デンプンの性質が清酒醸造で蒸米の消化性に及ぼす影響と物理的特性の分析による迅速な消化性の推定方法

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Influence of Starch Characteristics on Digestibility of Steamed Rice Grains under Sake-making Conditions, and Rapid Estimation Methods of Digestibility by Physical Analysis

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Abstract: Retrogradation of starch in steamed rice grains under sake-making conditions was examined. The enthalpy change derived from retrogradation of amylopectin increased during the *koji* and sake mash processes. Ethanol in the sake mash accelerated retrogradation of amylopectin. Enzyme digestibility and properties of endosperm starches of 136 Japanese rice samples used for sake production were examined. A digestion test of steamed rice grains was conducted after 24 h storage at 15°C to estimate more accurately digestibility during the sake-making process. The ratio of short-to-long chain amylopectin exhibited a significant correlation with enzyme digestibility, suggesting that the structure of amylopectin may be used as a predictor of digestibility. The gelatinization temperature of brown/milled rice and purified starch measured by DSC (differential scanning calorimetry) correlated well with the ratio of short-to-long chain amylopectin and with enzyme digestibility. Furthermore, the pasting temperature of milled rice as measured by RVA (Rapid Visco Analyser) correlated well with the ratio of short-to-long chain amylopectin and with enzyme digestibility. Therefore, DSC and RVA appear to be useful tools for estimating enzyme digestibility of steamed rice grains under sake-processing conditions because the measurements are done rapidly and require only small weights of brown/milled rice flour samples.

Key words: retrogradation, steamed rice grain, enzyme digestibility, differential scanning calorimetry, Rapid Visco Analyser

In previous studies, we have found that the velocity of starch retrogradation in steamed rice grains was highly dependent on the molecular characteristics of amylopectin and that samples with short-chain amylopectin exhibited good enzyme digestibility of steamed rice grains.[1,2] In sake mash, it is presumed that the retrogradation of amylopectin affects the digestibility of steamed rice grains and the efficiency of starch utilization. While the principle factors influencing enzyme digestibility have been well revealed, the effect of starch retrogradation in steamed rice grains during sake production has not been clarified.

Structural characteristics of rice starches are affected by environmental factors during grain filling.[3-9] This fact indicates that structural characteristics of starches vary with growing region even for the same cultivar. To our knowledge, these region-dependent structural differences in Japanese rice cultivars used for sake production have not been previously reported.

To evaluate enzyme digestibility of rice used for sake production, the national standard analysis method is usually used. This method involves time-consuming and labor-intensive operations, including adjusting moisture content, milling rice grains, steaming rice grains, enzyme digestion and analyzing digested solutions. Furthermore, the value of the data obtained is questionable because the possibility of retrogradation of the steamed rice grains is not taken into account. Thus, more effective methods for evaluating enzyme digestibility of rice used for sake production are sought by rice breeders and sake brewers. Starch structural characteristics correlate closely with the physical properties of starches, such as heat-induced gelatinization and cool-induced retrogradation as assessed using RVA (Rapid Visco Analyser) and DSC (differential scanning calorimetry).[1,2,10-14] RVA- and DSC-based methods have the advantages of accuracy, a requirement for small samples, and short measuring times, and they have been studied as methods for evaluating starchy foods and for breeding programs.[15-19] These methods appear to be suitable for estimating the digestibility of steamed rice grains used in sake production.

The present study was undertaken to clarify the influences of starch structure and retrogradation on digestibility of steamed rice grains under practical sake-making conditions. Initially, the retrogradation of starch in the steamed rice grains under such conditions was examined by DSC. The next step was to examine the structural characteristics of the starch and enzyme digestibility for 136 Japanese short grain rice samples under conditions simulating sake-making. Finally, on the basis of the RVA and DSC analyses, improved methods for rapidly estimating enzyme digestibility were proposed.

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MATERIALS AND METHODS

Materials. Samples used in this study are listed in Table 1. The moisture content of brown rice was adjusted to 13.8% (w/w) in a humidity chamber. Brown rice was milled to 70% of its original weight using a milling machine (TM-05; Satake Corporation, Higashi-Hiroshima, Japan) at a rate of 1000 rpm. The moisture content of the milled rice was adjusted to 13.5% (w/w) in a humidity chamber. Brown/milled rice flour was prepared using an automated crusher (AC1A; Satake). Endosperm starch was isolated from the rice grain flours by the alkali method.[20]

Enzyme digestibility of steamed rice grains. Ten grams of 70% milled rice grains were steeped in distilled water for 15–20 h 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, Yokohama, Japan) and then cooled to room temperature. The enzyme digestion test was conducted under the following three conditions. (A): digestion at 15°C for 24 h after storing steamed rice grains at 15°C for 3 h in a plastic bag. (B): digestion at 4°C for 48 h after storing steamed rice grains at 4°C for 1.5 h in a plastic bag. (C): digestion at 15°C for 24 h after storing steamed rice grains at 15°C for 24 h in a plastic bag. The enzyme digestibility of stored rice grains was evaluated, and the ratio of hydrolyzed starch to total starch was calculated according to previous studies.[1,2]

Analytical methods. Starch content was determined using the Megazyme Total Starch Assay Kit procedures (Megazyme International Ireland Ltd., Bray, Ireland, AACC Method 76-13, ICC Method No. 168). Gel filtration HPLC of isoamylase-debranched starch was performed as described previously.[2]

DSC thermal properties. The gelatinization temperature of brown and milled rice flours was determined by DSC (Pyrus Daiamond DSC, PerkinElmer Co., Ltd., USA). Samples (15 mg each) were weighed in sample pans, mixed with distilled water (30 mg), and sealed. Sample suspensions were then heated at a rate of 5°C/min from 5 to 120°C. Distilled water (32 mg) was used as a reference standard. Enthalpy change (ΔH) and peak temperature (T_P) were computed automatically.

Retrogradation of steamed rice samples. Retrogradation of steamed rice grains was examined under the following three conditions ((A)–(C)), using *Yamadanishiki* (milling ratio 70%, Hiroshima, harvested in 2005) and *Gohyakumangoku* (milling ratio 70%, Niigata, harvested in 2005) as steamed rice. (A) Retrogradation of steamed rice grains during *koji* making: ten grams of steamed rice grains were put in a petri dish and kept at 30°C/90% humidity for 24 h → 35°C/90% humidity for 9 h → 40°C/70% humidity for 13 h. (B) Retrogradation in sake mash: the small-scale sake production test (total rice weight 300 g) was performed according to Aramaki's method.[21] The proportion of raw materials was chosen according to Aramaki's report.[21] The mash temperature was as follows: 1st addition (*koji*) = 15°C, 2nd addition (steamed rice grains) = 7°C, with temperature increase at a rate of 1°C/day to a maximum of 13°C after the 2nd addition, and then holding at 13°C to the end of fermentation. The steamed rice grains in the sake mash except for *koji* were sampled. (C) Retrogradation in a buffer simulating a sake mash: ten grams of steamed rice grains were mixed with 50 mL of 0.1 M (pH 4.3) succinate buffer (±10% EtOH [v/v]) and incubated at 10°C for 20 days.

These stored rice samples ((A)–(C)) were dehydrated by addition of methanol and acetone as described by Ikawa *et al.*,[22] and their enthalpy changes derived from the amylopectin recrystallization peak were analyzed by DSC under the conditions noted above.

RVA pasting properties. Pasting properties of rice flours were measured using a RVA (RVA-3D; Newport Scientific Pty., Ltd., Australia). Each sample 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 then maintained at that temperature for 6 min. A constant rotating paddle (160 rpm) was used for mixing.

RESULTS AND DISCUSSION

Critical factors influencing digestibility of steamed rice grains: starch retrogradation of steamed rice grains under sake-making conditions.

To our knowledge, the retrogradation of starch in steamed rice grains in sake mash has not been reported previously, although the influence of starch retrogradation on the process of making sake has been researched.[23,24] In the present study, we examined the retrogradation of starch in steamed rice grains kept under sake-making conditions using DSC, a very sensitive detector of starch retrogradation.

Under *koji-making* conditions, the enthalpy change for the amylopectin retrogradation peak in steamed rice grains increased gradually and reached 4 J/g for 48 h (Fig. 1 (A)). As shown in Fig. 1 (B), retrogradation was also observed under conditions for sake mash, while the enthalpy change increased more slowly than for the *koji making* process and reached 6 J/g for 17 days. The fast retrogradation under *koji-making* conditions is probably due to a lower water content of the rice grains as previously reported.[25]

Although the amylopectin retrogradation of steamed rice grains was observed in the succinate buffer both with and without 10% ethanol, the starch retrogradation was accelerated by ethanol in the succinate buffer (Fig. 1 (C)). The concentration of ethanol can be as high as 20% in sake mash; therefore, starch retrogradation might proceed over time and be accelerated by increasing ethanol concentration. These two factors probably affect the decrease in digestibility of steamed rice grains under sake mash conditions, because the retrogradation of starch causes a decrease in enzyme digestibility.[1,2]

The retrogradation of *Gohyakumangoku* samples proceeded faster than those of *Yamadanishiki*, but the differences were small. Between the two samples, distinct differences in the structure of amylopectin were not observed (data not shown), which might account for the small differences, because the velocity of retrogradation closely re-

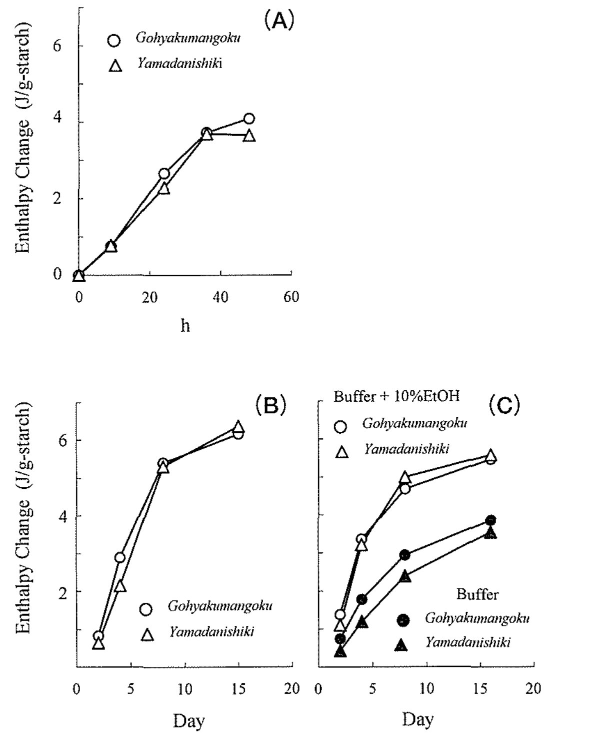


Fig. 1. Enthalpy changes derived from the amylopectin recrystallization peak of steamed rice samples under sake-making conditions.

(A) *koji* making, (B) in sake mash, (C) storage in 0.1 M succinic buffer (pH 4.3) with or without 10% (v/v) ethanol. Values are means of duplicate samples.

Table 1. Rice samples used in this study.[a]

Group 1: *Gohyakumangoku*, n = 18
[Chiba, Fukui (2), Fukushima, Gunma, Hyogo, Ibaraki, Ishikawa (2), Mie, Niigata (2), Shimane (2), Shizuoka, Tochigi, Tottori, Toyama]
Group 2: *Yamadanishiki*, n = 32
[Ehime (3), Fukui, Fukuoka, Hiroshima (4), Hyogo (2), Ibaraki, Ishikawa, Kagawa, Kochi (2), Kumamoto, Mie, Miyagi, Miyazaki, Okayama (2), Saga, Shimane, Shizuoka, Tochigi (3), Tokushima, Tottori, Toyama, Yamaguchi]
Group 3: *Koshihikari*, n = 31
[Aichi, Chiba, Fukui, Fukuoka, Fukushima, Gunma, Hiroshima (3), Hyogo, Ibaraki, Ishikawa, Kagawa, Kumamoto, Mie, Miyazaki (2), Nagano, Niigata (2), Okayama, Saga, Shimane (3), Tochigi (2), Tokushima, Tottori, Toyama, Yamagata]
Group 4: White-core rice, n = 24
Akitasakekomachi [Akita], *Dewasansan* [Yamagata], *Fusanomai* [Chiba], *Ginginga* [Iwate], *Ginpuu* [Hokkaido (2)], *Hanafubuki* [Aomori], *Hanakagura* [Miyazaki], *Hattan-nishiki–1* [Hiroshima (2)], *Hyogokitanishiki* [Hyogo], *Miyamanishiki* [Nagano], *Okuhomare* [Fukui (2)], *Oyamanishiki* [Toyama], *Sake8610–1* [Hyogo], *Senbonnishiki* [Hiroshima], *Shinriki* [Kumamoto], *Soraikusake170* [Hokkaido], *Suisei* [Hokkaido], *Tojinoyume* [Hyogo], *Toyamasake69* [Toyama], *Wakamizu* [Aichi], *Yumenokaori* [Fukushima]
Group 5: Non white-core rice, n = 31
Akebono [Okayama], *Akitsuho* [Kochi (2)], *Daichinohoshi* [Hokkaido], *Hanaomoi* [Aomori], *Hatsuboshi* [Chiba], *Hatsushizuku* [Hokkaido], *Hitomebore* [Iwate], *Kirara397* [Hokkaido (2)], *Matsuyamamii* [Ehime (2)], *Nakateshinsenbon* [Hiroshima], *Nipponbare* [Hiroshima (2), Hyogo, Ibaraki, Kyoto, Saitama, Shiga (3), Tokushima, Fukuoka], *Ooseto* [Kagawa (3), Tokushima], *Sasanishiki* [Miyagi Yamagata], *Syunyou* [Toyama]

[a]Cultivar names are in italics, production areas are in brackets, and sample numbers are in parentheses. All samples were harvested in 2005.

flects the ratio of short-to-long chain length of amylopectin.[2)]

The above results indicate that the retrogradation of starch needs to be considered in order to estimate the digestibility of steamed rice grains more accurately in sake mash as well as in the model digestion test.[1,2)]

Structural analysis of rice samples harvested in different regions of Japan.

One hundred and thirty-six rice samples harvested from different regions of Japan were collected (Table 1). Seventy-four of them are cultivars with a white core (*Yamadanishiki* 32, *Gohyakumangoku* 18, others 24) while the others are non-white core (*Koshihikari* 31, others 31). The structural characteristics of the starches were examined by gel-filtration chromatography for isoamylase-debranched starch. The proportions of FI (amylose), FIIa (ratio of long-length chains from amylopectin) and FIIb (ratio of short + intermediate-length chains from amylopectin) were calculated according to a previous report.[26)] The amylose (FI) content and the ratio of short-to-long chain length of amylopectin (FIIb/FIIa) varied among the samples in all cultivars and even within the same cultivars (Fig. 2).

The amylose content of *Koshihikari* cultivar was distributed around low values (13.2–17.7%; average, 15.2%). *Gohyakumangoku* had medium values (15.1–18.8%; average, 16.5%), while *Yamadanishiki* had high values (16.9–20.4%; average, 17.6%). The amylose content of the other white core rice samples were 14.7–19.3% (average 17.5%) while the other non-white core samples had values of 14.2–20.3% (average 16.9%). *Gohyakumangoku* had low ratio values of short-to-long chain length of amylopectin (FIIb/FIIa) (2.79–3.08; average 2.89), while *Yamadanishiki* had relatively high values (2.95–3.40; average 3.02). Values for *Koshihikari* ranged from low to high (2.83–3.25; average 3.05).

As shown in Fig. 2, the amylose content showed a high positive correlation with the ratio of short-to-long chain length of amylopectin in each cultivar (*Gohyakumangoku*; r = 0.863, *Yamadanishiki*; r = 0.860, *Koshihikari*; r = 0.889, white core rice; r = 0.827, non-white core rice; r = 0.693). Cool temperatures during grain filling enhance *Wx* gene expression and accumulation of amylose,[5,27,28)] and also cause an increase in the short-chain content of amylopectin.[6,7,9)] The samples of this study were harvested in different climatic regions of Japan; therefore the amylose content and amylopectin chain length distribution were probably affected by temperature differences during grain filling.

The FIIb/FIIa values for *Yamadanishiki* were relatively higher than those for *Gohyakumangoku* (Fig. 2 (A)). *Gohyakumangoku* is an early-ripening variety and its milky stage starts in late July, and therefore grain filling occurs at a higher temperature relative to *Yamadanishiki* which is a late-ripening variety whose milky stage starts in late August. We speculate that this difference in timing

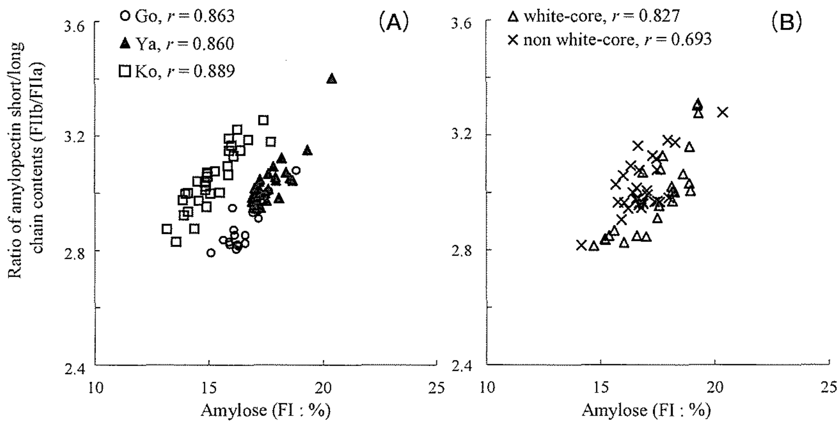


Fig. 2. Amylose content *vs.* ratio of short/long chain amylopectin of rice samples used in this study.

(A) ○Go, *Gohyakumangoku* (n = 18); ▲Ya, *Yamadanishiki* (n = 32); □Ko, *Koshihikari* (n = 31), (B) △, white core (n = 24); ×, non-white core (n = 31). p (0.01) = 0.590 (n = 18), 0.449 (n = 32), 0.456 (n = 31), 0.515 (n = 24), 0.456 (n = 31). Values are means of triplicate samples.

of the milky stage is likely the cause of the differences in the distribution of FIIb/FIIa values between *Yamadanishiki* and *Gohyakumangoku*. The distribution of FIIb/FIIa values for *Koshihikari* is broader than for the other varieties, which might be due to a larger growing region with different temperature conditions during grain filling. The amylose content of *Koshihikari* is lower than for the other varieties, as previously reported.[29] Genetic differences related to amylose biosynthesis may distinguish *Koshihikari* from other varieties.

These results indicate that structural characteristics of the starches varied widely among harvest areas even within the same rice cultivar.

Relationship between enzyme digestibility and amylopectin structure.

As shown in Fig. 1, starch retrogradation of steamed rice grains proceeded under sake-making conditions. Therefore, enzyme digestibility during the sake mash may change gradually from the early to the late stage of the mash. An enzyme digestion test was undertaken to estimate the efficiency of starch utilization under simulated sake-making conditions to estimate the real digestibility in the sake mash. The following three test conditions were evaluated. (A): digestion at 15°C for 24 h followed by storage at 15°C for 3 h = the national standard analysis method condition. (B): digestion at 4°C for 48 h followed by storage 4°C for 1.5 h. (C): digestion at 15°C for 24 h followed by storage at 15°C for 24 h. The representative enthalpy changes for amylopectin retrogradation under these conditions without addition of enzyme are shown in Table 2. The order of degree of retrogradation was (C) >> (B) > (A). In condition (C), the enthalpy change for retrogradation at 15°C for 24 h was 2.9 J/g corresponding to that at 5 days after the 2nd addition in sake mash as shown in Fig. 1 (B). The digestion test using condition (C) is likely a reasonable reflection of digestibility at the proceeded sake mash stage. On the other hand, the enthalpy change under conditions (A) and (B) was small and may reflect digestibility at an early stage of the sake mash.

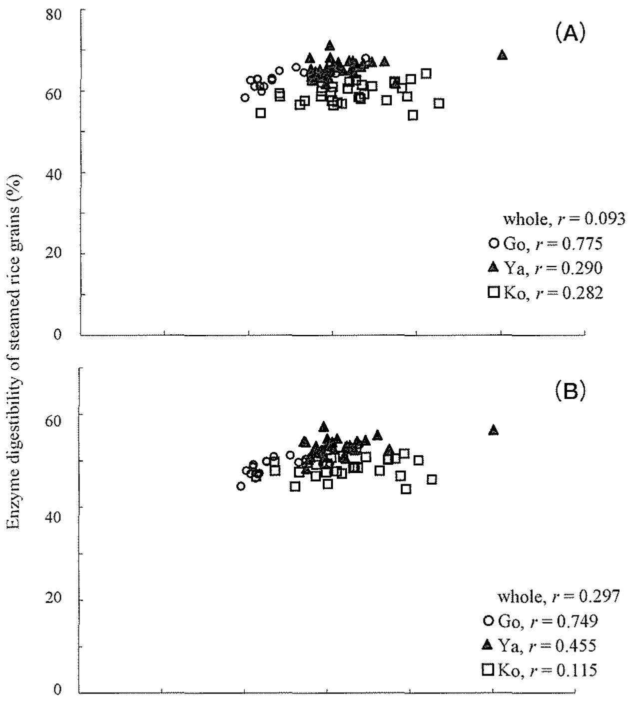
Table 2. Enthalpy changes for the amylopectin recrystallization peak from steamed rice grains held under enzyme digestion conditions.

Condition of retrogradation	Enthalpy change (J/g-starch)
(A) Storage at 15°C for 3 h in a plastic bag	0.0
Storage at 15°C for 3 h in a plastic bag + Storage at 15°C for 24 h in buffer	0.6
(B) Storage at 4°C for 1.5 h in a plastic bag	0.0
Storage at 4°C for 1.5 h in a plastic bag + Storage at 4°C for 48 h in buffer	1.9
(C) Storage at 15°C for 24 h in a plastic bag	2.8
Storage at 15°C for 24 h in a plastic bag + Storage at 15°C for 24 h in buffer	4.2

Yamadanishiki (Hiroshima) was used for measuring the retrogradation of amylopectin. Values are means of duplicate samples.

Enzyme digestibility (the ratio of hydrolyzed starch) of *Gohyakumangoku, Yamadanishiki* and *Koshihikari* was examined under conditions (A) and (B), and all samples were examined under condition (C) (Fig. 3). The order of enzyme digestibility was (A) > (B) >> (C), indicating that the retrogradation affected enzyme digestibility. Under condition (A), where retrogradation did not proceed very far, only *Gohyakumangoku* showed a significant positive correlation between the ratio of short-to-long chain amylopectin (FIIb/FIIa) and enzyme digestibility, but the correlation was weak in the other cultivars (Fig. 3 (A)). Under condition (B), the correlation coefficient increased slightly (Fig. 3 (B)). On the other hand, further retrogradation was observed under condition (C), where enzyme digestibility showed a highly significant positive correlation with the FIIb/FIIa ratio in each group (*Gohyakumangoku*; r = 0.959, *Yamadanishiki*; r = 0.950, *Koshihikari*; r = 0.919, white core; r = 0.931, non-white core; r = 0.809). The correlation coefficient for all samples was high (r = 0.709).

Wakai *et al.*[30] tested enzyme digestibility of steamed rice grains after storage at 4°C for 24 h in a plastic bag,



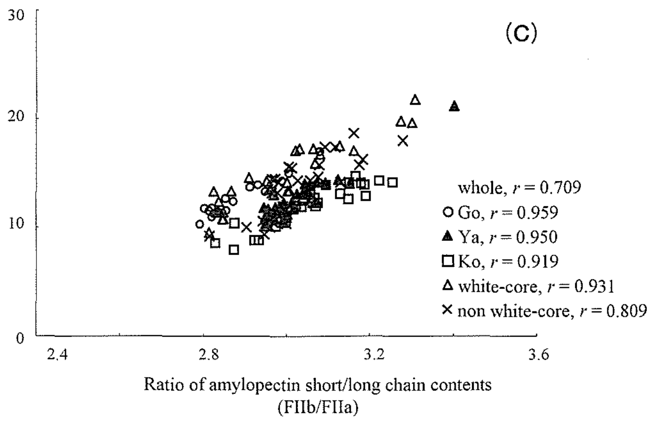


Fig. 3. Relationship between enzyme digestibility of steamed rice grains and ratio of short/long chain of amylopectin.

(A) digestion at 15°C for 24 h after storing at 15°C for 3 h in plastic bag, (B) digestion at 4°C for 48 h after storing at 4°C for 1.5 h in plastic bag, (C) digestion at 15°C for 24 h after storing at 15°C for 24 h in plastic bag. ○Go, *Gohyakumangoku* (n = 18); ▲Ya, *Yamadanishiki* (n = 32); □Ko, *Koshihikari* (n = 31); △, white core (n = 24); ×, non-white core (n = 31). p (0.01) = 0.590 (n = 18), 0.449 (n = 32), 0.456 (n = 31), 0.515 (n = 24), 0.456 (n = 31), 0.220 (n = 136). Values are means of duplicate samples.

which condition is similar to our condition (C) (Fig. 3). In their study,[30] enzyme digestibility showed a highly significant correlation with the sake cake ratio which indicates how much sake residue remains after the sake has been filtrated from sake mash. Their results suggested that condition (C) was the preferred condition for evaluating digestibility under sake-making conditions. Based on Wakai's report and our present results, the structure of amylopectin may have a significant influence on enzyme digestibility in the practical sake-making process. The observed correlation under conditions (A) and (B) was very weak, indicating that amylopectin structure does not have a major affect on enzyme digestibility when retrogradation does not proceed (Fig. 3). The weak correlation observed under conditions (A) and (B) might be due to the fast digestion that occurred after a short period of retrogradation. Under these conditions, other factors such as the histological structure of the rice grains might have greater affect on enzyme digestibility.

From the present results, we conclude that the retrogradation of starch in the steamed rice grains occurs under sake-making conditions, and that the digestibility is likely highly dependent on the structure of the amylopectin. The structure of starches in the various rice grains varied as a function of region where the rice was grown, even for the same cultivar. Therefore, the amylopectin structure appears to be the critical factor affecting digestibility of steamed rice grains during sake production.

Estimating enzyme digestibility from physical properties of starch:

Relationship between enzyme digestibility and gelatinization temperature measured by DSC.

The estimation of the digestibility of steamed rice grains in sake mash based on structural characteristics of amylopectin is problematic, because it requires a precise chemical analysis of the amylopectin. However, starch structural characteristics correlate with physical properties, including gelatinization temperature measured by DSC, a technique in which experimental data can be obtained rapidly and accurately by using small amounts of samples.[1,2,10–12,14] Therefore, we tried to evaluate enzyme digestibility in sake mash by using DSC.

The thermal properties of purified starch, brown rice flour and milled rice flour used in the present study were analyzed using DSC (Fig. 4). The means and range of the T_p for purified starch, milled rice flour and brown rice flour were as follows: *Yamadanishiki*, 66.3°C (61.3–67.8°C), 66.9°C (62.0–67.9°C), 68.8°C (63.5–69.7°C); *Gohyakumangoku*, 67.9°C (65.2–69.5°C), 68.8°C (66.6–70.0°C), 70.3°C (67.9–71.6°C); *Koshihikari*, 67.9°C (64.9–70.1°C), 69.3°C (66.6–71.9°C), 70.8°C (68.5–73.2°C), respectively. For the same sample, the T_p for brown rice flour was high, while values were moderate and low for milled rice flour and purified starch, respectively. In rice flour, starch swelling might be limited by the endosperm cell walls, protein, or other constituents. Prevention of swelling of the starch by these factors could result in high gelatinization temperatures.

As shown in Fig. 4, T_p values exhibited highly negative correlations with the ratio of short-to-long chain amylopectin (FIIb/FIIa) in both purified starch and milled/brown rice flour. The whole and range for the r values are as follows: purified starch, −0.818 and −0.966–−0.919; milled rice flour, −0.748 and −0.966–−0.938; brown rice flour, −0.755 and −0.973–−0.897. While the structural characteristics of amylopectin have been reported to correlate with the gelatinization temperature of purified starch measured by DSC,[1,2,12,14] the present results also indicate that the molecular structure of rice amylopectin can be estimated accurately by DSC even from brown and milled rice flour without need for purification of starch.

As shown in Fig. 5, the T_p measured by DSC corre-

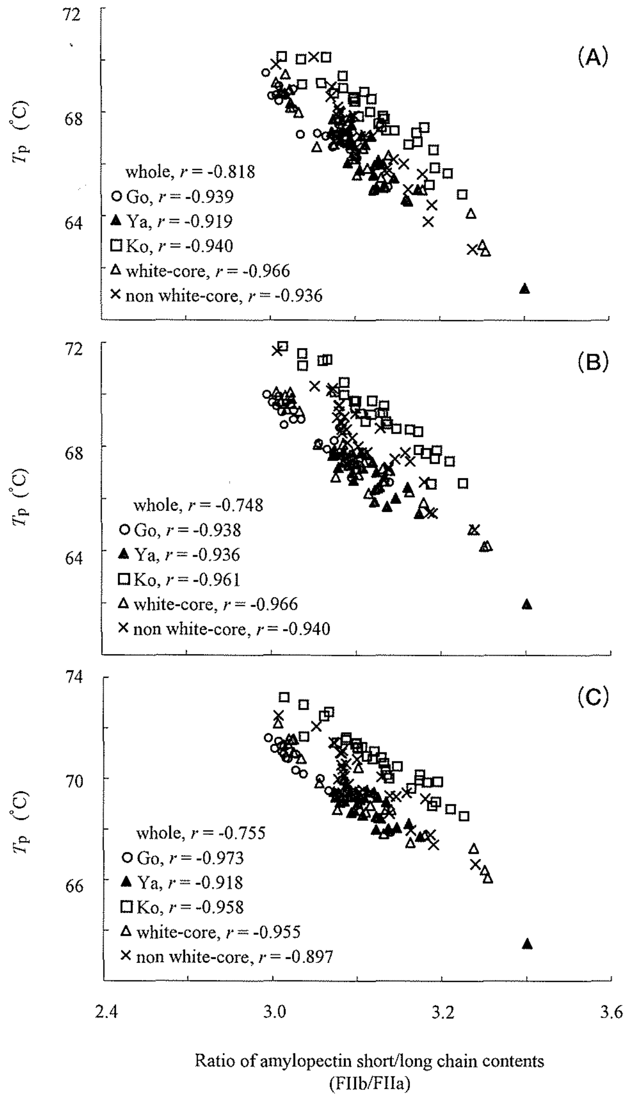


Fig. 4. Ratio of short/long chain amylopectin of rice samples *vs.* T_p measured by DSC.

(A) purified starch, (B) 70% milled rice flour, (C) brown rice flour. OGo, *Gohyakumangoku* (n = 18); ▲Ya, *Yamadanishiki* (n = 32); □Ko, *Koshihikari* (n = 31); Δ, white core (n = 24); ×, non-white core (n = 31). Values are means of duplicate samples.

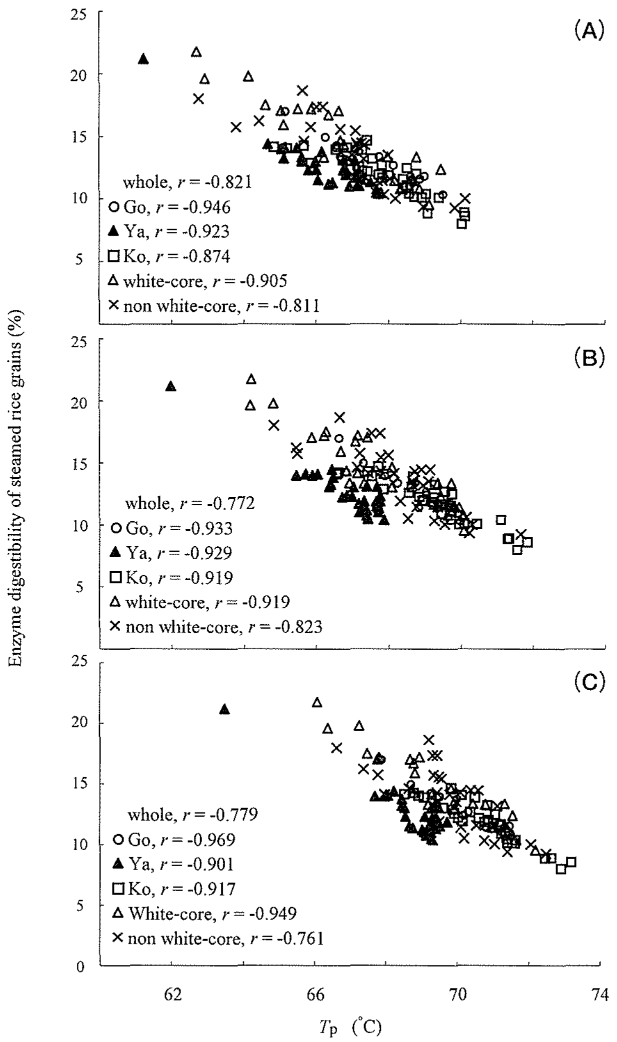


Fig. 5. T_p measured by DSC *vs.* enzyme digestibility of rice grains.

(A) purified starch, (B) 70% milled rice flour, (C) brown rice flour. OGo, *Gohyakumangoku* (n = 18); ▲Ya, *Yamadanishiki* (n = 32); □Ko, *Koshihikari* (n = 31); Δ, white core (n = 24); ×, non-white core (n = 31). The conditions for enzyme digestibility are defined in Fig. 3 (C).

lated well with enzyme digestibility of steamed rice grains after 24 h storage (condition (C) in Fig. 3), which accurately reflects sake-making conditions. The T_p exhibited a highly significant and negative correlation with enzyme digestibility in each cultivar. The correlation coefficients for each group were as follows: *Yamadanishiki*: r_{starch} = −0.923, $r_{\mathrm{milled\ rice}}$ = −0.929, $r_{\mathrm{brown\ rice}}$ = −0.901; *Gohyakumangoku*: r_{starch} = −0.946, $r_{\mathrm{milled\ rice}}$ = −0.933, $r_{\mathrm{brown\ rice}}$ = −0.969; *Koshihikari*: r_{starch} = −0.874, $r_{\mathrm{milled\ rice}}$ = −0.919, $r_{\mathrm{brown\ rice}}$ = −0.917; other white core rice: r_{starch} = −0.905, $r_{\mathrm{milled\ rice}}$ = −0.919, $r_{\mathrm{brown\ rice}}$ = −0.949; other non-white core rice: r_{starch} = −0.811, $r_{\mathrm{milled\ rice}}$ = −0.823, $r_{\mathrm{brown\ rice}}$ = −0.761; all samples: r_{starch} = −0.821, $r_{\mathrm{milled\ rice}}$ = −0.772, $r_{\mathrm{brown\ rice}}$ = −0.779.

These results indicate that enzyme digestibility of rice used for sake-making can be estimated precisely by gelatinization temperature using DSC and only one grain of brown/milled rice because the small amount of sample (5–15 mg) is enough for the DSC analysis.

Relationship between enzyme digestibility and pasting temperature measured by RVA.

RVA is also a useful tool for examining properties of starch. Pasting temperature of RVA measurement is a similar parameter to gelatinization temperature measured by DSC. The means and range of pasting temperatures for 70% milled rice flour from *Yamadanishiki*, *Gohyakumangoku* and *Koshihikari* were 66.1°C (61.3–67.5°C), 68.0°C (65.2–69.3°C) and 67.5°C (65.3–69.3°C), respectively. Those of other white-core and other non white-core rice flour samples were 67.0°C (64.8–69.1°C) and 67.0°C (64.8–69.6°C), respectively. As shown in Fig. 6, the pasting temperature showed a highly negative correlation with the ratio of short-to-long chain amylopectin (all samples; r = −0.790, each variety; r = −0.914–−0.852). These results indicate that the pasting temperature of milled rice measured by RVA is also useful for estimating amylopectin structure using samples consisting of milled rice flour.

As shown in Fig. 7, the pasting temperature measured by RVA correlated well with enzyme digestibility of steamed rice grains after 24 h storage (condition (C) in

Fig. 3). The correlation coefficients for each group were as follows: *Yamadanishiki*, $r = -0.863$; *Gohyakumangoku*, $r = -0.870$; *Koshihikari*, $r = -0.898$; other white core rice, $r = -0.869$; other non-white core rice, $r = -0.766$; all samples, $r = -0.708$. When the pasting temperature was higher, the enzyme digestibility of steamed rice grains was lower. On the other hand, when the pasting temperature was lower, enzyme digestibility was higher. Therefore, RVA analysis appears to be a useful tool for evaluating enzyme digestibility of steamed rice grains.

The national standard analysis method of rice for sake-making is usually used to evaluate digestibility of rice used in sake production. This method is time-consuming

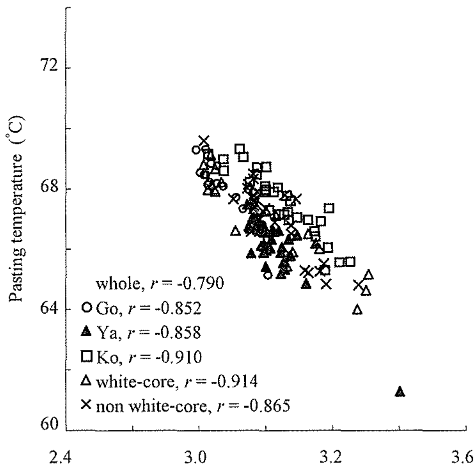


Fig. 6. Ratio of short/long chain amylopectin of rice samples *vs.* pasting temperature of 70% milled rice flour measured by RVA.

○Go, *Gohyakumangoku* (n = 18); ▲Ya, *Yamadanishiki* (n = 32); □Ko, *Koshihikari* (n = 31); △, white core (n = 24); ×, non-white core (n = 31).

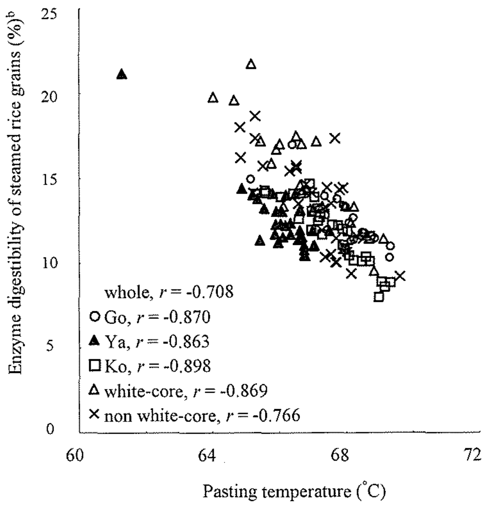


Fig. 7. Pasting temperature of 70% milled rice flour measured by RVA *vs.* enzyme digestibility of rice grains.

○Go, *Gohyakumangoku* (n = 18); ▲Ya, *Yamadanishiki* (n = 32); □Ko, *Koshihikari* (n = 31); △, white core (n = 24); ×, non-white core (n = 31). The conditions for enzyme digestibility are defined in Fig. 3 (C).

and labor-intensive as it involves adjusting moisture content, milling rice, enzyme digestion and analysis of the digests. In the present study, we determined that retrogradation of starch occurs in steamed rice grains under sake-making conditions. Enzyme digestibility under conditions that simulated the sake-making process (condition (C)) were found to correlate closely not only with amylopectin structure but also with gelatinization temperature as measured by DSC or pasting temperature as assessed by RVA. Therefore, DSC and RVA appear to have great potential utility estimating digestibility of steamed rice grains in sake mash because accurate data can be obtained rapidly using small samples without pretreatments such as purification of starch or isoamylase digestion. DSC is particularly powerful because a sample consisting of a single grain of brown rice (*ca.* 10 mg) is adequate, an advantage that lends itself to early use in breeding programs.

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REFERENCES

1) M. Okuda, I. Aramaki, T. Koseki, H. Satoh and K. Hashizume: Structural characteristics, properties, and *in vitro* digestibility of rice. *Cereal Chem.*, **82**, 361–368 (2005).
2) M. Okuda, I. Aramaki, T. Koseki, N. Inouchi and K. Hashizume: Structural and retrogradation properties of rice endosperm starch affect enzyme digestibility of steamed milled-rice grains used in sake production. *Cereal Chem.*, **83**, 143–151 (2006).
3) M. Asaoka, K. Okuno, Y. Sugimoto, J. Kawakami and H. Fuwa: Effect of environmental temperature during development of rice plants on some properties of endosperm starch. *Starch/Stärke*, **36**, 189–193 (1984).
4) M. Asaoka, K. Okuno and H. Fuwa: Effect of environmental temperature at the milky stage on amylose content and fine structure of amylopectin of waxy and nonwaxy endosperm starches of rice (*Oryza sativa* L.). *Agric. Biol. Chem.*, **49**, 373–379 (1985).
5) Y. Sano: Differential regulation of waxy gene expression in rice endosperm. *Theor. Appl. Genet.*, **68**, 467–473 (1984).
6) T. Umemoto, Y. Nakamura, H. Satoh and K. Terashima: Differences in amylopectin structure between two rice varieties in relation to the effects of temperature during grain-filling. *Starch/Stärke*, **51**, 58–62 (1999).
7) N. Inouchi, H. Ando, M. Asaoka, K. Okuno and H. Fuwa: The effect of environmental temperature on distribution of unit chains of rice amylopectin. *Starch/Stärke*, **52**, 8–12 (2000).
8) T. Koseki, M. Okuda, Y. Yonehara, K. Hachida, H. Iwata, I. Aramaki and K. Hashizume: Effects of high temperature during grain-filling on characteristics of rice for sake brewing. *J. Brew. Soc. Jpn.*, **99**, 591–596 (2004) (in Japanese).
9) Y. Yonehara, T. Koseki, M. Okuda, I. Aramaki and K. Hashizume: Effects of low temperature during grain-filling on characteristics of rice for sake brewing. *J. Brew. Soc. Jpn.*, **100**, 650–657 (2005) (in Japanese).
10) J. Jane, Y.Y. Chen, L.F. Lee, A.E. McPherson, K.S. Wong, M. Radosavljevic and T. Kasemsuwan: Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.*, **76**, 629–637 (1999).
11) Y. Nakamura, A. Sakurai, Y. Inaba, K. Kimura, N. Iwasawa and T. Nagamine: The fine structure of amylopectin in endosperm from Asian cultivated rice can be largely classified into two classes. *Starch/Stärke*, **54**, 117–131 (2002).
12) G.E. Vandeputte, R. Vermeylen, J. Geeroms and J.A. Delcour: Rice starches. I. Structural aspects provide insight into crystallinity characteristics and gelatinisation behaviour of granular

starch. *J. Cereal Sci*., **38**, 43–52 (2003).
13) G.E. Vandeputte, V. Derycke, J. Geeroms and J.A. Delcour: Rice starches. II. Structural aspects provide insight into swelling and pasting properties. *J. Cereal Sci.*, **38**, 53–59 (2003).
14) T. Horibata, M. Nakamoto, H. Fuwa and N. Inouchi: Structural and physicochemical characteristics of endosperm starches of rice cultivars recently bred in Japan. *J. Appl. Glycosci*., **51**, 303–313 (2004).
15) H. Toyoshima, H. Okadome, K. Ohtsubo, M. Suto, N. Horisue, O. Inatsu, A. Narizuka, M. Aizaki, T. Okawa, N. Inouchi and H. Fuwa: Cooperative test on the small-scale rapid method for the gelatinization properties test of rice flours with a rapid-visco-analyser (RVA). *Nippon Shokuhin Kagaku Kogaku Kaishi*., **44**, 579–584 (1997) (in Japanese).
16) H. Sato, S. Saito and T. Taira: Selection for high palatable rice lines by use of mido meter and rapid visco analyser. *Jpn. J. Crop Sci*., **72**, 390–394 (2003).
17) H. Sato, S. Saito and T. Yoshida: The hardness of glutinous rice cake, gelatinization and urea disintegration in waxy rice. *Jpn. J. Crop Sci*., **74**, 310–315 (2005).
18) M.X. Zhou and N.J. Mendham: Predicting barley malt extract with a rapid visco analyser. *J. Cereal Sci*., **41**, 31–36 (2005).
19) Y. Ikawa and C. Kouno (Kikuchi): Multiple evaluation of cooked rice retrogradation in early stage of storage. *J. Appl. Glycosci*., **2**, 293–298 (2005).
20) K. Yamamoto, S. Sawada and T. Onogaki: Effects of quality and quantity of alkaline solution on the properties of the rice starch. *J. Jpn. Soc. Starch Sci*., **28**, 241–244 (1981) (in Japanese).
21) I. Aramaki, R. Kanda, Y. Kikunaga, M. Yoshii, H. Iwata, M. Okuda, T. Koseki, M. Ogawa, T. Kumamaru, H. Satoh and K. Hashizume: Pasting and gelatinization properties of endosperm-mutant rice grains having a white core (Shinpaku) and analysis of their suitability for sake brewing. *J. Brew. Soc. Jpn*., **99**, 189–201 (2004) (in Japanese).
22) Y. Ikawa, C. Kikuchi, S. Kanehira, Y. Murakawa and S. Ijiri: Comparative study on evaluation methods for retrogradation of coocked rice at the early stage. *J. Appl. Glycosci*., **49**, 29–33 (2002) (in Japanese).
23) K. Miyoshi and K. Koyama: Retrogradation of steamed rice grains (part 1). *J. Brew. Soc. Jpn*., **70**, 277–281 (1975) (in Japanese).
24) K. Nagata and E. Nakazawa: Retrogradation of steamed rice grains (part 2). *J. Brew. Soc. Jpn*., **71**, 121–124 (1976) (in Japanese).
25) K.J. Zeleznak and R.C. Hoseney: The role of water in the retrogradation of wheat starch gels and bread crumb. *Cereal Chem*., **63**, 407–411 (1986).
26) M. Okuda, K. Kobayashi, T. Itani, I. Aramaki and K. Hashizume: Effects of milling ratio on properties of endosperm starches and rice flours from milled Japanese rice grains. *J. Appl. Glycosci*., **54**, 1–5 (2007).
27) F.D. Macdonald and J. Preiss: Partial purification and characterization of granule-bound starch synthases from normal and *waxy* maize. *Plant Physiol*., **78**, 849–852 (1985).
28) H. Hirano and Y. Sano: Enhancement of *Wx* gene expression and the accumulation of amylose in response to cool temperatures during seed development in rice. *Plant Cell Physiol*., **39**, 807–812 (1998).
29) I. Iwate: Breeding, production, physiology and quality of the famous Japanese rice variety Koshihikari. in *Specialty Rices of the World*. R.C. Claudhary, D.V. Tran and R. Duffy, eds., FAO Science Publishers, Enfield, pp. 243–284 (2001).
30) Y. Wakai, T. Mizuma, N. Miyazaki, T. Nagano and T. Yanagiuchi: Effect of properties of rice on suitability for Sake Brewing. *Seibutsukogaku*, **75**, 99–109 (1997) (in Japanese).

デンプンの性質が清酒醸造で蒸米の消化性に及ぼす影響と物理的特性の分析による迅速な消化性の推定方法

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清酒醸造条件下で蒸米中のデンプンの老化を調べた．アミロペクチンの老化に由来するエンタルピー変化量は，製麹およびもろみ工程中で増加した．清酒もろみ中のエタノールは，アミロペクチンの老化を促進した．清酒醸造に使用される日本産米 136 試料について酵素消化性とデンプン特性を調べた．清酒醸造条件での消化性をより正確に推定するために，15°C で 1 日放置した蒸米の酵素消化性試験を実施した．アミロペクチンの短鎖/長鎖比は酵素消化性と高い相関性を示し，このことからアミロペクチンの構造によって酵素消化性が予測できることが示された．玄米/精白米および精製デンプンの DSC (示差走査熱量計) による糊化温度はアミロペクチンの短鎖/長鎖比および酵素消化性と高い相関性を示した．さらに精白米の RVA (ラピッドビスコアナライザー) による糊化開始温度は，アミロペクチンの短鎖/長鎖比および酵素消化性と高い相関性を示した．以上の結果，DSC と RVA は，迅速にかつ少量の玄米あるいは精白米試料を用いて測定できるので，清酒醸造における蒸米の酵素消化性を推定するのに有用な装置であることが明らかとなった．