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Molecular structures of some wheat starches

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Molecular structures of amyloses and amylopectins from three varieties of Japanese wheat, Chihoku, Horoshiri, and Norin-61, and two wheat classes, Australian standard white (ASW) and Western white (WW), were characterized. WW amylose was the largest (number-average degree of polymerization $\overline{dp}_n$, 1570) and Chihoku amylose was the smallest ($\overline{dp}_n$ 830). ASW and Chihoku amyloses had higher amounts of branched molecules (~42% by mole) with lower average numbers of chains ($\overline{nc} \sim 13$), than those of others (~28% by mole, $\overline{nc} \sim 19$). The structural features of amylopectins by iodine affinity and chain-length distribution were also characterized by varieties and classes. The amylose contents of the starches were in the range of 21·7–27·4% for ASW and WW, respectively. A lower amylose content and a higher amount of the branched amylose molecule with a lower $\overline{nc}$ may produce better quality Japanese-type noodle.

INTRODUCTION

Starches from various plants show unique functional and physicochemical properties and may be distinguished from each other by morphological characteristics and microcrystalline structures. Starch is the main component of cereals, and its physicochemical properties appear to be important for functions of cereals. From this point of view, the properties, especially molecular structure, of rice (Takeda *et al.*, 1986, 1987*a*, 1989*a*, 1989*b*, 1993*a*; Juliano *et al.*, 1987; Hizukuri *et al.*, 1989) and corn (Takeda *et al.*, 1988, 1989*c*, 1993*b*; Takeda & Preiss, 1993) have been investigated and some varietal differences have been found. However, wheat starch and its amylose and amylopectin have not been well characterized (Atwell *et al.*, 1980; Medcalf and Gilles, 1965; Takeda *et al.*, 1987*b*), although some structural similarities of starches among some wheat varieties were reported (Lii & Lineback, 1977; Kobayashi *et al.*, 1986) and the properties of some wheat starches were also discussed in relation to their baking properties (D'Appolonia & Gilles, 1971; Kulp, 1973).

The quality of Japanese domestic wheat is inferior to that of Australian standard white (ASW) for the production and eating quality of Japanese-type noodles. This study attempts to relate the fine structures and some functions of three Japanese varieties of white wheats, ASW wheat and Western white (WW) wheat, as comparative standards, to their performance in noodles.

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MATERIALS AND METHODS

Starches were prepared as described previously (Hizukuri & Maehara, 1990) from the wheat flours of three Japanese varieties of wheat, Chihoku, Horoshiri, and Norin-61, which were cultivated in Hokkaido (the first two) and Gunma, and those of two foreign specimens of wheat, ASW (Australia) and WW (United States).

Sweet potato β-amylase was prepared by the method of Takeda & Hizukuri (1969) and further recrystallizated from aqueous ammonium sulfate. Crystalline *Pseudomonas* isoamylase was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama).

Fractionation of starch

Amylose and amylopectin were isolated by the methods of Lansky *et al.* (1949) with modifications (Takeda *et al.*, 1986). The heating process was performed under a

nitrogen atmosphere to prevent possible oxidation. The amyloses were purified by ultracentrifugation, followed by repeated recrystallizations from 10% aqueous 1-butanol.

β-limit dextrin of amylose (β-LD) and debranched amylopectin were prepared as described previously (Takeda *et al.*, 1987*b*; Hizukuri & Maehara, 1990). The purity of the amylose specimens was examined by gel-permeation chromatograph on a column with Toyopearl HW-75F (Takeda *et al.*, 1984).

Analytical methods

The iodine affinity (*ia*, I_2 g/100 g) was determined at 25°C by an automated amperometric titration (Takeda *et al.*, 1987*a*). The blue value and limiting viscosity number [η] (1 M KOH, 22·5°C) were determined as described previously (Suzuki *et al.*, 1981). The number-average degree of polymerization ($\overline{dp}_n$) of the amylose was determined by the modified Park–Johonson method (Hizukuri *et al.*, 1981). The addition of ferricyanide solution in the method was omitted erroneously in the original literature, but it was corrected elsewhere (Hizukuri *et al.*, 1983). The number-average chain length ($\overline{cl}_n$) of the amylose was determined by the rapid Smith-degradation method (Hizukuri *et al.*, 1981; Takeda *et al.*, 1984) coupled with fluorimetric enzyme assay of glycerol (Hizukuri *et al.*, 1981). The average number of chains per molecule ($\overline{nc}$) was calculated as $\overline{dp}_n/\overline{cl}_n$. The molar fractions (*MF*) of the branched and linear molecules were calculated using the following equations (Takeda *et al.*, 1987*b*):

$$MF_{\text{branched}} = (\overline{nc}_{\text{amylose}})/(\overline{nc}_{\beta\text{-limit dextrin}} - 1) \quad (1)$$

$$MF_{\text{linear}} = 1 - MF_{\text{branched}}. \quad (2)$$

The weight-average degree of polymerization ($\overline{dp}_w$) and degree of polymerization distribution of amylose were determined by gel-permeation HPLC on three tandem columns (Tosoh, TSKgel G6000PW, G4000PW, and G3000PW, 7·5 × 600 mm each) and monitored with a differential refractometer (R.i., Erma ERC-7512) and a low-angle laser-light-scattering photometer (Lalls, Tosoh LS-8) (Hizukuri & Takagi, 1984). Isoamylolysis of amylose was carried out under the conditions described previously (Hizukuri *et al.*, 1981). The $\overline{cl}_n$ of amylopectin was determined by the rapid Smith-degradation method (Hizukuri & Osaki, 1978) and by means of hydrolysis with isoamylase (Suzuki *et al.*, 1981). The weight-average chain length ($\overline{cl}_w$) and chain length distribution of the amylopectin were determined by HPLC using connected columns of Asahipak GS-320 (7·6 × 500 mm) × 2 (Asahi Chemical Industry Co., Ltd, Tokyo) and TSKgel G2000SW (7·5 × 600 mm) (Tosoh) with R.i. and Lalls (Tosoh LS-8000) as monitors (Hizukuri & Maehara, 1990). Carbohydrate and reducing terminal of debranched amylopectin were determined by the phenol–sulfuric acid method (Dubois *et al.*, 1956) and the Somogyi method (Somogyi, 1952) respectively. The latter used the calorimetric reagent the Nelson method (Nelson, 1944), but the heating time with the Somogyi reagent extended to 30 min (Hizukuri *et al.*, 1970). Phosphorus was determined as inorganic phosphorus (Itaya & Ui, 1966) after treatment with hot perchloric acid (Allen, 1940). Phosphorus attached to C-6 of the glucose residue was assayed as glucose-6-phosphate using glucose-6-phosphate dehydrogenase (Hizukuri *et al.*, 1970).

RESULTS

Structure of amylose

The purity of each amylose preparation was confirmed as free from amylopectin on HW-75F gel-chromatography (Takeda *et al.*, 1984) (data not shown). Each wheat amylose had unique properties as summarized in Table 1. The *ia* values (I_2 g/100 g) of the amyloses were similar (19·0–19·5) and slightly lower than those of corn (Takeda *et al.*, 1988) and rice (Takeda *et al.*, 1986, 1989*b*; Takeda & Preiss, 1993) amyloses (20·0–21·7), but similar to that of amylomaize amylose (19·4) (Takeda *et al.*, 1989*c*). The amyloses contained a very small amount of organic phosphorus. WW amylose showed the highest [η], $\overline{dp}_n$, and $\overline{dp}_w$ values, which were approximately twice the values of those of Chihoku amylose. To clarify these differences, the degree of polymerization distribution of the amyloses was examined by gel-permeation HPLC (Fig. 1). WW amylose includes two components with *dp* 5570 and 3180, judging from the flat tops of the original and isoamylolyzate curves. Chihoku amylose may also contain two components but their peak *dp* values (2020, 770) were much lower. The other amyloses showed a single sharp peak top with similar *dp* values (2530–2760). Thus WW amylose has the largest molecular size among these amyloses. Norin-61 and WW amyloses showed the widest apparent degree of polymerization distributions.

The amyloses were hydrolyzed (79–85%) by β-amylase, similar to those from rice and corn, suggesting they contained branched molecules. The $\overline{cl}_n$ values were in the range of 135–255 for Chihoku and WW, respectively. The $\overline{nc}$ ranged from 5·5 (Horoshiri) to 6·5 (Norin-61). The isoamylolysis of the amyloses produced a small amount of short chain fraction (SCF) (Fig. 1). Horoshiri amylose contained the highest amount of SCF (7·6%), which was almost twice the amounts for ASW (3·2%) and Chihoku (3·9%) (Table 1 and Fig. 1).

The branched nature of amylose was analyzed based on the structure of the β-LDs (Table 2). The *ia* values ranged from 15·3 (Chihoku) to 18·1 (Horoshiri), and

Table 1. Properties of wheat amyloses

Property	ASW	Chihoku	Horoshiri	Norin-61	WW
Iodine affinity (*ia*) (g/100 g)	19·5	19·5	19·5	19·0	19·0
Blue value	1·24	1·13	1·25	1·25	1·31
λ max (nm)	648	636	640	648	647
Limiting viscosity number ([η]), ml/g)	183	118	181	185	237
Number-average *dp* ($\overline{dp}_n$)	1180	830	1080	980	1570
Weight-average *dp* ($\overline{dp}_w$)	3480	2360	3660	4880	5450
$\overline{dp}_w/\overline{dp}_n$	2·95	2·84	3·39	4·98	3·47
Apparent *dp* distribution[a]	360–15 600	290–10 500	570–15 000	500–25 200	600–25 200
Short chain fraction (SCF) (%)	3·2	3·9	7·6	5·4	5·0
Number-average *cl* ($\overline{cl}_n$)	200	135	195	150	255
Average number of chains ($\overline{nc}$)	5·8	6·2	5·5	6·5	6·2
β-Amylolysis limit (β-AL) (%)	81	79	83	83	85
Organic phosphorus (ppm)	<1	3	<1	1	2

[a] The *dp* values of the subfractions (10% by weight) having the lowest and highest molecular weights.

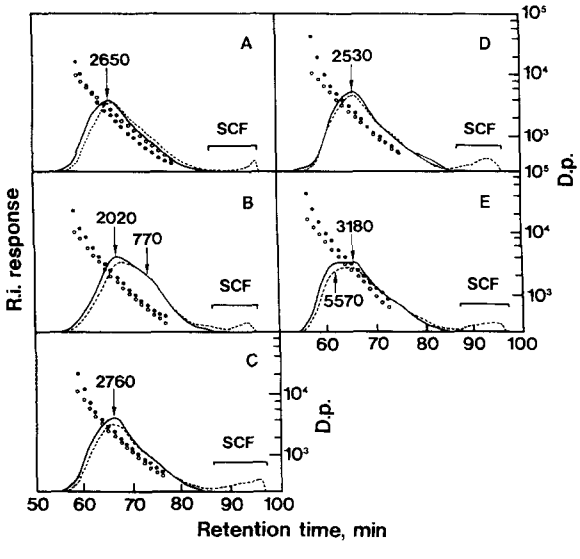

Fig. 1. Gel-permeation HPLC chromatograms of ASW (A), Chihoku (B), Horoshiri (C), Norin-61 (D), and WW (E) amyloses, and their isoamylolyzates on the connected columns of TSKgel G6000PW, G4000PW, and G3000PW. The conditions were as previously described (Hizukuri & Takagi, 1984). —— and ------, response of differential refractometer (R.i.) for the amyloses and isoamylolyzates, respectively; ● and ○, *dp* values of the amyloses and isoamylolyzates, respectively; SCF, short chain fraction; numbers with arrows are *dp* values of the peaks or shoulders.

were slightly lower than those of their parent amyloses. However, the values were much higher than those of wheat amylopectins (0·66–1·12) as described later, implying that the branched molecule was clearly distinguishable from amylopectins and proved that the branched nature was not due to contamination by amylopectin. The $\overline{dp}_n$ and $\overline{dp}_w$ of the β-LDs considerably differed among the varieties and standard specimens of wheat. WW β-LD had the largest $\overline{dp}_n$ and $\overline{dp}_w$ whereas Chihoku β-LD the smallest, similar to their parent amyloses. The $\overline{nc}$ values of ASW and Chihoku β-LDs, i.e. of branched molecules, were lowest (about 13), while those of Norin-61 and WW β-LDs were highest (about 20), and Horoshiri β-LD had a medium $\overline{nc}$ (16·5). Amounts of the branched molecule were distinctive by the varieties and standard specimens (Table 3), as follows. ASW and Chihoku amyloses contained much higher amounts of the branched molecule (0·40, 0·44 by molar fraction) than the others (0·26–0·29).

Structure of amylopectin

Table 4 summarizes the properties of the wheat amylopectins. The *ia* values of the amylopectins were in the range of 0·66–1·12 for WW and Chihoku, respectively. These values were in a similar range to that of rice (Hizukuri *et al.*, 1993) and normal corn (Takeda *et al.*, 1988; Takeda & Preiss, 1993). WW amylopectin had the lowest *ia* value, being almost half those for Chihoku and Horoshiri. Their [η] and $\overline{dp}_n$ values were between 116 (Chihoku) and 148 (Norin-61) ml/g, and between 5000 (Chihoku) and 9400 (WW), respectively, indicating that the wheat amylopectins were characteristic in molecular size.

To investigate the chain-length distribution, debranched amylopectins with isomylase were analyzed by gel-permeation HPLC, showing tetramodal distributions, and were separated into fractions of long chain (LC), B_3–B_1 and A, in order of elution (Hizukuri, 1986) (Fig. 3), and some differences in carbohydrate amounts and $\overline{cl}_w$ of the fractions were found (Table 5). The fraction LC was confirmed to be a real component, because each fraction separated by gel-permeation chromatography gave a similar blue value to that of amylopectin (0·115) as shown in Fig. 4, and the debranched amylopectin with one-tenth the amount of isoamylase showed the same $\overline{dp}_n$ value as that obtained under normal conditions. In addition, no fraction LC was detected on the debranched material after precipitation with 1-butanol as reported previously (Hizukuri, 1986). The $\overline{cl}_w$ of the fraction B_3 of Horoshiri and WW was 91, being longer by about 25% than those of ASW (68) and Chihoku (70). The amounts of the fraction LC were in the range

Table 2. Properties of β-limit dextrins from wheat amyloses

Property	ASW	Chihoku	Horoshiri	Norin-61	WW
ia (g/100 g)	16·9	15·3	18·1	16·8	17·2
Blue value	1·25	1·14	1·25	1·21	1·31
λ max (nm)	640	630	645	640	645
$\overline{dp}_n$	930	700	1050	1010	1430
$\overline{dp}_w$	3080	1670	3720	4120	5880
$\overline{dp}_w/\overline{dp}_w$ (amylose)	0·89	0·71	1·02	0·84	1·08
$\overline{dp}_w/\overline{dp}_n$	3·31	2·39	3·54	4·08	4·11
Apparent *dp* distribution	490–13 800	100–7 300	410–15 700	520–20 600	29 000
$\overline{cl}_n$	71	50	67	50	69
$\overline{nc}$	13·0	12·9	16·5	20·2	20·7

Table 3. Molar fractions of linear and branched molecules in wheat amyloses

Molecule	ASW	Chihoku	Horoshiri	Norin-61	WW
Branched	0·40	0·44	0·29	0·29	0·26
Linear	0·60	0·56	0·71	0·71	0·74

Table 4. Properties of wheat amylopectins

Property	ASW	Chihoku	Horoshiri	Norin-61	WW
ia (g/100 g)	0·89	1·12	1·06	0·93	0·66
Blue value	0·098	0·115	0·109	0·099	0·078
λ max (nm)	552	560	556	551	547
[η] (ml/g)	145	116	130	148	145
$\overline{dp}_n$	6200	5000	5200	6700	9400
$\overline{cl}_n$					
Smith degradation	20	20	21	20	21
Isoamylolysis	20	20	20	19	20
β-AL (%)	57	58	58	59	56
Phosphorus (ppm)					
Organic	9	13	20	13	10
C-6[a]	<1	1	<1	<1	<1

[a] Phosphorus at C-6 of the glucosyl residue.

of 4–6%. Chihoku and Horoshiri amylopectins contained 25–50% higher amounts of the fraction LC than others. The amount of fraction A of Chihoku was lower (40%) than those of others (43–44%).

The amylopectins contained 9–20 ppm of organic phosphorus (Table 4). A very small amount (<1 ppm) of phosphorus was found to be located in C-6 of the glucose residue and the remainder might be attached to C-3 of the glucose residue (Tabata & Hizukuri, 1971).

Amylose content

The amylose contents of the wheat starches were calculated from the *ia* values of starch, amylose, and amylopectin (Table 6). The values were between 21·7% (ASW) and 27·4% (WW), and the highest content (WW) was about 1·25 times that of the lowest (ASW). These contents were lower by 1–4% than apparent contents conventionally calculated without consideration of amylopectin *ia* values.

DISCUSSION

Some wheat starches showed some differences from rice and corn starches in molecular structures. Chihoku and WW amyloses were smaller and larger molecules than rice and corn amyloses, respectively, but the other wheat amyloses were similar. The $\overline{nc}$ of the wheat amyloses were 1·5–2·5 times higher than those of corn (2·4–3·4) (Takeda *et al.*, 1988; Takeda & Preiss, 1993) and rice (2·3–4·2) (Hizukuri *et al.*, 1989), and slightly higher than that of a wheat amylose (4.8) reported previously (Takeda *et al.*, 1987*b*). The *ia* values of Chihoku and Horoshiri amylopectins were similar to those for corn (1·14) (Takeda *et al.*, 1988) and higher than for japonica rice (0·39–0·87). Those of the other wheat amylopectins coincided with those of indica rice amylopectins having an intermediate *ia* value (0·63–9·86, IR48 and IR64) (Takeda *et al.*, 1989*b*). The wheat amylopectins contained a similar amount of organic phosphorus (9–20 ppm) to rice and corn amylopectins (5–29 ppm), but

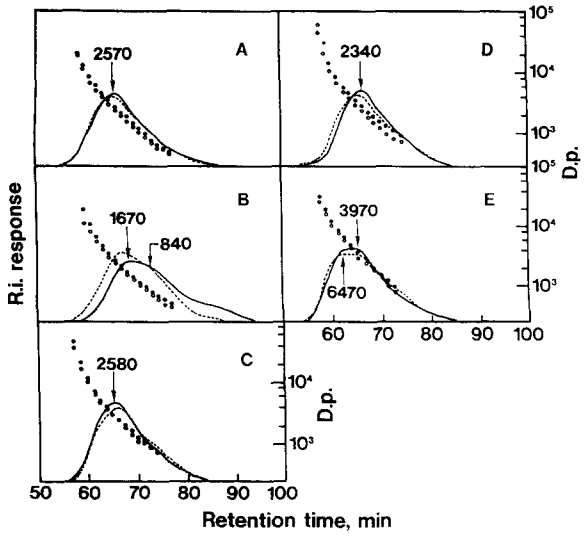

Fig. 2. Gel-permeation HPLC chromatograms of β-LDs of amyloses from ASW (A), Chihoku (B), Horoshiri (C), Norin-61 (D), and WW (E) on the connected columns of TSKgel G6000PW, G4000PW, and G3000PW. The conditions were as previously described (Hizukuri & Takagi, 1984). —— and ●, response of R.i. and *dp* of the β-LDs, respectively; numbers with arrows are *dp* values of the peaks or shoulders; ------, and ○, response of R.i. and *dp* of their parent amyloses, respectively (from Fig. 1 for comparison).

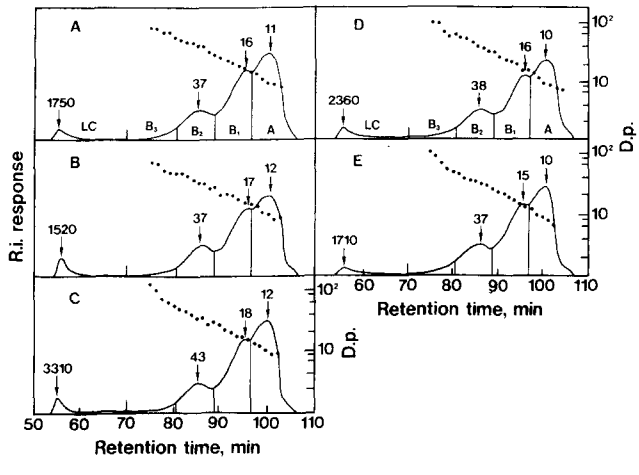

Fig. 3. Gel-permeation chromatograms of isoamylase-debranched amylopectins of ASW (A), Chihoku (B), Horoshiri (C), Norin-61 (D), and WW (E). The conditions were as previously described (Hizukuri & Maehara, 1990). ——, response of R.i.; ●, *dp*; numbers with arrows are *dp* values; arrows are *dp* values of the peaks.

most of the phosphorus was not attached to C-6 of the glucosyl residue, while phosphorus of corn and rice amylopectins was linked mostly to C-6.

For Japanese-type noodle making, ASW is generally superior in both processing performance and eating quality to Japanese wheats, among which Chihoku is best, Norin-61 is good, and Horoshiri is unsuitable. ASW flour showed a lower pasting temperature (Nagao *et al.*, 1977). Starch from various wheats showed a

Table 5. Carbohydrate amounts and weight-average *cl* ($\overline{cl}_w$) of the fractions of isoamylase-debranched amylopectins

	LC	B_3	B_2	B_1	A	Whole
$\overline{cl}_w$						
ASW	**1600**	68	41	21	11	85
Chihoku	**1100**	70	43	23	13	93
Horoshiri	**1700**	91	43	23	12	112
Norin-61	**1800**	79	41	20	10	94
WW	**1200**	91	43	22	10	72
Carbohydrate (% of total)						
ASW	4	4	16	33	43	100
Chihoku	6	4	18	32	40	100
Horoshiri	5	4	16	32	43	100
Norin-61	4	4	17	31	44	100
WW	4	4	16	33	43	100

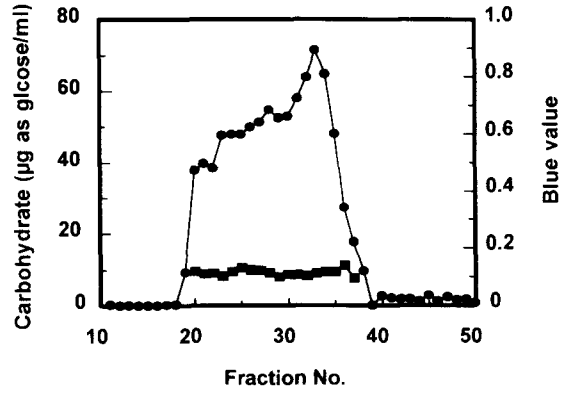

Fig. 4. Gel-permeation chromatogram on Toyopearl HW-75F (26 × 1000 mm) of Chihoku amylopectin. The conditions were as previously described (Takeda *et al.*, 1987*a*). ●, carbohydrate; ■, blue value.

different pasting temperature (Oda *et al.*, 1980; Endo *et al.*, 1988; Ito *et al.*, 1991), ASW's pasting temperature being lower than that of Norin-61 (Oda *et al.*, 1991). These observations may suggest that noodle quality depends on the physicochemical properties of starch, as well as protein content and its properties (Nagao, 1982). A low amylose content appears to be one of quality determinants, as suggested previously (Oda *et al.*, 1991), since ASW and Chihoku had a lower amylose content. ASW and Chihoku amyloses had a lower amount of SCF and comprised a higher amount of branched molecules with a lower $\overline{nc}$ value. Both the amylopectins had lower $\overline{cl}_w$ values (68–70) of the fraction B_3 than the others (79–90), while their amounts (4%) were the same (Table 5). These structural characteristics of amylose, especially, as well as amylopectin, may determine noodle quality. It is of interest that wheat starches have been suggested to have superior quality for bread making compared to those of other plant sources (Hoseney *et al.*, 1971). Therefore, it may be useful to pay attention to the effect of starch on the quality of bread and other wheat products.

Table 6. Iodine of wheat starches[a] and their amylose contents

Starch	*ia*	Amylose content
	g/100 g	%
ASW	4·86	21·7[b] (24·3)[c]
Chihoku	5·27	22·6 (26.4)
Horoshiri	5·61	24·7 (28·1)
Norin-61	5·27	23·7 (26·4)
WW	5·69	27·4 (28·5)

[a] Defatted by repeated dissolution in hot dimethyl sulfoxide and precipitation with ethanol.
[b] Calculated with the following equation: $(ia_{starch} - ia_{amylopectin})/(ia_{amylose} - ia_{amylopectin})$.
[c] Apparent content, calculated with the following equation: $(ia_{starch}/ia_{amylose=20})$.

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