Structural Properties of Amylopectins Corresponding to Physical Properties of Rice Starches Appropriate for Sake

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Abstract: Starches from a variety of Sake-rice (Yamadanishiki, Gohyakumangoku, Miyamanishiki, Kitamishiki and Wakamizu) which are suitable for Sake brewing were investigated by comparing them with Koshihikari starch to recognize their structural properties of amylopectins. The partial hydrolysate (about 15% hydrolysis) of starch with isoamylase was fractionated into three fractions (fr.1, fr.2 and fr.3) by gel permeation chromatography (GPC) with a Toyopearl HW-50S, and then the short-amylose chains derived mainly from the outer portion of the amylopectin collected in the fr.3 were analyzed by a HPAEC-system equipped with a pulsed amperometric detector (HPAEC-PAD). From comparison of the chain-length distributions between Sake-rice starches and that of Koshihikari, it was found that Sake-rice amylopectins were plentiful in longish chains around DP 13–23 and scarce in shortish chains around DP 6–12, suggesting a structural property susceptible to retrogradation. The pasting properties of Sake-rice starches analyzed by Rapid Visco Analyzer (RVA) showed higher pasting temperature (PT), higher setback (SB), and lower breakdown (BD) than Koshihikari starch, suggesting the outer portions of amylopectin had abundant longish debranched short side-chains. These results suggested that rice starches with amylopectins susceptible to retrogradation are adequate for Sake-rice.

Key words: Sake-rice, amylopectin, chain-length distribution, physical property, RVA, GPC, HPAEC-PAD

Physical properties of starch are important for the qualities of products based on starch and there are many studies on the relationships between information about the fine structures and physical analytical data.^{1–6} Rice grain, however, is a mixture of amylose, amylopectin, protein, lipid, mineral and so on, and it is not easy to understand their effects on the physical properties. Normal starches consist of amylopectin as the main component, generally 75–85%, and it could be suggested that the differences among fine structures of amylopectins reflect the physical properties of starches, especially starches of the same cultivars. So it is useful to obtain comprehensible analytical data for amylopectin.

Amylopectin is a highly branched and gigantic homopolysaccharide and complete elucidation of its fine structure is impossible at present. From these reasons, a partial hydrolysate of amylopectin with isoamylase has been studied in our laboratory.^{7–11} The rice starch of Reiho showed a tendency to retrograde more quickly than that of Koshihikari evaluated as good tasting, and the comparative study of Reiho and Koshihikari amylopectins conducted by the analysis method led a suggestion that longish chains were plentiful in the outer portion of Reiho amylopectin and shortish chains were plentiful in outer portion of that of Koshihikari.¹⁰ A convenient method has been developed for getting directly this information from starch without separation of amylopectin.⁷

The structural analysis for recognizing physical properties of starch should be verified through more examples. The typical Japanese brew, Sake, is generally produced

from Yamadanishiki and Gohyakumangoku which are well known as rice suitable for Sake brewing. Their physical properties are different from those of ordinary cooked rice.

In the present study, the pasting properties of some typical Sake-rice starches and the distributions of short-amylose chains in the outer portion of their amylopectins were studied by comparison with Koshihikari starch to inspect the correlation between the amylopectin fine structure and the physical property of rice starch.

MATERIALS AND METHODS

Materials. All rice cultivars used in this study were obtained from Food Research Center, Aichi Industrial Technology Institute, Japan, and were cultivated in the year 2000 in Japan.

Preparation of starches. Starch was isolated from rice grains by using a cold 0.2% sodium hydroxide solution according to the alkali method¹² with modification. The precipitate was washed with distilled water till the pH decreased to 7.0 and the protein could not be determined by the Bradford method.¹³ After being rinsed with ethanol, the starch was dried in the air, and then passed through a 100-mesh sieve.

Preparation of defatted starches. Starch (2 g) was suspended in dimethyl sulfoxide (40 mL), dissolved by shaking at 37°C for overnight, poured gently in 100 mL methanol and left to stand overnight. The precipitate collected on a glass-filter 3G-3 was rinsed successively with methanol and ether, and then dried in the air. The starch powder passed through a 100-mesh sieve was used.

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Preparation of 0.2% starch solution and partial hydrolysate by isoamylase. A 0.2% defatted starch solution (15 mL) was mixed with 15 mU of *Pseudomonas* isoamylase (Hayashibara Biochemical Laboratories, Japan) and shaken at 50 rpm at 30°C.⁷⁾ One isoamylase unit is defined as the amount of enzyme required to produce 1 μ mol of reducing sugar per min from waxy maize starch. In order to measure a time course of the debranching reaction, partial hydrolysates (2 mL) taken out continuously from the test tube at appropriate reaction times (0.5, 1, 1.5, 2, 2.5 and 3 h) were heated in a boiling water bath for 5 min to inactivate the enzyme. The reducing power was analyzed by a modified Park-Johnson method¹⁴⁾ and the degree of hydrolysis (%) was calculated by dividing the value of the reducing power of the partial hydrolysate with that of the complete hydrolysate. A complete debranching reaction was conducted with ten isoamylase units (10 mU/mL) and shaken for 3 days. A time course was prepared from the various partial hydrolysates in each rice starch and used to estimate an appropriate time for preparation of 15% partial hydrolysate, according to the previous method.⁷⁾

Iodine affinity and amylose content. Iodine affinities of defatted starches were determined according to the method described by Kasemsuwan *et al.*¹⁵⁾ Apparent amylose contents were calculated by dividing the iodine affinity of defatted starch by 20%.¹⁶⁾ Each analysis was replicated two times at least.

Gel permeation chromatography (GPC) of partial and complete hydrolysates. Ten milliliter of the hydrolysate was applied on a column (2.6 cm ID $\times$ 100 cm) of Toyopearl HW-50S (Tosoh Co., Ltd., Japan), and eluted with 50 mM NaCl containing 0.01% of NaN₃ by using a peristaltic pump on the flow rate of 40 mL/h. Carbohydrate in each fraction (5 mL) was estimated by the phenol-H₂SO₄ method.¹⁷⁾ The fractions eluted were classified into three fractions (fr.1, fr.2 and fr.3) and the fr.3 sample was concentrated by a rotary evaporator and lyophilized.

Chain-length distribution. Chain-length distribution was determined by HPAEC-PAD according to the method described previously.⁷⁾

Starch pasting properties. The pasting properties of starches sieved with 100-mesh were measured with Rapid Visco Analyzer (RVA, model RVA-3D, Newport Scientific, Australia). Moisture content was determined by drying at 110°C for 40 min to ensure slurry concentrations of 6, 8, 10 and 12% on dry weight basis. Total slurry weight was 30 g. A starch suspension was held at 30°C for 6 min, and subsequently heated to 95°C at a rate of 13°C/min, held at 95°C for 6.5 min, cooled down to 30°C at a rate of 10.8°C/min, and finally held at 30°C for 9 min. The suspension was stirred at 160 rpm throughout the experiment. Each analysis took 32.5 min.^{18,19)}

RESULTS AND DISCUSSION

Iodine affinity and amylose content.

Apparent amylose contents of the defatted starches varied from 12.9% (Kitanishiki) to 18.1% (Miyamanishiki) as shown in Table 1. Yamadanishiki and Gohyakumangoku showed the same content (16.7%). There were no

Table 1. Iodine affinities and amylose contents of rice starches.

Starch	Iodine affinity IA (g/100g)	Apparent amylose content (%) [*]
Yamadanishiki	3.35	16.7
Gohyakumangoku	3.34	16.7
Miyamanishiki	3.62	18.1
Kitanishiki	2.58	12.9
Wakamizu	2.86	14.3
Koshihikari	2.79	13.9

^{*}Calculated by (Iodine affinity IA/20) $\times$ 100.

Table 2. Composition of each fraction of complete hydrolysates of rice starches by isoamylase eluted on GPC Toyopearl HW-50S.

Starch	Total saccharide ratio (%)		
	Amylose	Amylopectin	
	fr.1	fr.2	fr.3
Yamadanishiki	16.5	22.1	61.4
Gohyakumangoku	16.9	23.4	59.7
Miyamanishiki	17.3	24.5	58.3
Kitanishiki	13.1	23.0	64.0
Wakamizu	14.9	28.7	56.5
Koshihikari	14.0	22.9	63.1

obvious differences between Koshihikari and Kitanishiki and Wakamizu in amylose contents.

GPC of partial and complete hydrolysates on Toyopearl HW-50S.

GPC profiles of the partial and complete hydrolysates of starches are shown in Fig.1. The fractions eluted were classified into three fractions (fr.1, fr.2 and fr.3) by referring elution pattern of completely debranched rice starch in each case, and the data for the three fractions are shown in Table 2. An approximate content of amylose was also estimated from the fr.1 and it was found that the results were similar to the apparent amylose contents determined by the iodine affinity method. On the partial hydrolysate, halfway hydrolysate of amylopectin was eluted in fr.1 and the debranched short side-chains in the amylopectin released were accumulated in fr.3. The debranched short side-chains in amylopectin could be considered to be very minor in the partial hydrolysate⁷⁾ and almost all chains eluted in the lower molecular side at fr.3 could be speculated to be chains released from the outer layer of the amylopectin.

Chain-length distributions of the fr.3.

Chain-length distributions of fr.3 in the GPC of partial hydrolysates were analyzed by HPAEC-PAD and those of Yamadanishiki, Gohyakumangoku, Miyamanishiki, Kitanishiki and Wakamizu were compared with that of Koshihikari (Fig.2). From the results, it could be estimated that amylopectins of Sake-rice starches have characteristic structures with a large amount of longish chains (DP 13–23) and a small amount of shortish chains (DP 6–12).

Starch pasting properties.

As shown in Fig.3, RVA viscograms obtained for rice defatted starch at different slurry concentrations indicated

Fig. 1. Gel permeation chromatography (GPC) of partial (●) and complete hydrolysates (○) of rice starches with isoamylase on the column of Toyopearl HW-50S.

The three fractions (fr.1, fr.2 and fr.3) were divided by considering the three peaks on the elution pattern of the complete hydrolysate.

Fig. 2. Differences of debranched short side-chains in amylopectin between fr.3 of Sake-rice starches and fr.3 of Koshihikari starch.

Fig. 3. Rapid viscogram patterns of Yamadanishiki starch at various concentrations.

P, peak viscosity (maximum paste viscosity); H, hot paste viscosity (minimum paste viscosity); C, cold paste viscosity (final viscosity).

Fig. 4. Viscogram parameters of Sake-rice starches and Koshihikari starch at various concentrations.

Symbols P, H and C are explained in Fig. 3.

a variety of patterns. When the slurry was heated, the granules began to swell and paste at pasting temperature (PT, the temperature when the viscosity reached one-twentieth of the difference between the initial viscosity and the maximum viscosity). The viscosity increased and reached the peak viscosity (P, maximum paste viscosity achieved in the heating stage of the profile) until the

Table 3. Pasting viscosity parameters of rice starches at different concentrations.

Starch	PT ^{*1} (°C)				P ^{*2} (RVU)				H ^{*3} (RVU)			
	6%	8%	10%	12%	6%	8%	10%	12%	6%	8%	10%	12%
Yamadanishiki	86.3	72.4	71.0	69.7	60	157	331	635	38	73	117	157
Gohyakumangoku	89.5	75.1	73.3	72.1	61	162	345	646	39	83	130	179
Miyamanishiki	90.6	73.4	71.6	69.8	53	148	322	599	33	76	135	177
Kitanishiki	84.6	74.3	66.3	65.5	70	177	352	663	45	91	127	176
Wakamizu	82.9	75.2	73.3	72.5	81	201	408	740	43	86	119	169
Koshihikari	68.9	68.0	66.3	65.5	80	193	402	737	43	79	118	164

Starch	C ^{*4} (RVU)				BD ^{*5} (RVU)				SB ^{*6} (RVU)			
	6%	8%	10%	12%	6%	8%	10%	12%	6%	8%	10%	12%
Yamadanishiki	105	196	311	421	23	84	213	479	67	124	193	265
Gohyakumangoku	109	216	332	457	22	79	215	467	70	132	202	279
Miyamanishiki	115	222	369	474	20	72	187	423	82	146	234	297
Kitanishiki	109	205	317	430	25	86	226	488	64	115	190	312
Wakamizu	111	202	307	386	38	115	289	571	67	116	188	217
Koshihikari	113	199	308	379	37	114	284	573	70	120	190	215

Slurry concentration was adjusted by dry weight basis of starch. ^{*1} Pasting temperature (PT), ^{*2} peak viscosity (P), ^{*3} hot paste viscosity (H), ^{*4} cold paste viscosity (C), ^{*5} breakdown (BD), P-H, ^{*6} setback (SB), C-H.

Table 4. Pasting viscosity parameters* of rice starches at 200 RVU of peak viscosity.

Starch	Slurry (%)	H (RVU)	C (RVU)	BD (RVU)	SB (RVU)
Yamadanishiki	8.6	88	235	112	147
Gohyakumangoku	8.5	96	250	104	154
Miyamanishiki	8.7	99	279	101	180
Kitanishiki	8.3	95	229	105	134
Wakamizu	8.0	83	204	117	121
Koshihikari	8.0	85	206	119	124

Symbols H, C, BD and SB are explained in Table 3. *All parameters were calculated at 200 RVU of peak viscosity by using the figures shown in Fig. 4.

swollen granules burst, and then decreased quickly to the base level of hot paste viscosity (H, minimum paste viscosity achieved after holding at the maximum temperature). When the gelatinized starch was cooled with tap water, the viscosity increased again through retrogradation. C is the cold paste viscosity (or final viscosity, the viscosity at the end of run). P-H is the breakdown (BD), and C-H is the setback (SB). The PT, P, H, C, BD and SB for starches tested at 6, 8, 10 and 12% slurries are summarized in Table 3. These data seem to show tendencies in the physicochemical properties of Sake-rice starch. Many PT, BD and SB values of Sake-rice starches were higher, lower and higher than that of Koshihikari, respectively. As the SB values could be speculated to be related with retrogradation, the higher SB values of Sake-rice starches at high concentrations led to the similar speculation for Sake-rice amylopectins in HPAEC-PAD. However, they had somewhat complicated and incoherent contents indicated with shaded boxes in Table 3.

To understand more clearly the correlation between the pasting property and amylopectin fine structure, the viscosity parameters of P, H and C were plotted against the slurry concentration according to a report by K.R. Bhat-tacharya *et al.*²⁰⁾ and V. Singh *et al.*⁴⁾ Using the curves shown in Fig. 4, the values of slurry concentration, H, C,

BD and SB were calculated at a peak viscosity of 200 RVU and summarized in Table 4. It was found that the BD of Wakamizu was almost no different from that of Koshihikari, and those of other Sake-rice starches were lower than that of Koshihikari. According to the results of Xian-Zhong Han *et al.*,²⁾ the proportion of long chains of amylopectin was negatively correlated and the proportion of short chains of amylopectin was positive correlated with BD. The BD value of Sake-rice agreed with that. Miyamanishiki, Gohyakumangoku and Yamadanishiki showed clearly higher values for SB, and Kitanishiki, Wakamizu and Koshihikari showed lower values for SB. The SB value had a positive correlation with amylose content of starch, as discussed,²¹⁾ but it is also important to consider the characteristics of amylopectin structure. It could be understood that the typical Sake-rice starches have unique amylopectins with plentiful longish chains and scarce shortish chains.

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清酒に適した米澱粉の物性に対応する アミロペクチンの構造特性

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酒米に使用されている山田錦, 五百万石, 美山錦, 北錦, 若水の澱粉について, アミロペクチンの構造特性を理解するため, コシヒカリと比較した。澱粉溶液に直接イソアミラーゼを作用させて約 15% 部分加水分解物を調製し, Toyopearl HW-50S によるゲルクロマトグラフィーで 3 区分 (fr.1, fr.2, fr.3) に分けた。fr.3 に分画されたアミロペクチンの外層から主に切り出された短鎖アミロースを HPAEC-PAD で分析した。酒米とコシヒカリの鎖長分布を比較したところ, 酒米のアミロペクチンは DP 13-23 付近の長めの鎖長が多く, DP 6-12 付近の短めの鎖長が少なく, 老化しやすい構造特性であると推測した。次に RVA を使用し酒米の物性特性を調べた結果, 酒米は高い糊化開始温度, 高いセットバック値, 低いブレイクダウン値を示し, アミロペクチンの外層部には長めの短鎖アミロースが多く存在すると推察された。これらの結果から, 老化しやすいアミロペクチン構造を有した米澱粉が酒米に適していると考えられた。