CHARACTERIZATION AND COMPARISON OF CEREAL STARCHES

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ABSTRACT

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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 amyllopectin 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 d.p. of approximately 11-25 and 52-60 were obtained from each amyllopectin. Average unit-chain lengths of the amyllopectins ranged from 15 for triticale to 26 for rye. The ratio of A-chains to B-chains in each amyllopectin, 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

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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 amyllopectin. 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 amyllopectin has recently been reviewed (9). The use of those
enzymes has led to the postulation of a revised structure for amyllopectin and
glycogen (10) in which the arrangement of A-chains and B-chains 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 amyllopectin
(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 amyllopectin components
that could be related to differences in functional properties. The ratios of A-
chains and B-chains in amyllopectins 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).

3Terminology:
1. A-chains are those chains in amyllopectin 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 dextrans are β-amylose-limit dextrans formed by treating amyllopectin with sweet potato β-amylase.
3. ϕ-Dextrans are muscle phosphorylase-limit dextrans formed by treating amyllopectin with rabbit muscle
phosphorylase a.
4. ϕ, β-Dextrans are ϕ-amylase-limit, phosphorylase-limit dextrans formed by treating ϕ-dextrans 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 amyllopectin 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 amyllopectin 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 mM 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 mM 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 (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 (\(c.I.\)) 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 \(\beta\)-Amylase Limit Dextrins and \(\phi\), \(\beta\)-Dextrins

\(\beta\)-Amylase-limit dextrins (\(\beta\)-limit dextrins) were prepared by treating
amylopectin (25 mg/ml in 100 mM acetate buffer, pH 4.8) with 380 units/ml
sweet potato \(\beta\)-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 (\(\phi\)-dextrins) were prepared by treating amylopectin (25 mg/ml in
80 mM sodium phosphate buffer, pH 6.8, containing 40 mM \(\beta\)-mercaptoethanol
with 4 units/ml of rabbit muscle phosphorylase a (Sigma Chemical Co., St.
Louis, Mo.) at 30°C for 24 hr (23,24). \(\phi\), \(\beta\)-Dextrins were prepared by treating \(\phi\)-
dextrin (20 mg/ml) with 380 units/ml of sweet potato \(\beta\)-amylase in 100 mM
sodium acetate buffer, pH 4.8, at 37°C for 24 hr (10,24).

Debranching with Pullulanase

Pullulanase from \textit{Aerobacter aerogenes} was prepared and purified as

<table>
<thead>
<tr>
<th>Source</th>
<th>Starch</th>
<th>Amylose</th>
<th>Amylopectin</th>
</tr>
</thead>
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<tr>
<td>Scout</td>
<td>4.62</td>
<td>19.88</td>
<td>0.81</td>
</tr>
<tr>
<td>Omar</td>
<td>4.39</td>
<td>19.56</td>
<td>0.77</td>
</tr>
<tr>
<td>Kalyan sona</td>
<td>4.36</td>
<td>19.34</td>
<td>0.82</td>
</tr>
<tr>
<td>\textit{T. dicoccum}</td>
<td>4.73</td>
<td>19.49</td>
<td>0.76</td>
</tr>
<tr>
<td>Soft wheat</td>
<td>4.84</td>
<td>19.67</td>
<td>0.78</td>
</tr>
<tr>
<td>Durum</td>
<td>4.61</td>
<td>19.23</td>
<td>0.69</td>
</tr>
<tr>
<td>Triticale</td>
<td>4.62</td>
<td>19.01</td>
<td>0.68</td>
</tr>
<tr>
<td>Rye</td>
<td>4.91</td>
<td>19.42</td>
<td>0.74</td>
</tr>
</tbody>
</table>
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 mM 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 isomylase 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 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 amyllopectin 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):

<table>
<thead>
<tr>
<th>Using φ, β-Dextrins</th>
<th>Reducing Power Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoamylase</td>
<td>B-chains</td>
</tr>
<tr>
<td>Pullulanase</td>
<td>B + A-chains</td>
</tr>
</tbody>
</table>

Difference: A-chains

<table>
<thead>
<tr>
<th>Using β-Limit Dextrins</th>
<th>Reducing Power Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoamylase</td>
<td>B + 1/2 A-chains</td>
</tr>
<tr>
<td>Pullulanase</td>
<td>B + A-chains</td>
</tr>
</tbody>
</table>

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 mM 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>
<thead>
<tr>
<th>Source</th>
<th>Birefringence End Point Temperature Range °C</th>
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</thead>
<tbody>
<tr>
<td>Scout</td>
<td>54.5°—60.0°—64.5°</td>
</tr>
<tr>
<td>Omar</td>
<td>54.0°—60.5°—62.0°</td>
</tr>
<tr>
<td>Kalyan sona</td>
<td>53.0°—58.5°—61.5°</td>
</tr>
<tr>
<td>T. dicoccum</td>
<td>52.0°—58.0°—63.0°</td>
</tr>
<tr>
<td>Soft wheat</td>
<td>53.0°—58.0°—63.0°</td>
</tr>
<tr>
<td>Durum</td>
<td>50.0°—55.0°—60.5°</td>
</tr>
<tr>
<td>Triticale</td>
<td>53.0°—56.5°—61.5°</td>
</tr>
<tr>
<td>Rye</td>
<td>50.0°—53.5°—58.0°</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>c.l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scout</td>
<td>19</td>
</tr>
<tr>
<td>Omar</td>
<td>18</td>
</tr>
<tr>
<td>Kalyan sona</td>
<td>17</td>
</tr>
<tr>
<td>T. dicoccum</td>
<td>19</td>
</tr>
<tr>
<td>Soft wheat</td>
<td>20</td>
</tr>
<tr>
<td>Durum</td>
<td>17</td>
</tr>
<tr>
<td>Triticale</td>
<td>15</td>
</tr>
<tr>
<td>Rye</td>
<td>26</td>
</tr>
</tbody>
</table>
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 amylpectin 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 mM 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 amylpectin 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 mM 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 amylase reportedly has an iodine affinity of 20.0% when determined potentiometrically (29), we used that value for the wheat starches we studied; amylase 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 amylase 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 d.p. of 52-60; the second peak, material of 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>
<thead>
<tr>
<th>Source</th>
<th>Ratio of A-Chains to B-Chains Using</th>
<th>β-Limit Dextrin</th>
<th>ϕ, β-Dextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scout</td>
<td></td>
<td>1.51</td>
<td>1.48</td>
</tr>
<tr>
<td>Omar</td>
<td></td>
<td>1.59</td>
<td>1.54</td>
</tr>
<tr>
<td>Kalyan sona</td>
<td></td>
<td>1.48</td>
<td>1.44</td>
</tr>
<tr>
<td>T. dicoccum</td>
<td></td>
<td>1.21</td>
<td>1.19</td>
</tr>
<tr>
<td>Soft wheat</td>
<td></td>
<td>1.73</td>
<td>1.64</td>
</tr>
<tr>
<td>Durum</td>
<td></td>
<td>1.89</td>
<td>1.78</td>
</tr>
<tr>
<td>Triticale</td>
<td></td>
<td>2.11</td>
<td>2.02</td>
</tr>
<tr>
<td>Rye</td>
<td></td>
<td>1.81</td>
<td>1.71</td>
</tr>
</tbody>
</table>
had a 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 d.p. of the components. The major peak with the lower d.p. may well represent the unit chains of amylpectin. It is not yet known whether the component with higher d.p. arises from the same polysaccharide as does the component with the lower d.p. (9). These results further confirm the correctness of the model of amylpectin 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 (c.l.) of the amylpectins—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 amylpectin had the longest c.l., 26, and triticale amylpectin the shortest c.l., 15. The wheat starches had a relative limited 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 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 amylpectins—determined enzymatically using both $\beta$-limit dextrans and $\phi$, $\beta$-
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 amyllopectins. 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 amyllopectin, having an A-chain to B-chain ratio of 2.1, apparently has a greater extent of multiple branching than do the other amyllopectins, particularly the amyllopectin from *T. dicoccum*. The hard wheat and club wheat amyllopectins had lower A-chain to B-chain ratios than did the soft wheat and durum amyllopectins. Thus, the fine structures of those amyllopectins 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 \( \beta \)-amylysis of the debranched \( \beta \)-limit dextrins and \( \phi, \beta \)-dextrins. Because total debranching would release all linear chains, the chains should be completely degraded to maltose by \( \beta \)-amylase. The degree of \( \beta \)-amylysis of the debranched polysaccharides (Table V) ranged from 98 to 101% for both dextrins, indicating that debranching was essentially complete.

**\( \beta \)-Amylysis Limits of Amyloses**

The iodine affinities for the amyloses (Table I) showed small differences, ranging from 19.0% for triticale amyllose to 19.9% for Scout amyllose. The values indicated that the amyloses were quite pure. The \( \beta \)-amylysis limits of the amyloses (Table VI) ranged from 77% for rye and club wheat (Omar) amyloses to 82% for durum wheat amyllose. All wheat amyloses, except durum amyllose, had \( \beta \)-amylysis 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 \( \beta \)-amylase may be attributed to a small proportion of \((1 \rightarrow 6)\)-\( \alpha \)-D-glucosidic linkages (branching) present in the amylose molecule (33,34).

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