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Note

Properties of hot-water-extractable amylose

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Amylose, defined as a (1→4)-α-D-glucan component in starch, is prepared conventionally by complete dispersion of starch in hot water followed by crystallisation with hydrophilic organic solvents[1-3]. However, these amylose preparations, even after repeated recrystallisations, include 27–70% of slightly branched molecules[4-8]. The latter molecules are differentiated from amylopectin in that (*a*) they have a small number of branches, (*b*) the average chain lengths, (c.l.) of their beta-limit dextrins are 60–200, which is much higher than those of amylopectins, (*c*) they form crystalline complexes with 1-butanol, (*d*) the iodine affinities of their beta-limit dextrins are similar to, or slightly less than, those of the parent amyloses, and (*e*) the molecular weights of their beta-limit dextrins are similar to those of the parent amyloses, which are much less than those of amylopectins. Thus, they have properties that are similar to those of amylose and have been termed[9] branched amylose or a third component of starch. Here, the linear and branched amylose are denoted, as l- and b-amylose, and together they constitute the amylose fraction. No effective method is yet available that will separate the l- and b-amylose from each other. However, pure l-amylose can be obtained[10-14] by the aqueous leaching of starch at slightly above the gelatinisation temperature. The application of this procedure to wheat and potato starches is now described.

Table I shows the structural properties of the amylose fractions extracted with hot water from wheat and potato starches. One recrystallisation appeared to be necessary to purify the wheat amylose fraction extracted at 70°. For wheat starch, the large molecules were extracted with increased temperatures in the range 60–80° and the yield was also increased from 2.1 to 8.1%. Their beta-amylolysis limits were in the range 83–87%, which suggested the presence of the small proportions of α-(1→6) linkages. The average chain length (c.l.) and the average number of chains (n.c.) were also increased from 230 to 290 and from 2.0 to 2.7, respectively, by increasing the temperature. It was expected that pure l-amylose would be extractable at 60°, but the resulting fraction contained one branch linkage on average. Thus, molecules that had shorter c.l. and fewer branches were extracted at lower temperatures, and had less chains than those (4.8) in the fraction[4] obtained by the complete dispersion method. Arbuckle and Greenwood[12] obtained amylose with a 98% beta-amylolysis limit by aqueous leaching at 70°, but the

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TABLE I

Properties of wheat and potato amyloses extracted at 60–80°

Temperature (°)	*Number of recrystal-lisations*	*Yield*[a] (%)	$\overline{D.p._n}$	$\overline{C.l.}$	$\overline{N.c.}$	*Beta-amylolysis limit* (%)	*Blue value*	λ_{max}[b] (*nm*)
Wheat								
60	1	2.1	4.70	230	2.0	87	1.32	640
70	0	4.8	500	220	2.3	86	1.33	645
70	1	4.5	580	250	2.3	88	1.35	645
70	2	4.2	560	270	2.1	88	1.37	645
80	1	8.1	790	290	2.7	83	1.39	645
Potato								
60	1	3.1	4360	850	5.1	89	1.46	665
80	1	7.2	6990	670	10.1	90	1.49	670

[a] From starch. [b] Iodine-stained solution.

amylose that was extracted even at 60° had a considerably lower beta-amylolysis value. The reason for this discrepancy might be due to differences in the experimental conditions.

The yields of fractions extracted at 60° and 70° from potato starch were 3.1 and 7.2%, respectively, which corresponded to 16 and 37%, respectively, of the total amylose fraction. These minor amylose fractions were not pure l-amylose and contained 5.1 and 10.1 chains per molecule, respectively. The $\overline{d.p._n}$ of these amylose fractions were as high as that of the amylose fraction prepared by the dispersion method[15]. The beta-amylolysis value (89%) of the sample obtained by leaching at 70° was much lower than those (96–100%) reported by previous workers for amyloses extracted at this temperature from potato[11] and other sources[14].

The branching was determined quantitatively on the basis of the $\overline{n.c.}$ value (equal to the number of branches + 1), and was estimated also from the beta-amylolysis limit. However, the beta-amylolysis limit is not directly proportional to the number of the branches because it is influenced by the location of the branches and the fine structure of the molecule. For example, the potato amylose fraction that had higher $\overline{c.l.}$ and $\overline{n.c.}$ values gave similar or slightly higher values for the beta-amylolysis limits than those of the wheat amylose fractions, suggesting that they differed in the fine structure of the b-amylose and the proportion of l- to b-amylose. A technique for the quantitative separation of the l- and b-amyloses would be of considerable value, but it seems to be difficult.

EXPERIMENTAL

Materials. — Wheat starch was prepared from wheat flour[16]. One potato starch was that (Kenebec) used in a previous study[15] and the other (Eniwa) was prepared by a

similar procedure. Beta-amylase was prepared[17] from sweet potato and was recrystallised from aqueous ammonium sulfate. Reagents were the highest grade available commercially.

Preparation of amylose. — An aqueous suspension of wheat or potato starch (20 g/100 mL) was poured slowly into 6.7mM phosphate buffer (pH 6.7, 1900 mL) maintained at a constant temperature with gentle stirring and under nitrogen. The extractions were continued for 1 (wheat) or 3 h (potato). Each suspension was centrifuged at 8000*g* for 10 min, and the amylose fraction was recovered from the supernatant solution by centrifugation at 8500*g* for 10 min after crystallisation by the addition of 1-butanol (190 mL) to the solution and cooling slowly to room temperature. The crystalline amylose was washed by suspending it in a small amount of 1-butanol-saturated water and centrifugation. The amylose was recrystallised from 1-butanol-saturated water (500 mL) by heating under nitrogen and cooling to room temperature. The product was dehydrated with ethanol, washed with ether, and dried over $CaCl_2$ under reduced pressure.

Analytical methods. — $\overline{D.p._n}$, $\overline{c.l.}$, and $\overline{n.c.}$ were determined by the assay of reducing and nonreducing residues[16], and other analytical procedures were carried out as described[18].

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