Structural and Retrogradation Properties of Rice Endosperm Starch Affect Enzyme Digestibility of Steamed Milled-Rice Grains Used in Sake Production

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ABSTRACT

Structural and physicochemical characteristics of endosperm starch from milled rice grains of seven Japanese cultivars used in sake production were examined. Amylose content was 15.2–20.2%, number-average degree of polymerization (DP_n) of amylose was 900–1,400, and the ratio of short-to-long chain amylopectin was 2.7–3.5, respectively. The degree of retrogradation of purified starch stored for seven days at 4°C after gelatinization was 20–31%. The degree of retrogradation correlated negatively with the ratio of short-to-long chain amylopectin. The effect of holding time after steaming on enzyme digestibility and starch retrogradation of steamed rice grains was investigated. The longer the holding time after steaming, the greater the extent of retrogradation, and the less the degree of enzymatic digestibility. The decreased rate of enzyme digestibility correlated with amylopectin chain length distribution. Samples with short-chain amylopectin exhibited a slow decrease in enzyme digestibility. It was determined that the structure and retrogradation properties of endosperm starch in Japanese rice cultivars affect the decreasing rate of enzyme digestibility of the steamed, milled rice grains.

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Sake is a traditional alcoholic beverage in Japan made principally from rice and water. Enzyme digestibility of the steamed rice grains is a critical property in sake production because the rate of the alcohol fermentation is dependent on the digestion step. It is important to elucidate the factors influencing enzyme digestibility, in part to aid in the development of new cultivars, and in the evaluation of rice quality for sake production. Among the many factors that affect enzymatic digestion of the steamed rice grains, recent research has been directed toward starch itself, the largest component of rice grains (Wakai et al 1997; Mizuma et al 2003; Aramaki et al 2004). Wakai et al (1997) and Mizuma et al (2003) reported that the amylose content of rice grains correlated negatively with enzyme digestibility, while Aramaki et al (2004) reported a negative correlation between the content of amylopectin long chains (DP 35–41) and enzyme digestibility. In spite of these previous studies, the relationship between starch properties and enzyme digestibility of steamed rice for sake production remains poorly understood.

Among the many short-grain rice cultivars harvested in Japan, two types of cultivars, sake-type and cooking-type, are commonly used in sake production. Although cooking-type rice cultivars are mainly used for staple foods, some of them are preferred for use in sake. The molecular structure of the endosoperm starch in the cooking-type cultivars has been well characterized (Takeda et al 1986, 1987; Yoshio et al 1995). On the other hand, sake-type rice cultivars have never been used for staple foods and it is well accepted that they are especially suitable for sake brewing (Yoshizawa 2004). Sake-type rice is a large grain, nonglutinous rice cultivar and often has white, opaque tissue at the center of the grain (white core). These rice grains absorb water quickly and form a large amount of sugar upon saccharification by amylase (Yanagiuchi et al 1996). Because the mycelia of *Aspergillus oryzae* grows in the white-core cavity as well as on the surface of the grains, they are especially suitable for koji mold production (Iemura and Fujita 1982). No significant differences in protein, starch, or lipid content have been reported between sake-type and cooking-type rice cultivars (Yoshizawa 2004). However, little has been reported on the structural or physicochemical characteristics of starch in sake-type rice other than the study of Chen et al (2003).

The amylose/amylopectin ratio and amylopectin chain length distribution, the two most commonly used structural parameters of starches, have significant effects on starch retrogradation and staling of starchy foods (Yao et al 2002; Fitzgerald 2004). The influence of amylose/amylopectin ratio on the texture of cooked rice is well known (Chikubu et al 1985; Takahashi et al 1998) and differences in amylopectin structure affect rice cake hardening rates (Okamoto et al 2002). Amylose-free starch containing a high level of short-chain amylopectin produced by genetic modification exhibited no syneresis and excellent freeze-thaw stability (Jobling et al 2002). In the production of sake, steamed rice grains are digested under conditions that easily retrograde starch: a low moisture content (≈30–40%) of steamed rice grains; cool temperatures (≈7–15°C), and long periods (≈15–40 days) in the *moromi* mash. Therefore, it is likely that starch retrogradation might affect enzyme digestibility of steamed rice grains because starch retrogradation suppresses the normal swelling of gelatinized starch caused by absorption of water.

In our previous report, the relationship between structural characteristics and enzyme digestibility of steamed rice grains stored for 3 hr was examined in endosperm starch rice mutants with an amylose content and amylopectin chain length distribution different from that in normal rice cultivars (Okuda et al 2005). The results indicated that the more digestible rice samples had starch with a lower amylose content and a greater amount of short chain amylopectin. Differential scanning calorimetry (DSC) analysis showed that the retrogradation properties of the starch were related to enzyme digestibility of steamed rice grains. Because endosperm starch mutants are not cultivated commercially, it is important to investigate this relationship in both commonly used sake-type and cooking-type commercial rice cultivars.

In the present study, structural characteristics and physicochemical properties of starch were examined in sake-type and cooking-type rice cultivars used for sake production in Japan. The effect of a 0.5–24 hr holding period shortly after steaming on enzyme digestibility and starch retrogradation of steamed rice grains was also examined. In addition, enzyme digestibility and retrogradation of steamed rice grains were examined as a function of the structural characteristics of starches under conditions that mimicked sake production.

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MATERIALS AND METHODS

Materials

Seven Japanese short-grain cultivars were harvested during the 2001 growing season in Japan. Yamadanishiki (grown at Hiroshima), Ginpuu and Hatsusizuku (grown at Hokkaido), Gohyakumangoku (grown at Ishikawa), and Okuhomare (grown at Fukui) are sake-type rices and Nipponbare (grown at Hiroshima) and Koshihikari (grown at Chiba) are cooking-type rices. The moisture content of rice grains was measured by the decrease in weight after 3 hr of heating at 135°C according to the method of Ito et al (1988). The moisture content of brown rice was adjusted to 13.8% (w/w) in a humidity chamber, and 1,000 kernel grains were weighed. The ratio of white-core grains (%) was determined as the ratio of the number of white-core grains to the total number of grains. Brown rice (150 g) was milled to 70% of its original weight using a milling machine (TM-05; Satake, Higashihiroshima, Japan) at a rate of 1,000 rpm. The moisture content of the milled rice was adjusted to 13.5% (w/w) in a humidity chamber. Rice flour was prepared from the milled rice using an automated crusher (AC1A; Satake) and was then passed through a 300-µm mesh sieve. Starch was isolated from 70% milled rice grain flour by the alkali method (Yamamoto et al 1981). Fractionation of the rice endosperm starch into amylose and amylopectin was performed under a nitrogen atmosphere to avoid oxidative degradation as described in Lansky et al (1949) and modified by Takeda et al (1986). Amylose was purified by ultracentrifugation, and purity was determined by gel-filtration chromatography (Takeda et al 1984) on Toyopearl HW-75S resin (Toso, Tokyo, Japan).

Analytical Methods

Starch content was determined using Total Starch Assay Kit procedures (Megazyme, Bray, Ireland) (Approved Method 76-13, AACC International 2000) (ICC Method No. 168). Protein content (N × 5.95) was determined according to the Dumas method using an automated elemental analyzer (vario EL III, Hanau, Germany).

Gel-filtration HPLC of isoamylase-debranched starch and amylopectin was performed according to Cheethman and Tao (1997) as modified by Okuda et al (2005). The molecular size distribution of amylose was analyzed as described in Okuda et al (2005). The number-average degree of polymerization (DP_n) of amylose was determined by a modification of the Park-Johnson method (Hizukuri et al 1981).

Physicochemical Analysis

Pasting properties of starches were analyzed by a Rapid Visco Analyser (RVA-3D, Newport Scientific, Australia) according to Jane et al (1999) with the following modifications. Each starch suspension (9%, w/w, dry weight basis; 28 g total weight) was equilibrated at 50°C for 1 min, heated at a rate of 5°C/min to 95°C, maintained at that temperature for 5 min, and then cooled to 50°C at a rate of 5°C/min, and maintained at that temperature for 6 min. A constant rotating paddle (160 rpm) was used.

The gelatinization and retrogradation properties of samples were determined by differential scanning calorimetry (DSC) (Micro DSC III, Setram, France). Starch (200 mg) was weighed in sample pans, mixed with distilled water (500 mg), and sealed. The starch suspension was heated at a rate of 1°C/min from 20 to 120°C. Distilled water (595 mg) was used as the reference standard.

TABLE I
Properties of Brown Rice and Milled Rice Grains of Japanese Rice Cultivars Used in Sake Production

	Brown Rice		Milled Rice[a]	
	1,000 Kernel Weight (g)	Ratio of White-Core Grains (%)	Protein Content[b] (%)	Total Starch[c] (%)
Sake-type				
Yamadanishiki	26.5	79.6	5.0 ± 0.1	92.8
Ginpuu	25.8	90.6	5.6 ± 0.1	92.5
Hatsusizuku	25.1	1.4	5.6 ± 0.1	92.6
Gohyakumangoku	25.5	90.6	5.2 ± 0.0	91.7
Okuhomare	31.1	98.8	5.8 ± 0.1	91.4
Cooking-type				
Nipponbare	22.9	0.6	5.1 ± 0.0	92.5
Koshihikari	21.2	0.1	5.2 ± 0.0	91.6

[a] Moisture free basis.
[b] Values are means of triplicate measurements ± standard deviations.
[c] Values are means of duplicate measurements.

TABLE II
Molecular Structural Characteristics of Starch, Amylopectin, and Amylose Purified from Japanese Rice Cultivars Used in Sake Production[a]

		Starch[b]			Amylopectin[c]			Amylose
	Sample	FI (%)	FIIa (%)	FIIb (%)	FIIa' (%)	FIIb' (%)	FIIb'/FIIa'	DP_n[d]
Sake-type	Yamadanishiki	17.8 ± 0.5	20.2 ± 0.1	61.9 ± 0.4	23.9 ± 0.3	76.1 ± 0.3	3.2 ± 0.1	1,058 ± 29
	Ginpuu	19.2 ± 0.6	19.3 ± 0.1	61.4 ± 0.4	23.5 ± 0.8	76.5 ± 0.8	3.3 ± 0.1	1,309 ± 55
	Hatsusizuku	20.2 ± 0.4	19.0 ± 0.2	60.8 ± 0.5	22.4 ± 0.1	77.6 ± 0.1	3.5 ± 0.0	1,478 ± 35
	Gohyakumangoku	15.6 ± 0.2	22.9 ± 0.1	61.5 ± 0.1	26.6 ± 0.1	73.4 ± 0.1	2.8 ± 0.0	1,004 ± 36
	Okuhomare	15.5 ± 0.3	23.3 ± 0.2	61.1 ± 0.5	27.0 ± 0.5	73.0 ± 0.5	2.7 ± 0.1	956 ± 58
Cooking-type	Nipponbare	18.1 ± 0.3	20.4 ± 0.0	61.5 ± 0.3	24.5 ± 0.3	75.5 ± 0.3	3.1 ± 0.1	1,128 ± 54
	Koshihikari	15.2 ± 0.2	21.5 ± 0.1	63.2 ± 0.1	24.7 ± 0.2	75.3 ± 0.2	3.0 ± 0.0	1,100 ± 40

[a] Values are means of triplicate measurements ± standard deviations.
[b] Proportion of chains in debranched starch measured by gel-filtration chromatography. Fraction I (FI) = long linear chains from amylose; Fraction IIa (FIIa) and IIb (FIIb) = long-length and short + intermediate-length chains from amylopectin, respectively.
[c] Proportion of chains in debranched amylopectin measured by gel-filtration chromatography (GFC). Fraction IIa' (FIIa') and IIb' (FIIb') = long-length and short + intermediate-length chains from amylopectin, respectively. FIIb'/FIIa' = ratio of FIIb' to FIIa'.
[d] Number-average degree of polymerization of amylose measured by modified Park-Johnson method.

Enthalpy change (ΔH), gelatinization onset temperature (T_o), and peak temperature (T_p) were computed automatically. The retrogradation study was performed using the same method with the sample used for the gelatinization study after storage at 4°C for seven days. Degree of amylopectin retrogradation was determined as the ratio of retrogradation enthalpy change (ΔH_1 of dissociating retrogradaded starch) to gelatinization enthalpy change (ΔH_1 of starch gelatinization).

Enzyme Digestibility of Steamed Rice Grains

Milled rice grains 70% (10 g) were steeped in distilled water for 15–20 hr at 15°C after which the steeped rice grains were separated from the water. The steeped rice grains were then steamed for 45 min using a steam boiler (M-11, Eishin Electric, Japan) and then cooled to room temperature. The steamed rice grains were put in a plastic bag and kept at 15°C for 0.5–24 hr. The enzyme digestibility of stored rice grains was examined as described in Okuda et al (2005). The ratio of hydrolyzed starch to total starch was calculated using a set formula (Table VI, a).

Retrogradation of Steamed Rice Samples

Steamed rice grains (10 g) were put in a plastic bag and kept at 15°C for 0.5–24 hr. The stored rice samples were dehydrated by addition of methanol and acetone according to the method of Ikawa et al (2002), and the thermal properties were analyzed by DSC under the conditions noted above.

RESULTS

Properties of Rice Samples

Table I shows 1,000 kernel weight of brown rice (g), the ratio of white-core grains (%), total starch, and crude protein in milled rice grains of Japanese sake-type and cooking-type rice samples, both of which are used in sake production in Japan. The 1,000 kernel weight of brown rice varied from 21.2 to 31.1 g and that of the sake-type rice was higher than that of the cooking-type. The ratio of white-core grains varied from 0.1 to 98.8 %. Sake-type rice samples, except for Hatsusizuku, had high white-core ratio values, while the cooking-type rice samples had low values. No significant differences in starch content or crude protein content were observed among samples.

Structural Analysis

Starch, amylopectin, and amylose were purified from 70% milled rice grain samples and the structural characteristics were examined. The gel-filtration chromatograms of isoamylase debranched starch and amylopectin are shown in Fig. 1. The FIIa' and FIIb' peaks in the chromatograms of debranched purified amylopectin closely matched the FIIa and FIIb peaks of debranched starches in all the samples tested. FI was identified as a true amylose fraction because it was found only in the debranched starches. The proportions of FI, FIIa, and FIIb were calculated from the respective peak areas of debranched starches, and those of FIIa' and FIIb' were calculated from the respective peak areas of purified amylopectins debranched by isoamylase (Table II). The proportions of FI varied from 15.2 to 20.2%. Koshihikari, Gohyakumangoku, and Okuhomare had low amylose contents (15.2, 15.5, and 15.6%, respectively), while Ginpuu and Hatsusizuku had high amylose contents (19.2 and 20.2%, respectively). The proportion of FIIa varied from 19.0 to 23.3% and that of FIIa' varied from 22.4 to 27.0%. Hatsusizuku had the lowest value, while Okuhomare had the highest. The FIIb'/FIIa' ratio varied from 2.7 to 3.5. Hatsusizuku had the highest FIIb'/FIIa' ratio, while Okuhomare had the lowest. The samples with high FIIb'/FIIa' ratios, such as Ginpuu and Hatsusizuku, had a high proportion of FI, while the samples with low FIIb'/FIIa' ratios had a small proportion of FI.

The molecular size distribution of amylose (Fig. 2) purified from endosperm starch was analyzed by gel-filtration chromatography (GFC). The molecular weights of all samples ranged from 10^4 to $>10^6$. The peak shapes of Ginpuu and Hatsusizuku were sharp, and the ratios of high-to-low molecular weight chains were high. On the other hand, the peak shapes of Gohyakumangoku and Okuhomare were broad, and the ratios of small-to-large molecular weight chains were high. The DP_n values for amylose were 900–1,400 (Table II). The DP_n values correlated closely with the peak shapes obtained by GFC analysis. Hatsusizuku had a large amount of high molecular weight amylose and high DP_n values; Okuhomare had low amounts of high molecular weight amylose and low DP_n values. The DP_n values correlated well with amylose content FI values ($r = 0.867$).

These results reveal a great similarity in the structure and composition of starch from sake-type and cooking-type rice cultivars.

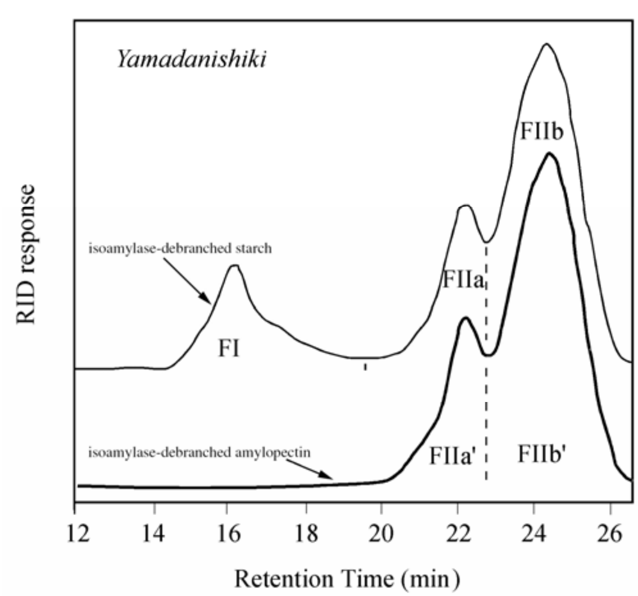

Fig. 1. Representative chain length distribution of isoamylase-treated starch and amylopectin by gel-filtration chromatography.

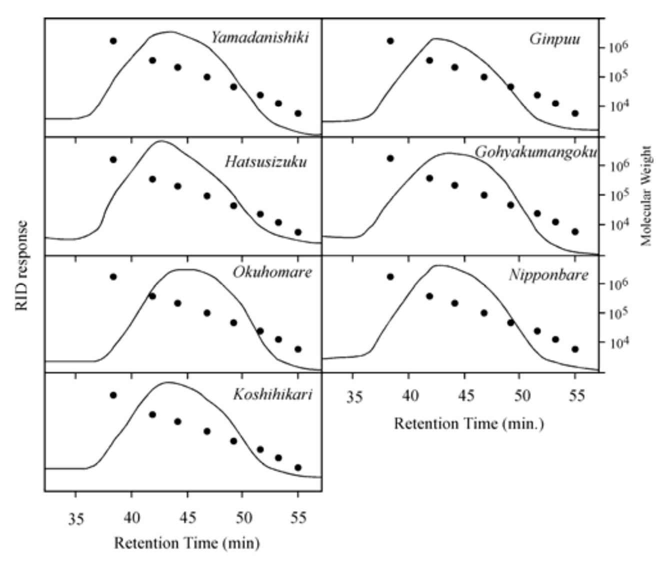

Fig. 2. Gel-filtration chromatograms of amylose purified from rice grains derived from Japanese cultivars used for sake production. Differential refractometry (solid line). Relative molecular size standard (Pullulan Shodex P-82) (dots).

Pasting Properties and Thermal Properties

The pasting properties of purified endosperm starches by RVA are summarized in Table III. With respect to peak viscosity and breakdown that reflect swelling power and fragility of swollen granules, Ginpuu and Hatsusizuku had low values of 1,723 and 1,806 cP, respectively. On the other hand, Gohyakumangoku, Okuhomare, and Koshihikari had high values of 2,473, 2,424, and 2,369 cP, respectively.

In setback, which reflects the retrogradation of amylose-lipid complex reassociation, Ginpuu and Hatsusizuku had high values of 988 and 942 cP, respectively. On the other hand, those of Koshihikari and Okuhomare had low values of 612 and 660 cP, respectively. RVA parameters correlated with the amylose content (FI) (peak viscosity, $r = -0.944$; breakdown, $r = -0.993$; setback, $r = 0.923$), agreeing with the finding of Zeng et al (1997).

The thermal properties of native starch are summarized in Table IV. The native starches showed two transitions. The first peak (peak 1) indicates a melting of the crystalline region that is mainly derived from amylopectin (Morrison et al 1993), while the second peak (peak 2) indicates a melting of the amylose-lipid complexes (Kugimiya et al 1980). In the first peak, the onset temperature (T_{o1} 50.1–58.5°C), peak temperature (T_{p1} 58.8–65.2°C), and enthalpy change (ΔH_1 10.2–13.0 J/g) differed among the samples. The T_{p1} of Hatsusizuku and Ginpuu, with short-chain amylopectin, was low, while that of Gohyakumangoku and Okuhomare, with long-chain amylopectin, was high. The T_{p1} results are in good agreement with those reported by Asaoka et al (1984). The ΔH_1 of Ginpuu, with a high amylose content, had the lowest value, while that of Gohyakumangoku, with a low amylose content, was the highest. In the second peak, onset T_{o2} (90.6–92.5°C) and peak T_{p2} (98.2–102.0°C) did not differ much among samples and did not correlate with amylose content or the number average degree of polymerization of amylose (DP_n). On the other hand, the enthalpy change (ΔH_2) range was 2.7–4.0J/g among the cultivars and correlated weakly with amylose content (FI) ($r = 0.583$).

The thermal properties of retrograded starch stored at 4°C for seven days are summarized in Table V. The retrograded starch showed two peaks in the 30–60°C range derived from recrystallization of amylopectin and 90–110°C derived from reassociation of the amylose-lipid complex. Onset thermal transition temperatures of dissociating retrograded starches were 31.2–32.6°C and were lower than the onset gelatinization temperature of native starches (50.1–58.5°C). The ΔH_1 differed among cultivars (2.1–4.0 J/g); Ginpuu had the the lowest and Gohyakumangoku had the

TABLE III
RVA[a] Pasting Properties of Starches Purified from Rice Grains Derived from Japanese Rice Cultivars Used in Sake Production[b]

	Sample	Peak Viscosity (cP)	Breakdown (cP)	Setback (cP)	Pasting Temp (°C)
Sake-type	Yamadanishiki	2,080 ± 12	1,380 ± 8	935 ± 11	64.5 ± 0.6
	Ginpuu	1,723 ± 9	1,127 ± 5	988 ± 2	61.9 ± 07
	Hatsusizuku	1,806 ± 8	955 ± 13	942 ± 11	63.7 ± 0.7
	Gohyakumangoku	2,473 ± 7	1,643 ± 12	711 ± 27	65.6 ± 0.3
	Okuhomare	2,424 ± 51	1,699 ± 49	660 ± 30	65.8 ± 0.2
Cooking-type	Nipponbare	2,172 ± 41	1,348 ± 30	823 ± 45	64.0 ± 0.4
	Koshihikari	2,369 ± 54	1,791 ± 45	612 ± 3	63.8 ± 0.1

[a] Rapid Visco Analyser.
[b] Values are means of triplicate measurements ± standard deviations.

TABLE IV
DSC[a] Gelatinization Properties of Starches Purified from Rice Grains Derived from Japanese Rice Cultivars Used in Sake Production[b]

		First Peak			Second Peak		
	Sample	T_{o1} (°C)	T_{p1} (°C)	ΔH_1 (J/g)	T_{o2} (°C)	T_{p2} (°C)	ΔH_2 (J/g)
Sake-type	Yamadanishiki	52.81 ± 0.17	60.24 ± 0.34	10.63 ± 0.24	91.42 ± 1.06	98.18 ± 0.54	3.87 ± 0.24
	Ginpuu	50.11 ± 0.20	58.77 ± 0.12	10.20 ± 0.09	90.56 ± 2.57	99.03 ± 0.14	4.03 ± 0.28
	Hatsusizuku	51.01 ± 0.08	59.69 ± 0.19	11.60 ± 0.26	92.46 ± 1.22	98.22 ± 0.14	3.36±0.36
	Gohyakumangoku	57.49 ± 0.69	65.24 ± 0.07	12.96 ± 1.21	91.22 ± 2.98	100.68 ± 0.50	2.89 ± 0.20
	Okuhomare	58.47 ± 0.19	65.21 ± 0.17	12.64 ± 0.25	92.44 ± 1.17	101.87 ± 0.45	2.73±0.07
Cooking-type	Nipponbare	55.69 ± 0.64	62.53 ± 0.58	12.77 ± 0.12	91.84 ± 1.70	102.01 ± 0.16	3.16 ± 0.13
	Koshihikari	55.53 ± 0.11	64.45 ± 0.10	11.14 ± 0.31	91.37 ± 0.52	98.93 ± 0.22	3.38 ± 0.62

[a] Differential scanning calorimetry.
[b] Values are means of triplicate measurements ± standard deviations. T_o = onset temperature; T_p = peak temperature; ΔH = enthalpy.

TABLE V
DSC[a] Retrogradation Properties of Starches from Japanese Rice Cultivars Used in Sake Production Stored at 4°C for Seven Days After Gelatinization

		First Peak[b]			
	Sample	T_{o1} (°C)	T_{p1} (°C)	ΔH_1 (J/g)	Retrogradation (%)[c]
Sake-type	Yamadanishiki	32.23 ± 0.41	44.48 ± 0.45	2.25 ± 0.17	21.04 ± 1.17
	Ginpuu	31.63 ± 0.44	44.10 ± 0.29	2.09 ± 0.10	20.58 ± 1.52
	Hatsusizuku	32.27 ± 0.18	44.42 ± 0.81	2.41 ± 0.16	21.28 ± 1.89
	Gohyakumangoku	32.44 ± 0.32	45.08 ± 1.32	3.99 ± 0.17	31.17 ± 2.09
	Okuhomare	31.21 ± 0.07	46.59 ± 0.32	3.53 ± 0.08	29.54 ± 0.10
Cooking-type	Nipponbare	32.63 ± 1.16	45.38 ± 0.98	3.08 ± 0.22	25.43 ± 0.76
	Koshihikari	31.50 ± 0.40	46.92 ± 1.53	2.52 ± 0.02	22.87 ± 1.71

[a] Differential scanning calorimetry.
[b] Values are means of triplicate measurements ± standard deviations. T_o = onset temperature; T_p = peak temperature; ΔH = enthalpy.
[c] Retrogradation(%) = ΔH_1 (retrogradaded starch) × 100/ΔH_1 (native starch).

highest. The degree of retrogradation ranged from 20.6% for Ginpuu to 31.2% for Gohyakumangoku and exhibited a significant negative correlation with the ratio of short-to-long chain amylopectin (Fig. 3).

Enzyme Digestibility of Rice Grains After Steaming

Enzyme digestibility of steamed rice grains stored for 0.5–24 hr at 15°C was examined because storing simulated the cooling step before mashing in sake production (Table VI). At 0.5 hr after steaming, Yamadanishiki and Gohyakumangoku had high digestibility values (71.3 and 65.9%, respectively), while Hatsusizuku and Nipponbare had low values (57.4 and 58.8%, respectively). After 3–24 hr, enzyme digestibility of all cultivars decreased as previously reported (Miyoshi and Koyama 1975). However, the rate of decreasing enzyme digestibility differed among cultivars. Nipponbare and Hatsusizuku had lower values than other cultivars after 3 hr (51.5 and 52.5%, respectively) and Okuhomare had the lowest value after 6–24 hr. Yamadanishiki had high values after 3–24 hr. Ginpuu and Hatsusizuku had high values after 24 hr (18.3 and 16.6%, respectively).

Relative enzyme digestibility (the ratio of enzyme digestibility to that at 0.5 hr after steaming) was calculated to eliminate factors other than starch and to investigate the decreasing rate of the enzyme digestibility of steamed rice grains (Table VI). The values for Ginpuu, Hatsusizuku, and Yamadanishiki were high, while those of Okuhomare and Gohyakumangoku were low after 3–24 hr. As shown in Fig. 3, the degree of amylopectin retrogradation had a negative correlation with the ratio of short-to-long chain amylopectin (FIIb'/FIIa'). Thus, the cultivars that exhibited a slow retrogradation of starch underwent a slow decrease in enzyme digestibility after steaming.

The correlation coefficients for seven samples relating to enzyme digestibility of steamed rice grains after 0.5–24 hr are shown in Table VII. At 0.5 hr after steaming, the enzyme digestibility of the steamed rice grains did not exhibit a significant correlation with any other parameters. However, after 9–11 hr, three parameters (T_{p1}, ΔH_1, and retrogradation) showed significant negative correlations. After 24 hr, many structural/physicochemical parameters (FI, FIIb'/FIIa', DP_n, peak viscosity, setback, pasting temperature, T_{p1}, retrogradation) also showed significant correlations. In contrast, protein content and the ratio of white-core grain did not. The sign of the correlation coefficient for amylopectin structure, amylose content, RVA parameters, and DSC parameters after 0.5 hr changed from negative to positive after >3–6 hr, and the significance of these parameters increased. The correlation coefficients relating relative enzyme digestibility after 3–24 hr to amylose content (FI), the ratio of short/long chain amylopectin (FIIb'/FIIa'), DP_n, and setback were significant and positive but were negative with respect to peak viscosity, T_{p1}, ΔH_1, and retrogradation (Table VII). The sign of the correlation coefficients for these parameters did not change after 0.5–24 hr.

Retrogradation of Rice Grains After Steaming

To clarify the reason for the observed decrease in enzyme digestibility of rice grains after steaming, starch retrogradation of the steamed rice grains was examined using DSC. Representative DSC heat flow curves for rice samples stored for 0.5–24 hr at 15°C after steaming are shown in Fig. 4. The peak areas at 90–110°C, which correspond to the melting of the amylose-lipid complex did not change after steaming. On the other hand, the peak area at 30–60°C, which corresponds to recrystallization of amylopectin, increased as a function of the poststeaming holding time for all samples. The increasing rate of enthalpy changes in amylopectin recrystallization differed among the cultivars (Fig. 5).

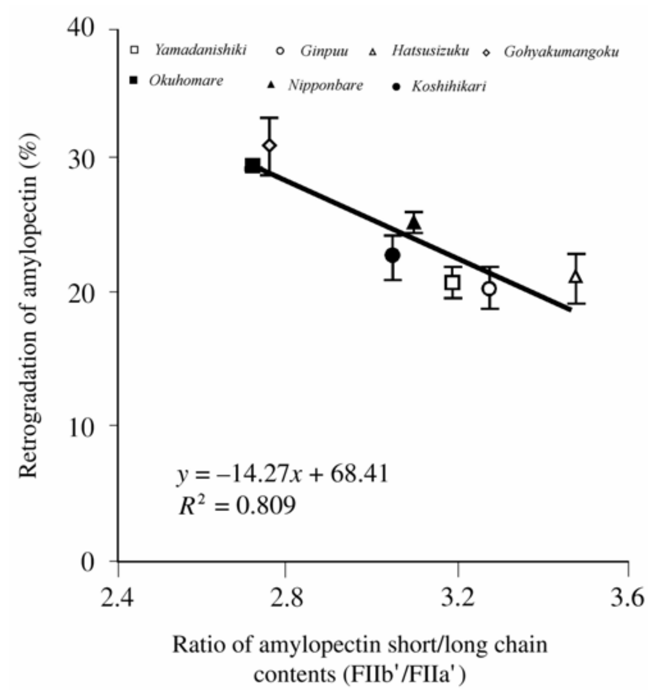

Fig. 3. Relationship between amylopectin structure and degree of amylopectin retrogradation in gelatinized starch stored at 4°C for seven days.

TABLE VI
Enzyme Digestibility[a] (%) and Relative Value[b] (%) (in parentheses) of Steamed Rice Grains After Steaming and Storage at 15°C for 0.5–24 hr in Plastic Bags[c]

	Holding Period After Steaming (hr)					
Sample	**0.5**	**3**	**6**	**9**	**11**	**24**
Sake-type						
Yamadanishiki	71.3 ± 0.2 (100)	66.7 ± 0.5 (93.6)	58.1 ± 1.2 (81.5)	49.6 ± 0.9 (69.5)	43.6 ± 0.8 (61.2)	15.4 ± 1.0 (21.6)
Ginnpuu	61.3 ± 1.4 (100)	56.7 ± 0.5 (92.5)	50.3 ± 1.1 (82.1)	44.1 ± 0.2 (72.0)	38.5 ± 1.5 (62.9)	18.3 ± 0.9 (29.9)
Hatsusizuku	57.4 ± 0.8 (100)	52.5 ± 0.7 (91.4)	46.6 ± 0.8 (81.2)	39.3 ± 1.2 (68.5)	34.7 ± 1.2 (60.4)	16.6 ± 0.3 (28.9)
Gohyakumangoku	65.9 ± 1.1 (100)	56.4 ± 0.5 (85.6)	46.5 ± 0.9 (70.6)	35.2 ± 0.6 (53.5)	28.9 ± 1.6 (44.0)	9.5 ± 0.8 (14.5)
Okuhomare	63.8 ± 0.3 (100)	53.3 ± 0.3 (83.6)	40.7 ± 0.6 (63.9)	28.8 ± 1.3 (45.1)	24.3 ± 0.5 (38.1)	7.4 ± 1.1 (11.6)
Cooking-type						
Nipponbare	58.8 ± 0.6 (100)	51.5 ± 0.8 (87.6)	43.0 ± 1.1 (73.1)	33.2 ± 0.8 (56.5)	28.2 ± 1.6 (48.0)	10.2 ± 1.2 (17.3)
Koshihikari	63.4 ± 0.6 (100)	55.9 ± 2.5 (88.2)	47.1 ± 0.6 (74.3)	36.8 ± 1.7 (58.0)	31.5 ± 1.6 (49.7)	8.9 ± 0.6 (14.0)

[a] Enzyme digestibility of rice grains after steaming = ratio of hydrolyzed starch to total starch (degree of starch conversion [%]) calculated as:
Degree of starch conversion (%) = (hydrolyzed starch [glucose equivalents]/total starch in milled rice grains [glucose equivalents]) × 100
= (TSC × [vol 1 + vol 2]/rice dry weight {mg} × total starch {%}/100 × 180/162) × 100
where TSC is total sugar concentration (mg/mL); vol 1 is volume of enzyme solutions (mL); vol 2 is volume of absorbed water after steaming (mL); 180/162 is adjustment from anhydro glucose to free glucose.
[b] Relative value (%) = (enzyme digestibility of rice grains after steaming/enzyme digestibility [0.5 hr]) × 100.
[c] Values are means of triplicate measurements ± standard deviation.

After 3 hr, the enthalpy changes of amylopectin recrystallization ranged from 0.15 J/g for Ginpuu to 0.31 J/g for Gohyaku-mangoku, while after 24 hr, the values ranged from 2.62 J/g for Ginpuu to 4.44 J/g for Okuhomare. The cultivars with high short-chain contents of amylopectin exhibited low increasing rates of enthalpy change, while those with low short-chain contents of amylopectin exhibited high increasing rates of enthalpy change.

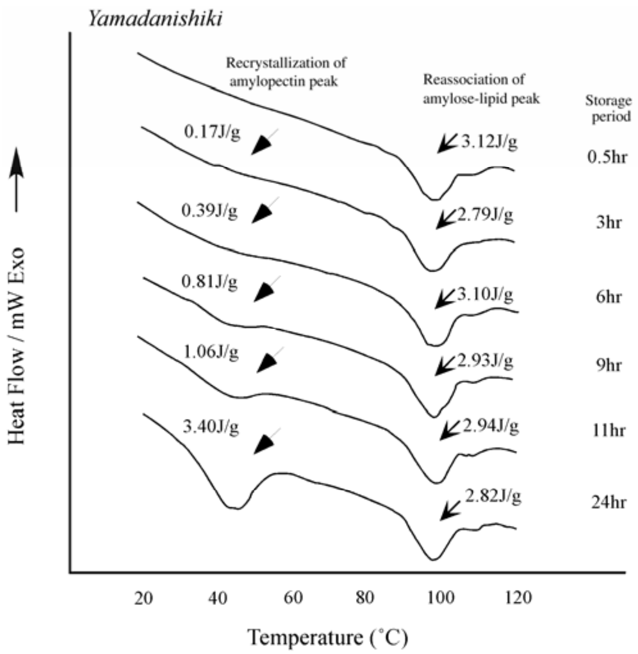

Fig. 4. Representative differential scanning calorimetry (DSC) heat flow curves of steamed rice samples stored at 15°C for 0.5–24 hr in plastic bags. Recrystallization of amylopectin peak values are enthalpies (ΔH_1). Reassociation of amylose-lipid peak values are enthalpies (ΔH_2). Values are means of duplicate samples.

The enthalpy change rates were similar to the results shown in Fig. 3, which indicate a significant negative correlation between retrogradation of amylopectin and the ratio of short-to-long chain amylopectin (FIIb'/FIIa'), although the holding time in the experiment for the data shown in Fig. 5 was shorter than for the data shown in Fig. 3. The enthalpy change of amylopectin recrystallization also had a high correlation with the log of the relative enzyme digestibility of steamed rice samples after 3–24 hr for all cultivars (Fig. 6). The results shown in Figs. 5, 6, and Table VII indicate that the enzyme digestibility of steamed rice grains is affected significantly by the degree of retrogradation after steaming, and that the decreasing rate of enzyme digestibility is closely related to amylopectin chain distribution.

DISCUSSION

The structural characteristics of starches derived from rice cultivars used for sake production in Japan were examined. A similar degree of variability in the structural and physical characteristics of starch was observed in both sake-type and cooking-type rice cultivars. Such properties are affected by genotype and environmental factors during grain filling. Cool temperatures during grain filling enhance *Wx* gene expression and accumulation of amylose (Hirano and Sano 1998). Cool temperatures also cause an increase in the short-chain content of amylopectin (Umemoto et al 1999; Inouchi et al 2000). The starch characteristics of the present samples seem to be related to the temperature during grain filling because the rice was harvested in different climatic regions of Japan. The relationship between starch characteristics and temperature during grain filling is supported by the fact that the amylose content was directly proportional to the short-chain content of amylopectin. Ginpuu and Hatsusizuku in particular, harvested at Hokkaido in northern Japan, which is cool even in summer, had a high ratio of amylopectin FIIb'/FIIa' and a high amylose content, suggesting a cool temperature effect during grain filling.

TABLE VII
Correlation Coefficients Relating Enzyme Digestibility of Steamed Rice Grains Stored at 15°C for 0.5–24 hr to Structural Characteristics or Physical Property Parameters of Rice Starch Samples[a]

	Holding Period After Steaming (hr)					
	0.5	3	6	9	11	24
ED[b]						
Protein	–0.416	–0.442	–0.422	–0.294	–0.251	–0.164
Ratio of white-core grain	0.587	0.427	0.211	0.130	0.093	0.039
FI	–0.468	–0.046	0.289	0.501	0.545	0.869*
FIIb'/FIIa'	–0.389	0.077	0.441	0.629	0.676	0.844*
DP_n	–0.657	–0.263	0.119	0.350	0.397	0.765*
Peak viscosity	0.386	–0.059	–0.397	–0.615	–0.658	–0.943**
Setback	–0.129	0.286	0.560	0.722	0.746	0.942**
Pasting temp.	0.423	0.035	–0.296	–0.499	–0.524	–0.719
T_{p1} of native starch	0.184	–0.275	–0.582	–0.763*	–0.799*	–0.969**
ΔH_1 of native starch	–0.200	–0.559	–0.744	–0.830*	–0.851*	–0.741
Retrogradation(%)	0.119	–0.329	–0.616	–0.756*	–0.800*	–0.788*
RV[c]						
Protein	–	–0.179	–0.168	–0.099	–0.065	–0.267
Ratio of white-core grain	–	–0.123	–0.214	–0.128	–0.141	–0.088
FI	–	0.732	0.771*	0.779*	0.792*	0.926**
FIIb'/FIIa'	–	0.858*	0.900**	0.881**	0.899**	0.886**
DP_n	–	0.622	0.724	0.717	0.728	0.881**
Peak viscosity	–	–0.819*	–0.845*	–0.871*	–0.884*	–0.979**
Setback	–	0.837*	0.831*	0.858*	0.862*	0.917**
Pasting temp	–	–0.688	–0.743	–0.759*	–0.754*	–0.766*
T_{p1} of native starch	–	–0.914**	–0.908**	–0.930**	–0.943**	–0.955**
ΔH_1 of native starch	–	–0.845*	–0.793*	–0.823*	–0.835*	–0.654
Retrogradation (%)	–	–0.910**	–0.890**	–0.885**	–0.909**	–0.765*

[a] Parameters are as noted in Tables I–V. ** and * = $P < 0.01$ and 0.05, respectively; $n = 7$.
[b] ED = enzyme digestibility as noted in Table VI.
[c] RV = relative value of enzyme digestibility as noted in parentheses in Table VI.

Almost all of the sake-type rice cultivars had 1,000-kernel weights of >25 g and a white-core structure but did not differ from cooking-type rice in starch structure (Tables I, II). Chen et al (2003) reported that the outer portions of rice amylopectin from sake-type rice had a high content of long chains (≈DP 13–23) and a low content of short chains (≈DP 6–12) relative to the cooking-type rice cultivar Koshihikari. The differences between their findings and our results might be due to the use of different cultivars and different analytical methods.

Previously, viscoelasticity and retrogradation of steamed rice grains have been closely correlated with enzyme digestibility (Wakai et al 1995). The properties of steamed rice grains might be influenced by the physical properties of the gelatinized starches such as swelling power and retrogradation because starch is the largest component of the rice endosperm. In the present study, both RVA pasting properties and DSC gelatinization properties were examined to understand the physical properties of purified starch from Japanese rice cultivars used in sake production (Tables III, IV). Sake-type rice starch did not have a specific RVA pasting property or DSC gelatinization property compared with cooking-type rice starch, although the values differed among the samples. The DSC parameters for melting the amylose-lipid complex (T_{o2}, T_{p2}, ΔH_2) were similar among the samples, although the amylose structural characteristics differed. Suzuki et al (1985) reported that the DP_n values of amylose or the molecular weight distribution were related to starch retrogradation properties in potato, tapioca, and kuzu. (DP_n values for amylose were 4,070, 3,220, and 1,590, respectively). In the present study, the DP_n of amylose or the molecular weight distribution did not seem to affect the retrogradation properties of starch because the DP_n of amylose was 900–1,400, while the differences in retrogradation were negligible.

In sake production, the mash fermentation is typically conducted at 7–15°C. Therefore, steamed rice grains are put into the mash tank after cooling. Sometimes the cooling period is longer than 3 hr, and in such a case a decrease in enzyme digestibility occurs (Miyoshi and Koyama 1975; Wakai et al 1997). However, the factors that decrease enzyme digestibility are not well understood because time-dependent changes of starch retrogradation have not been detected (Miyoshi and Koyama 1975). To simulate the cooling steps before mashing in the present study, enzyme digestibility of steamed rice grains and retrogradation of starch were examined using DSC for detecting starch retrogradation. Enzyme digestibility decreased after steaming in all cultivars (Table VI) as previously reported by Miyoshi and Koyama (1975). The decrease in digestibility might be due to amylopectin recrystallization rather than reassociation of the amylose-lipid complex (Figs. 4 and 5). For this reason, the enthalpy changes for amylopectin recrystallization exhibited a significant correlation with relative enzyme digestibility of the steamed rice grains (Fig. 6). Furthermore, the degree of decreasing enzyme digestibility after steaming differed among cultivars (Table VI). Physical changes in steamed rice grains after 0.5–24 hr might be due to recrystallization of amylopectin as well as the decrease in enzyme digestibility (Fig. 4). In a previous study of endosperm starch mutants (Okuda et al 2005), amylose content exhibited a significant negative correlation with enzyme digestibility of steamed rice grains stored at 15°C for 3 hr. Amylose-lipid reassociation seemed to suppress enzyme digestibility because enthalpy changes for the amylose-lipid reassociation were related to amylose content. On the other hand, in the present study, amylose content exhibited a weak negative correlation with enzyme digestibility at 0.5 hr after steaming but the sign of the correlation coefficients became positive after 6–24 hr (Table VI).

We offer the following possible explanations for the observation that amylose content did not correlate significantly and negatively with enzyme digestibility. First, the differences in amylose content among samples were small. In a previous study (Okuda et al 2005), the amylose content of samples was 4–26%; ΔH_2 for amylose-lipid reassociation was 0.9–3.7 J/g. In the present study, amylose content of samples was 15–20%; ΔH_2 for amylose-lipid reassociation was 2.8–3.7 J/g. Second, although amylose content and short chain content of amylopectin are competitive parameters with respect to enzyme digestibility, amylose content was closely linked to the ratio of short-to-long chain amylopectin content in the present samples.

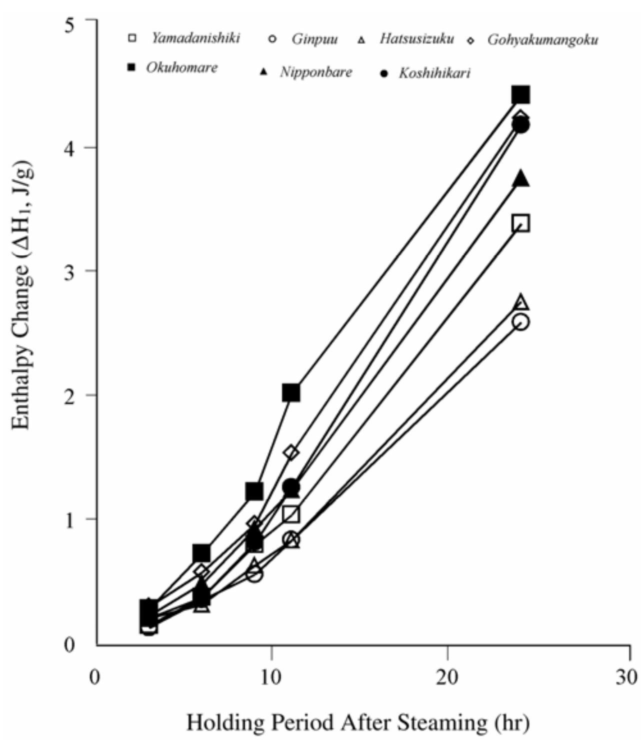

Fig. 5. Effect of holding period after steaming on enthalpy changes derived from amylopectin recrystallization peak of rice samples stored at 15°C. Values are means of duplicate samples.

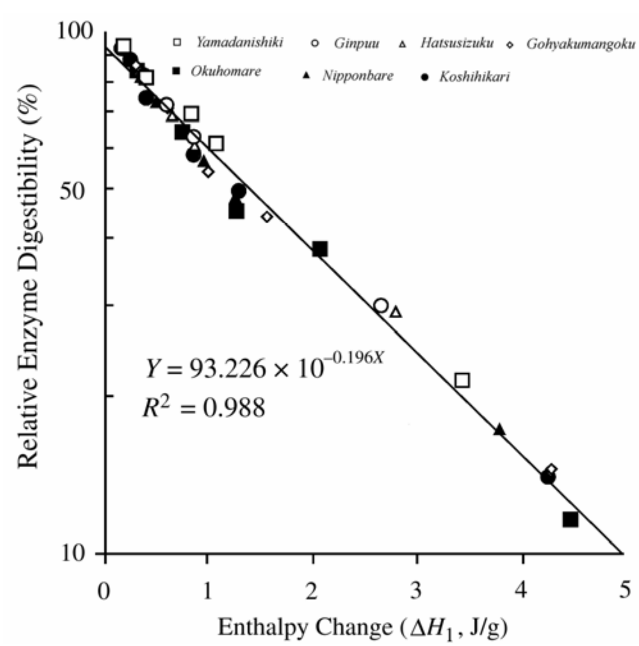

Fig. 6. Relationship between relative digestibility and enthalpy of amylopectin recrystallization of steamed rice grains after 3–24 hr.

The enthalpy change for amylopectin recrystallization in steamed rice grains was small (2.6–4.4 J/g) even after 24 hr, and this value did not indicate a high degree of retrogradation. On the other hand, relative enzyme digestibility after 24 hr decreased to a great extent (11.6–29.9%) (Table VI). The reason for the decrese over time might be due to the continued retrogradation of amylopectin during enzyme digestion. In addition, because the steamed rice grains were digested without pasting or crushing, hardening or a decrease in water absorbability might have affected enzyme digestibility. As Nagata et al (1976) previously reported that water absorbability of steamed rice grains decreased after steaming and exhibited a positive correlation with enzyme digestibility, the retrogradation of amylopectin was predicted to cause a decrease in water absorbability of steamed rice grains.

The rate of starch retrogradation in steamed rice grains seemed to be rapid. The values for enthalpy changes in amylopectin recrystallization of steamed rice grains stored at 15°C for one day (2.6–4.4 J/g) were about the same as for retrogradated starch stored at 4°C for seven days (2.1–4.0 J/g) (Fig. 5, Table V). As reported by Zeleznak et al (1986), recrystallization of starch gels is profoundly influenced by gel moisture. Using DSC enthalpy values as a crystallinity index in aged gels, crystallinity reached maximum levels in gels of 50–60% starch and disappeared altogether in very dilute (10%) or very concentrated (80%) starch gels. In the present study, because steamed rice grains contain ≈60% starch, the observed rapid retrogradation might be due to the moisture content of the starch.

Based on the present results and our previous work (Okuda et al 2005), we conclude that in sake production using Japanese short-grain rice cultivars, when the period after steaming is short, the enzyme digestibility of the rice grains seems to be affected by amylose content and other factors such as the histological structure of the rice grains. On the other hand, when the period following steaming is prolonged, the enzyme digestibility of the rice grains might be significantly affected by amylopectin structure. No clear differences between sake-type and cooking-type rice cultivars were observed with respect to these properties. Therefore, to achieve good enzyme digestibility, avoidance of retrogradation of the steamed rice grains and use of rice cultivars with a high content of short-chain amylopectin are recommended because steamed rice grains are digested under conditions that can easily cause starch retrogradation during sake production.

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