and Gilles (4) concluded that wheat starch was generally

[1]Contribution No. 915-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan, KS 66506. Presented at the 60th Annual Meeting, Kansas City, Mo., Oct. 1975.
[2]Respectively: Research Associate and Professor, Department of Grain Science and Industry, Kansas State University.

Copyright © 1977 American Association of Cereal Chemists, Inc., 3340 Pilot Knob Road, St. Paul, Minnesota 55121. All rights reserved.

superior in baking quality to starches from other sources. However, researchers disagree on the relative breadmaking quality of starches from different wheat varieties. Harris and Sibbitt (5,6) found that starches from different varieties of wheat varied greatly in their breadmaking quality; Kulp (7), D'Appolonia and Gilles (8), and Hoseney *et al.* (3) found that the differences were minor. In reconstitution studies, Hoseney *et al.* (3) noted that starch from durum wheats tended to give bread with a lower loaf volume and that starch from club wheats tended to give higher loaf volume than did the control hard wheat starch. The higher loaf volume obtained with the club wheat starch seemingly resulted from genetic factors rather than from growing conditions.

In recent years, the availability of enzymes with known specificity has greatly facilitated the study of the structure of amylose and amylopectin. The application of pullulanase (pullulan 6-glucanohydrolase, EC 3.2.1.41) and isoamylase (glycogen 6-glucanohydrolase, EC 3.2.1.68) to the investigation of the fine structure of amylopectin has recently been reviewed (9). The use of those enzymes has led to the postulation of a revised structure for amylopectin and glycogen (10) in which the arrangement of A-chains and B-chains[3] differs from the traditional Meyer structure. The use of pullulanase and isoamylase allows determination of the relative ratio of A-chains and B-chains in an amylopectin (11), thus facilitating a comparison of the structures of starch components from different sources.

This investigation was initiated to determine physical and biochemical properties of selected cereal starches in an effort to discover whether there are significant differences in structure of the amylose and amylopectin components that could be related to differences in functional properties. The ratios of A-chains and B-chains in amylopectins isolated from the starches were determined enzymatically to allow us to compare the fine structure of the components.

MATERIALS AND METHODS

Starch Isolation

Starches were isolated from flours from hard wheat (Scout and Kalyan sona varieties), *Triticum dicoccum* (Emmer), club wheat (Omar variety), durum, soft wheat, triticale, and rye. Kalyan sona, a hard spring wheat, and *T. dicoccum* were obtained from India. Flours were milled from the grains on a Buhler experimental mill. Prime starches were isolated from the wheat and triticale flours by the dough-kneading procedure (12). The liquid slurry obtained from hand-washing the dough ball was centrifuged (2,000 × *g*, 15 min). The tailings layer was removed by scraping with a spatula. The prime starch was reslurried in water and centrifuged two or three additional times. The recovered prime starch was air-dried at 40°–45°C. Prime starch was isolated from rye flour by the procedure of Berry *et al.* (13).

[3]Terminology:
1. A-chains are those chains in amylopectin which are unbranched, and B-chains are those chains to which another chain is attached by an α-D-(1 → 6)-linkage.
2. β-Limit dextrins are β-amylase-limit dextrins formed by treating amylopectin with sweet potato β-amylase.
3. φ-Dextrins are muscle phosphorylase-limit dextrins formed by treating amylopectin with rabbit muscle phosphorylase a.
4. φ, β-Dextrins are β-amylase-limit, phosphorylase-limit dextrins formed by treating φ-dextrins with sweet potato β-amylase.

Scanning Electron Microscopy (SEM)

Starches, sprinkled onto double-sided Scotch tape attached to specimen stubs, were coated *in vacuo* successively with about 60 Å of carbon and about 100 Å gold-palladium. Samples were viewed and photographed using an ETEC Autoscan scanning electron microscope operating at 20 kV accelerating voltage. Images were photographed on Polaroid film, type 55 P/N.

Birefringence End Point Temperature Range

Birefringence end point temperature ranges were determined for each starch using a polarizing microscope with a Kofler hot stage. Temperatures were recorded that corresponded to loss of birefringence by 2, 50, and 98% of the starch granules observed in the field, designating them as initial, midpoint, and end point values, respectively (14).

Iodine Affinity

Starches were defatted (24 hr, methanol) prior to determining their iodine affinity. Iodine affinities for whole starch, amylose, and amylopectin were determined by potentiometric titration (15) using a Beckman pH meter with a platinum electrode calibrated with a voltage reference cell.

Brabender Viscosity Curves

Brabender viscosity curves were determined using a Brabender Viscoamylograph®, according to the procedure described by Mazurs *et al.* (16) and 8.5% starch concentrations (w/v).

Starch Fractionation

Starches were fractionated into amylose and amylopectin by Schoch's procedure (17). Amylose was initially precipitated with n-pentanol, then recrystallized from 1-butanol. To isolate it, amylose was dehydrated with acetone and vacuum-dried at 40°C. Amylopectin was collected by repeatedly precipitating the supernatant gel with methanol.

Debranching Amylopectin with Isoamylase

Cytophaga isoamylase (Lytic enzyme L1, Gallard-Schlesinger, Carle Place, New York) was purified by chromatography on a column of DEAE-cellulose as described by Gunja-Smith *et al.* (18). The purified isoamylase had an activity of 1.2 units/ml.

Amylopectin (20 mg) was debranched with 0.12 units of isoamylase in 5 ml of 100 m*M* acetate buffer, pH 5.5, for 24 hr at 37° C. The digest was heated in boiling water for 5 min to inactivate the enzyme. Insoluble material was removed by centrifugation (10,000 × *g*, 20 min).

Gel Filtration of Debranched Amylopectin

The solution containing the debranched amylopectin was chromatographed on a jacketed column of Bio-Gel P-10 (2.5 × 75 cm). The carbohydrate was eluted with 10 m*M* sodium phosphate buffer, pH 7.0, containing 0.02% sodium azide at 30°C. Fractions were automatically collected at 15-min intervals (4 ml), and carbohydrates were located in the effluent fractions by assaying an aliquot of each fraction using the phenol-sulfuric acid procedure (19). The absorbance was determined at 490 nm.

The average degree of polymerization ($\overline{d.p.}$) of material in effluent fractions was determined by dividing the total carbohydrate [phenol-sulfuric acid procedure (19)] by the reducing power [Nelson colorimetric copper procedure (20)] expressed as glucose.

Unit-Chain Lengths of Amylopectins

The average unit-chain lengths ($\overline{c.l.}$) of amylopectins were determined by dividing the total amount of polysaccharide (amylopectin) debranched by isoamylase by the amount of reducing groups released during the debranching (21). The total amount of polysaccharide was determined by the phenol-sulfuric acid procedure (19), using a standard curve prepared from D-glucose. Reducing sugars were determined by Nelson's colorimetric copper procedure (20) calibrated against D-glucose.

Preparing β-Amylase Limit Dextrins and φ, β-Dextrins

β-Amylase-limit dextrins (β-limit dextrins) were prepared by treating amylopectin (25 mg/ml in 100 m*M* acetate buffer, pH 4.8) with 380 units/ml sweet potato β-amylase (crystalline, Type I-B, Sigma Chemical Co., St. Louis, Mo.) at 37°C with dialysis to remove maltose (11,22). Muscle phosphorylase-limit dextrins (φ-dextrins) were prepared by treating amylopectin (25 mg/ml) in 80 m*M* sodium phosphate buffer, pH 6.8, containing 40 m*M* β-mercaptoethanol with 4 units/ml of rabbit muscle phosphorylase a (Sigma Chemical Co., St. Louis, Mo.) at 30° C for 24 hr (23,24). φ, β-Dextrins were prepared by treating φ-dextrin (20 mg/ml) with 380 units/ml of sweet potato β-amylase in 100 m*M* sodium acetate buffer, pH 4.8, at 37°C for 24 hr (10,24).

Debranching with Pullulanase

Pullulanase from *Aerobacter aerogenes* was prepared and purified as

TABLE I
Iodine Affinities (%) of Starches, Amyloses, and Amylopectins

Source	Iodine Affinity (%)		
	Starch	Amylose	Amylopectin
Scout	4.62	19.88	0.81
Omar	4.39	19.56	0.77
Kalyan sona	4.36	19.34	0.82
T. dicoccum	4.73	19.49	0.76
Soft wheat	4.84	19.67	0.78
Durum	4.61	19.23	0.69
Triticale	4.62	19.01	0.68
Rye	4.91	19.42	0.74

described by Mercier *et al.* (25). The purified enzyme had an activity of 15.1 units/ml.

β-Limit dextrins (2.0 mg) and ϕ, β-dextrins (2.0 mg) were debranched with 1.51 units of pullulanase in 1.0 ml of 20 m*M* sodium acetate buffer, pH 5.5, for 12 hr at 37°C. The digest was heated in boiling water for 5 min to inactivate the enzyme. Insoluble material was removed by centrifugation.

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 m*M* sodium acetate buffer, pH 4.8, for 24 hr at 37°C. Reducing power was measured by the Nelson colorimetric copper method (20) and expressed as glucose.

Determining the Ratio of A-Chains to B-Chains in Amylopectin

The ratio of A-chains to B-chains in amylopectin was determined enzymatically using both β-limit dextrins and ϕ, β-dextrins in the manner described by Marshall and Whelan (11). The ratio of A-chains to B-chains was calculated from the increase in reducing power following the action of debranching enzymes as shown here (9):

Using ϕ, β-Dextrins	*Reducing Power Measured*
Isoamylase	B-chains
Pullulanase	B + A-chains
	Difference: A-chains

Using β-Limit Dextrins	
Isoamylase	B + 1/2 A-chains
Pullulanase	B + A-chains
	Difference: 1/2 A-chains

B-Amylolysis Limits of Amyloses

Amylose (20 mg) was hydrolyzed with 750 units of sweet potato β-amylase in 5 ml of 100 m*M* acetate buffer, pH 4.8, at 37°C for 24 hr. The degree of β-amylolysis was determined by measuring the reducing sugars released, using Nelson's colorimetric copper method (20).

RESULTS AND DISCUSSION

Starch Isolation

As observed by scanning electron microscopy, the isolated starches had the same general shapes and sizes. The starches, rye excepted, had two general types of granules: small, spherical granules, and larger, lenticular granules, as has been generally recognized. Granule sizes ranged from 6 to 40 μ, except that granules of rye starch tended to range from 20 to 55 μ (perhaps small granules were lost during the centrifuging portion of the isolation procedure). Irregularities and dents were observed on the surfaces of some granules, probably because the granules were packed within the protein matrix. Hemispherical grooves (26,27) were observed only in rye starch granules.

Physical Characterization of Starches

As measured by potentiometric titration, iodine affinity values (Table I) for whole starch (defatted with methanol), amylose, and amylopectin ranged from

TABLE II
Birefringence End Point Temperature Ranges of Starches

Source	Birefringence End Point Temperature Range °C
Scout	54.5°–60.0°–64.5°
Omar	54.0°–60.5°–62.0°
Kalyan sona	53.0°–58.5°–61.5°
T. dicoccum	52.0°–58.0°–63.0°
Soft wheat	53.0°–58.0°–63.0°
Durum	50.0°–55.0°–60.5°
Triticale	53.0°–56.5°–61.5°
Rye	50.0°–53.5°–58.0°

TABLE III
Average Unit-Chain Lengths ($\overline{c.l.}$) of Amylopectins

Source	$\overline{c.l.}$
Scout	19
Omar	18
Kalyan sona	17
T. dicoccum	19
Soft wheat	20
Durum	17
Triticale	15
Rye	26

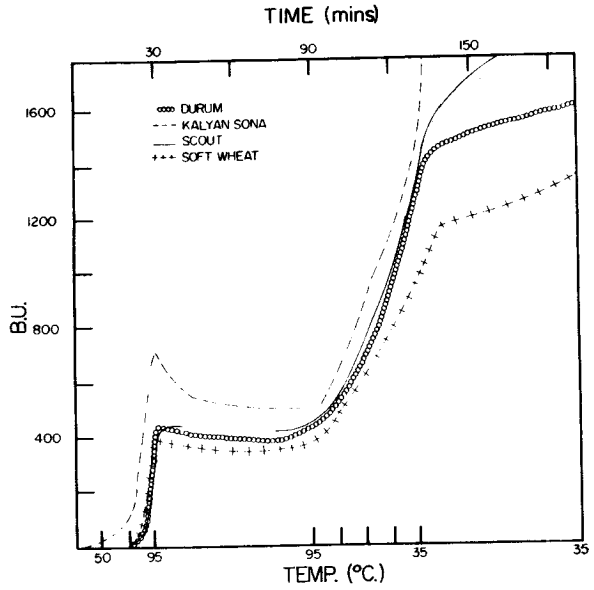

Fig. 1. Brabender viscosity at 8.5% (w/v) starch concentrations.

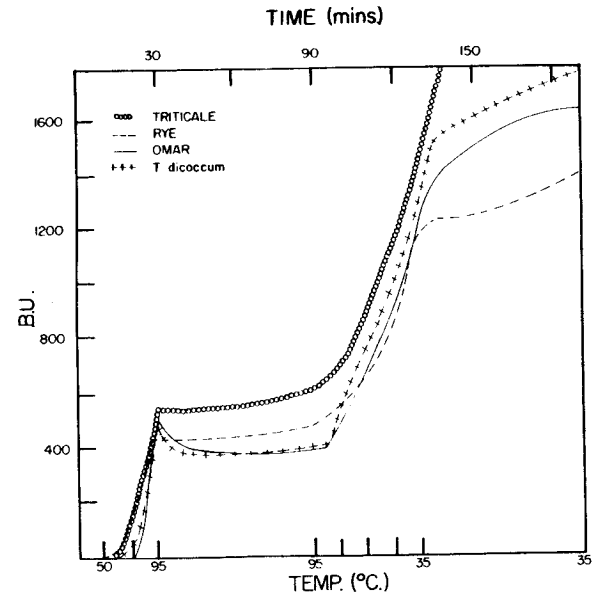

Fig. 2. Brabender viscosity at 8.5% (w/v) starch concentrations.

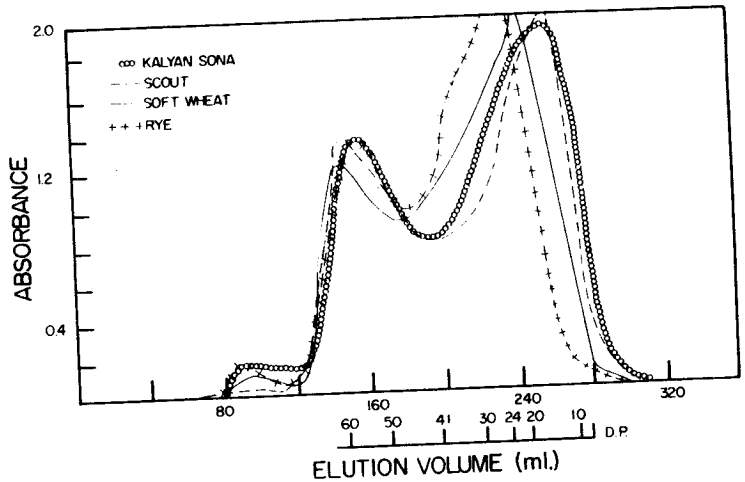

Fig. 3. Fractionation of isoamylase-debranched amylopectin from Scout starch, Kalyan sona starch, soft wheat starch, and rye starch on a column (2.5 × 75 cm) of Bio-Gel P-10 eluted with 10 m*M* sodium phosphate buffer, pH 7.0, containing 0.02% sodium azide at a flow rate of 16 ml/hr at 30°C. Carbohydrate components were located by assaying aliquots of effluent fractions using the phenol-sulfuric acid procedure (19).

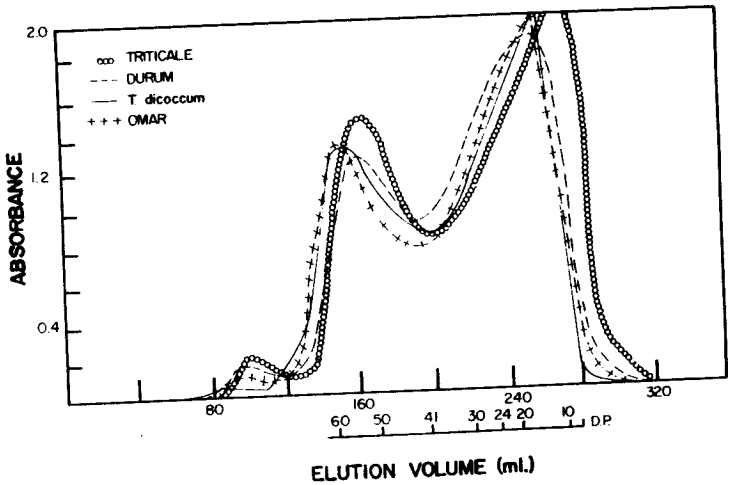

Fig. 4. Fractionation of isoamylase-debranched amylopectin from triticale starch, durum starch, Omar (club wheat) starch, and *T. dicoccum* starch on a column (2.5 × 75 cm) of Bio-Gel P-10 eluted with 10 m*M* sodium phosphate buffer, pH 7.0, containing 0.02% sodium azide at a flow rate of 16 ml/hr at 30°C. Carbohydrate components were located by assaying aliquots of effluent fractions using the phenol-sulfuric acid procedure (19).

4.36% for Kalyan sona starch to 4.91% for rye starch. The values agreed with those reported by Deatherage *et al.* (28). Because pure wheat starch amylose reportedly has an iodine affinity of 20.0% when determined potentiometrically (29), we used that value for the wheat starches we studied; amylose contents ranged from 21.8% for Kalyan sona to 24.3% for soft wheat. Those values agreed well with the range of 23.4 to 27.6% reported for the amylose content of starches from 17 wheat varieties representing hard red spring, hard red winter, durum, and soft wheats (30).

Temperature ranges (birefringence end point temperatures) at which starches gelatinize are indicated in Table II, which gives temperatures at which 2, 50, and 98% of the granules in the field lost birefringence. Initial temperatures for wheat starches ranged from 50.0° to 54.5°C, and final temperatures from 60.5° to 64.5°C; durum and Scout starches had the lowest and highest temperatures, respectively. The values were similar to those previously reported for wheat starches by Medcalf and Gilles (30). The birefringence end point temperature ranges for triticale and rye starches agreed with the 55.2° to 61.5°C and 50.0° to 59.6°C ranges, respectively, reported by Klassen and Hill (31).

The Brabender viscosity curves obtained using 8.5% starch concentrations (Figs. 1 and 2) were similar to those expected of normal cereal starches (32). Some differences were noted between the amylograms of the various starches. Triticale and Kalyan sona starches had relatively higher hot-paste viscosities than did the other starches. Triticale, Kalyan sona, and Scout starches possessed much higher cold-paste viscosities than did the other starches.

Structural Studies of Amylopectins

Iodine affinities of the amylopectins (Table I) did not differ significantly. When the amylopectins were debranched with isoamylase and then chromatographed on a column of Bio-Gel P-10, two major chain populations of carbohydrate were observed for each sample (Figs. 3 and 4). The first major peak contained material with a $\overline{d.p.}$ of 52-60; the second peak, material of $\overline{d.p.}$ 11-25. The third minor peaks, observed at the void volume of the column, were considered to consist of small amounts of incompletely debranched material (9). A similar pattern was obtained when waxy maize amylopectin was debranched with isoamylase and chromatographed on Sephadex G-50 (10); the two peaks

TABLE IV
Ratio of A-Chains to B-Chains in Amylopectins

Source	Ratio of A-Chains to B-Chains Using	
	β-Limit Dextrin	φ, β-Dextrin
Scout	1.51	1.48
Omar	1.59	1.54
Kalyan sona	1.48	1.44
T. dicoccum	1.21	1.19
Soft wheat	1.73	1.64
Durum	1.89	1.78
Triticale	2.11	2.02
Rye	1.81	1.71

had a $\overline{d.p.}$ of about 20 and above 50. The starches appeared to be very similar, having very nearly the same relative proportions of the two major carbohydrate fractions, but perhaps differing slightly in the $\overline{d.p.}$ of the components. The major peak with the lower $\overline{d.p.}$ may well represent the unit chains of amylopectin. It is not yet known whether the component with higher $\overline{d.p.}$ arises from the same polysaccharide as does the component with the lower $\overline{d.p.}$ (9). These results further confirm the correctness of the model of amylopectin proposed by Gunja-Smith *et al.* (10), in that a Meyer-type structure would require there to be a continuous distribution of chain lengths ranging from a high to a low d.p.

The average unit-chain lengths ($\overline{c.l.}$) of the amylopectins—calculated from the total amount of polysaccharide debranched by isoamylase divided by the amount of reducing groups liberated during the debranching (21)—revealed (Table III) that rye amylopectin had the longest $\overline{c.l.}$, 26, and triticale amylopectin the shortest $\overline{c.l.}$, 15. The wheat starches had a relative limited $\overline{c.l.}$ range of 17 (Kalyan sona, durum) to 20 (soft wheat). The average c.l. agreed reasonably well with the chain profiles for the component of lower $\overline{d.p.}$, as observed in Figs. 3 and 4.

Table IV shows the ratios of the number of A-chains and B-chains in the amylopectins—determined enzymatically using both β-limit dextrins and φ, β-

TABLE V
β-Amylolysis (%) of β-Limit Dextrins and φ,β-Dextrins Debranched with Isoamylase and Pullulanase

Source	β-Amylolysis (%) of Debranched	
	β-Limit Dextrin	φ,β-Dextrin
Scout	101	101
Omar	99	101
Kalyan sona	102	101
T. dicoccum	98	99
Soft wheat	99	100
Durum	100	101
Triticale	101	101
Rye	99	101

TABLE VI
β-Amylolysis (%) of Amyloses

Source	β-Amylolysis %
Scout	78.0
Omar	77.3
Kalyan sona	79.1
T. dicoccum	78.1
Soft wheat	78.0
Durum	81.6
Triticale	78.1
Rye	76.6

dextrins. Values derived from both dextrins showed excellent agreement. Marshall and Whelan (11) reported an A-chain to B-chain ratio of 1.5 for an unidentified wheat sample; values obtained by us closely agree with that value. Table IV indicates significant variation in the ratio of A-chains to B-chains within the amylopectins. Because A-chains are unbranched and B-chains are those to which another chain is attached, increasing A-chain to B-chain ratios indicate an increasing extent of multiple branching. Triticale amylopectin, having an A-chain to B-chain ratio of 2.1, apparently has a greater extent of multiple branching than do the other amylopectins, particularly the amylopectin from *T. dicoccum*. The hard wheat and club wheat amylopectins had lower A-chain to B-chain ratios than did the soft wheat and durum amylopectins. Thus, the fine structures of those amylopectins apparently have definite differences, as reflected by the ratio of A-chains to B-chains.

The completeness of debranching was determined by measuring the degree of β-amylolysis of the debranched β-limit dextrins and ϕ, β-dextrins. Because total debranching would release all linear chains, the chains should be completely degraded to maltose by β-amylase. The degree of β-amylolysis of the debranched polysaccharides (Table V) ranged from 98 to 101% for both dextrins, indicating that debranching was essentially complete.

β-Amylolysis Limits of Amyloses

The iodine affinities for the amyloses (Table I) showed small differences, ranging from 19.0% for triticale amylose to 19.9% for Scout amylose. The values indicated that the amyloses were quite pure. The β-amylolysis limits of the amyloses (Table VI) ranged from 77% for rye and club wheat (Omar) amyloses to 82% for durum wheat amylose. All wheat amyloses, except durum amylose, had β-amylolysis limits ranging from 77 to 79%, indicating that any structural differences in these wheat amyloses are probably limited. Amylose's incomplete conversion to maltose by β-amylase may be attributed to a small proportion of (1 → 6)-α-D-glucosidic linkages (branching) present in the amylose molecule (33,34).

Literature Cited

1. HARRIS, R. H. The baking qualities of gluten and starch prepared from different wheat varieties. Baker's Dig. 16(4): 217 (1942).
2. SANDSTEDT, R. M. The function of starch in the baking of bread. Baker's Dig. 35(3): 36 (1961).
3. HOSENEY, R. C., FINNEY, K. F., POMERANZ, Y., and SHOGREN, M. D. Functional (breadmaking) and biochemical properties of wheat flour components. VIII. Starch. Cereal Chem. 48: 191 (1971).
4. MEDCALF, D. G., and GILLES, K. A. The function of starch in dough. Cereal Sci. Today 13: 382 (1968).
5. HARRIS, R. H., and SIBBITT, L. D. The comparative baking qualities of starches prepared from different wheat varieties. Cereal Chem. 18: 585 (1941).
6. HARRIS, R. H., and SIBBITT, L. D. The comparative baking qualities of hard red spring wheat starches and glutens as prepared by the gluten-starch blend baking method. Cereal Chem. 19: 763 (1942).
7. KULP, K. Physicochemical properties of starches of wheats and flours. Cereal Chem. 49: 697 (1972).
8. D'APPOLONIA, B. L., and GILLES, K. A. Effect of various starches in baking. Cereal Chem. 48: 625 (1971).
9. MARSHALL, J. J. Application of enzymic methods to the structural analysis of polysaccharides: Part I. Advan. Carbohyd. Chem. Biochem. 30: 257 (1974).
10. GUNJA-SMITH, Z., MARSHALL, J. J., MERCIER, C., SMITH, E. E., and WHELAN, W. J. A revision of the Meyer-Bernfeld model of glycogen and amylopectin. FEBS Letters 12: 101 (1972).
11. MARSHALL, J. J., and WHELAN, W. J. Multiple branching in glycogen and amylopectin. Arch. Biochem. Biophys. 161: 234 (1974).
12. WOLF, M. J. Wheat starch isolation. Methods Carbohyd. Chem. 4: 6 (1964).
13. BERRY, C. P., D'APPOLONIA, B. L., and GILLES, K. A. The characterization of triticale starch and its comparison with starches of rye, durum, and HRS wheat. Cereal Chem. 48: 415 (1971).
14. WATSON, S. A. Determination of starch gelatinization temperature. Methods Carbohyd. Chem. 4: 240 (1964).
15. SCHOCH, T. J. Iodimetric determination of amylose. Potentiometric titration: Standard method. Methods Carbohyd. Chem. 4: 157 (1964).
16. MAZURS, E. G., SCHOCH, T. J., and KITE, F. E. Graphical analysis of the Brabender viscosity curves of various starches. Cereal Chem. 36: 534 (1959).
17. SCHOCH, T. J. Preparation of starch and starch fractions, p. 5. In: Methods in Enzymology, ed. by S. P. Colowick and N. O. Kaplan, Vol. 3. Academic Press: New York (1957).
18. GUNJA-SMITH, Z., MARSHALL, J. J., SMITH, E. E., and WHELAN, W. J. A glycogen-debranching enzyme from *Cytophaga*. FEBS Letters 12: 96 (1970).
19. DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350 (1956).
20. NELSON, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375 (1944).
21. GUNJA-SMITH, Z., MARSHALL, J. J., and SMITH, E. E. Enzymatic determination of the unit chain length of glycogen and related polysaccharides. FEBS Letters 13: 309 (1971).
22. WHELAN, W. J. Hydrolysis with β-amylase and preparation of the β-amylase limit dextrin of amylopectin. Methods Carbohyd. Chem. 4: 261 (1964).
23. LEE, E. Y. C., CARTER, J. H., NIELSON, L. D., and FISCHER, E. H. Purification and properties of yeast amylo-1,6-glucosidase-oligo-1,4 → 1,4-glucan transferase. Biochem. 9: 2347 (1970).
24. WALKER, G. J., and WHELAN, W. J. The mechanism of carbohydrase action: 8. Structures of the muscle-phosphorylase limit dextrins of glycogen and amylopectin. Biochem. J. 76: 264 (1960).
25. MERCIER, C., FRANTZ, B. M., and WHELAN, W. J. An improved purification of cell-bound pullulanase from *Aerobacter aerogenes*. Eur. J. Biochem. 26: 1 (1972).
26. BUTTROSE, M. S. Submicroscopic development and structure of starch granules in cereal endosperms. J. Ultrastructure Res. 4: 231 (1960).
27. EVERS, A. D. Scanning electron microscopy of wheat starch. III. Granule development in the endosperm. Staerke 23: 157 (1971).
28. DEATHERAGE, W. L., MacMASTERS, M. M., and RIST, C. E. A partial survey of amylose content in starch from domestic and foreign varieties of corn, wheat, sorghum, and from other starch-bearing plants. Trans. Amer. Ass. Cereal Chem. 13: 31 (1955).
29. MONTGOMERY, E. M., and SENTI, F. R. Separation of amylose and amylopectin of starch by an extraction sedimentation procedure. J. Polym. Sci. 28: 1 (1958).
30. MEDCALF, D. G., and GILLES, K. A. Wheat starches. I. Comparison of physicochemical properties. Cereal Chem. 42: 558 (1965).
31. KLASSEN, A. J., and HILL, R. D. Comparison of starch from triticale and its parental species. Cereal Chem. 48: 647 (1971).
32. SCHOCH, T. J., and MAYWALD, E. C. Preparation and properties of various legume starches. Cereal Chem. 45: 564 (1968).
33. KJOLBERG, O., and MANNERS, D. J. Studies on carbohydrate-metabolizing enzymes. 9. The action of isoamylase on amylose. Biochem. J. 86: 258 (1963).
34. BANKS, W., and GREENWOOD, C. T. The fine structure of amylose: The action of pullulanase as evidence of branching. Arch. Biochem. Biophys. 117: 674 (1968).

[Received February 17, 1976. Accepted May 25, 1976]