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One Pot Cooking of Rice Grains for Preparation of Rice-Gel Samples Using a Small-Scale Viscosity Analyzer

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Abstract: Rice-gel prepared by the following three steps: rice grain cooking, shearing of the cooked rice, and cooling for gel formation, is expected as a novel food ingredient for modification of various food products such as bread and noodles. To meet the demand for high-throughput systems for research and developments on the new rice gels, herein we established a mini-cooking system for preparation of rice gel samples from grains using a small-scale viscosity analyzer (Rapid Visco Analyzer; RVA). Polished rice grains (4 g) were cooked with 22 mL of water in a canister, and the paddle equipped in the canister was rotated at 2,000 rpm for 30 min (80 °C was used as a representative) to shear the cooked rice. The sheared paste was cooled to 10 °C at 160 rpm, and the initial gelation property was evaluated by viscosity analysis within the RVA. Alternatively, the sheared paste was transferred to an acrylic mold and kept at 4 °C for 0, 1, 3, and 5 days for determination of the hardness with a compression test. Compressive forces required to penetrate 20 % thickness for three tested rice cultivars were measured, and the trend of the value shifts during preservation is similar to the corresponding trend obtained in 300-g grain scale laboratory tests, whereas the individual values were halved in the former. This small cooking method could offer a useful assay system for a rapid evaluation in the breeding programs and in the high-throughput screening of additives for the modification of properties.

Key words: rice gel, rice grain, Rapid Visco Analyzer, small-scale preparation

INTRODUCTION

Rice is the major staple food in many Asian countries. It has been consumed as cooked grain rice and rice flour ingredients for various foods such as rice noodles, rice bread, and rice pudding. Starch is the main carbohydrate and energy source in rice grain and generally eaten after gelatinization not only for making it more digestible but also for forming sol or gel structures to give the foods unique structures, textures, or other characteristics. Tightly packed constituents (*i.e.*, amylose and amylopectin) in a semi-crystalline granule loosen by water absorption and gelatinization, and start to rearrange by inter- or intra-molecular interactions, which is inclusively called retrogradation.[1)] Some of the rearrangements by retrogradation are regarded as desirable for expressing unique characteristics, whereas others are taken as undesired by deteriorating food quality. Thus, it is important to evaluate and control the arrangements of starch constituents in both positive and negative aspects of food quality.

It has been recognized that the quality and added value of the products from rice grain are affected by both rice cultivar used as a raw material or an ingredient and changes caused by food processing or cooking. As for the former, plenty of rice cultivars with different amylose contents and amylopectin structures have been developed, widening the market by adaptation to specific demands of manufacturers and consumers.[2)] Food processing or cooking is an alternative determinant of food quality. Araki *et al*. (2009) reported that a milling process controls the amount of damaged starch in rice flour, which negatively correlates with specific loaf volume of one-loaf bread made from rice flour with wheat vital gluten.[3)] Also, for the manufacture of gluten-free rice bread, breakthroughs in preparation of rice batter with amorphous rice flour[4)] and with Pickering emulsion microstructure[5)] were proposed.

Recently, another novel process technology for rice gel production, which can be applied for a wide variety of products including rice bread[6)] and noodles,[7)] was proposed by Shibata *et al*. (2012);[8)] hereafter we use the term, "new

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Abbreviations: RVA, Rapid Visco Analyzer; HA1, high-amylose rice cultivar #1; HA2, high-amylose rice cultivar #2; Momi, Momiroman; Yume, Yumetoiro; Kosh, Koshihikari, Milk, Milky Queen; Koga, Koganemochi; Basm, Basmati Rice; Jasm, Jasmine Rice.

rice-gel" for the rice gel made by this process, to discriminate from conventional gels made from rice flour or starch. The process is composed of three individual steps: cooking of rice grain, high-speed shear treatment of the cooked rice, and cooling for gel formation. This breakthrough for widening the applicability of new rice-gel was discovered with high-amylose type rice cultivars. This suggests that adaptation of rice cultivar to the optimized process would be crucial, whereas the functions of amylose and amylopectin in grain for desirable gel formation are not clear. Therefore, to make this process more versatile, it is needed to accumulate fundamental data on the structure-function relationship of components in rice grain as well as the effects of each processing condition on the properties of products. For this purpose, a high-throughput (HTP) evaluation system is expected in the breeding program in which the amount of grain obtained from individual variety is generally small. In addition, the HTP system would be desirable for the rapid estimation of conditions for process optimization.

In this study, we report on the development of an HTP system for evaluation of new rice-gel production from rice grain by using Rapid Visco Analyzer (RVA). RVA, a powerful tool for evaluation of gelatinization and paste viscosity characteristics of milled flour,[9] is a one-pot apparatus for viscosity analysis with temperature and paddle-speed controllable system. Basically, flour of milled grain and distilled water are transferred into a test canister, a paddle is placed into the canister, and the canister is set in the apparatus for starting a program of heating/cooling. The paddle in the canister is rotated during the procedure to measure the viscosity of the slurry. This system enables to predict the sensory properties of cooked grain and processing properties of ingredients. It mimics the process of cooking, and parameters such as pasting temperature, peak viscosity, and setback viscosity could be used for prediction of properties during swelling of granules, breakdown of starch granule, and amylose retrogradation.

The versatility of RVA has been expanded by program optimization for certain purposes: Toyoshima *et al*. (1997) prolonged the holding time of the standard method at 93 °C from 4 to 7 min for detecting individual gelatinizing properties of rice flour samples.[10] Kapoor *et al*. (2004) used the apparatus for a small-scale test for cheese manufacture and compared the obtained data with those from pilot-scale manufacture.[11] In the meanwhile, rice-grain cooking in the canister of RVA and shearing of the cooked rice for one pot processing of rice-gel would be a unique attempt, to our best knowledge.

MATERIALS AND METHODS

Materials. Rice samples varying in amylose content were used. High-amylose rice cultivars #1 and #2 (HA1 and HA2, respectively) were grown at the Central Region Agriculture Research Center, NARO (Niigata, Japan). Momiroman (Momi) was obtained from a local farmer. Other rice cultivars and varieties, Yumetoiro (Yume), Koshihikari (Kosh), Milky Queen (Milk), Koganemochi (Koga), Basmati Rice (Basm), and Jasmine Rice (Jasm) were purchased at local markets. Isoamylase from *Pseudomonas* sp. was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). All other reagent grade chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Purified water (produced with a Milli-Q Integral/10, Merck-Millipore Ltd., Burlington, MA, USA) was used in all experiments.

Structural properties of the rice were characterized as follows: apparent amylose content was determined by iodine colorimetry,[12] amylose content by Concanavalin A (ConA) precipitation method using an amylose/amylopectin assay kit (Megazyme International Ireland), chain length distribution of amylopectin by high-performance anion exchange chromatography on a CarboPac PA-1 column equipped with a pulsed amperometric detector (HPAEC-PAD, Thermo Scientific Inc., Waltham, MA, USA) according to Nagamine and Komae (1996).[13]

Protocol design for new rice-gel preparation.

Cooking with a rice cooker. Polished rice grain (hereafter we use "rice grain") samples (100 g) were rinsed three times with water at room temperature followed by straining to remove excess water. After rinsing, samples were transferred to rice-cooker bowl and a total of 300 g of water, including those absorbed by the rice during washing, was added. The grain was cooked with a rice cooker (JBU-A, Tiger Cooperation, Osaka, Japan) using the "rice porridge mode." Temperature data logger (Superthermochron, KN Laboratories, Osaka, Japan) was used to collect temperature data during cooking.

RVA method for cooking rice. The method for cooking rice grain in an RVA (RVA4 equipped with a Thermocline for Windows Ver. 3.0, Newport Scientific, Inc., Warriewood, NSW, Australia) was developed according to the temperature data obtained during cooking in a rice cooker (RC in Fig. 1). Basically, the temperature was linearly raised, the heating rate was changed at inflection point, observed at 35, 80, and 90 °C during cooking with a rice cooker, to match the temperature profile. The temperature profile of above method is shown in Fig. 1 (RVA P). To confirm temperature profiles during rice cooking in RVA, 10 g of rice grain with 30 mL of water added (RVA 3x), or 5 g of rice grain with 25 mL of water added (RVA 5x), was cooked with an RVA using the above method. The method was confirmed by checking the actual temperature of the rice samples obtained during cooking in the RVA (RVA 3x and RVA 5x, respectively, in Fig. 1) using a temperature logger. Since the logger takes up some space in the canister, the center shaft and the blade of a paddle were removed, and only the upper part of the paddle was used during temperature logging. When the temperature logger was not put in the canister, an intact paddle was inserted, and the speed was set at 0 during cooking.

RVA method for high-speed shear. After cooking 4 g of rice grain sample with 22 mL of water added in the RVA canister according to the condition of RVA P in Fig. 1, the temperature was decreased to 80, 50, or 25 °C at 10 °C/min, then the speed was brought up to 2,000 rpm to give high-speed shear. The paddle speed was raised stepwise starting at 60 rpm and doubling every 5 s up to 480 rpm,

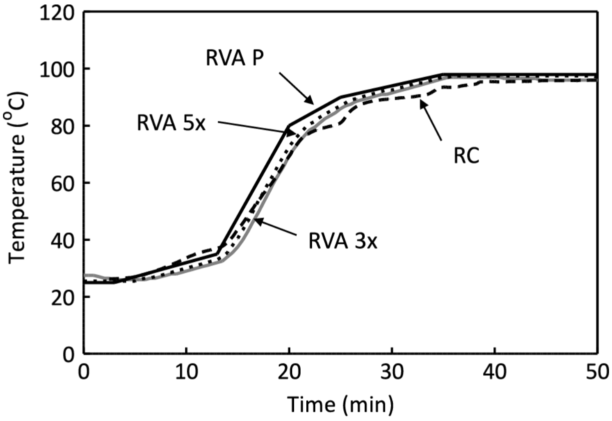

Fig. 1. Temperature profile during rice cooking.
Temperature profile during cooking with a rice cooker (RC) at porridge mode is shown in long dashed line (- - - -). Temperature program used in cooking with RVA is shown in solid black line (RVA P). Actual temperature change while cooking with RVA with 3-fold water (solid grey line, RVA 3x) or 5-fold water (dotted line, RVA 5x).

then to 2,000 rpm, and kept at the speed for a defined time.

RVA method for the measurement of viscosity development. After the high-speed shear treatment for 30 min, the paddle speed was brought down to 160 rpm for the measurement of viscosity development. The temperature was brought down to 10 °C at 10 °C/min and kept at 10 °C for 30 min. The temperature of the cooling unit was set to 7 °C, since setting at lower temperature resulted in the freezing of the water used for cooling. Viscosity value immediately after high-speed shear treatment was recorded as initial viscosity. Peak viscosity and final viscosity values were also recorded.

Continuous protocol from cooking rice to the measurement of viscosity. Four grams of rice grain samples were weighed in an RVA canister and 22 mL of distilled water was added. The canister was set onto the RVA with a paddle. The samples were cooked using the cooking method of RVA P in Fig. 1, followed by cooling at 10 °C/min to 80, 50, or 25 °C, high-speed shearing for 30 min, then cooling to 10 °C and viscosity measuring at 10 °C for 30 min at 160 rpm. The protocol lasted for a total of 119 min. A typical RVA method with shearing at 80 °C is shown in Table 1 and Fig. 2.

Determination of hardness of new rice-gel. The continuous protocol for 4-g rice grain was suspended immediately after the high-speed shearing step, and the canister was removed from the instrument. The rice paste was quickly poured into acrylic tubes with 36-mm diameter and 10-mm height while still hot using two spatulas and packed between two glass plates covered with parafilm to prevent the gel from sticking to the plate and kept at 4 °C for 1 h (0 day), 1, 3, or 5 days. The hardness of the rice gel samples was measured without removing from the acrylic tube using a rheometer (CR-500DX, Sun Scientific Co., Ltd., Tokyo, Japan) equipped with a 10-mm diameter cylindrical plunger. The rice gel was placed on a glass plate and uniaxially compressed with a plunger at a constant rate of deformation (1 mm/s) to 95 % of its original thickness. Compressive forces required to penetrate to 20 % thickness was

Table 1. An RVA method for continuous protocol from cooking rice to measurement of gel viscosity sheared at 80 °C.

	Time (h:mm:ss)	Type (Temp/Speed)	Value (°C or rpm)
1	0:00:00	Temp	25
2	0:00:00	Speed	0
3	0:03:00	Temp	25
4	0:13:00	Temp	35
5	0:20:00	Temp	80
6	0:25:00	Temp	90
7	0:35:00	Temp	98
8	0:50:00	Temp	98
9	0:51:48	Temp	80
10	0:51:48	Speed	60
11	0:51:53	Speed	120
12	0:51:58	Speed	240
13	0:52:03	Speed	480
14	0:52:18	Speed	2000
15	1:22:00	Speed	160
16	1:22:00	Temp	80
17	1:29:00	Temp	10
18	1:59:00	End	

It should be noted that this table is written in the format of an RVA program. Actual temperature and speed change are shown in Fig. 2.

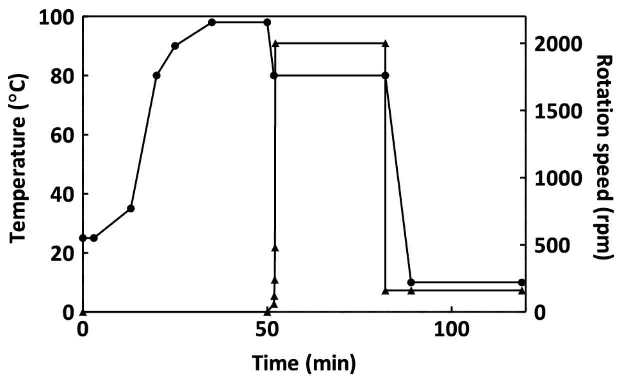

Fig. 2. An RVA method profile for continuous protocol from cooking rice to measurement of gel viscosity sheared at 80 °C: temperature (closed circle); rotation speed (closed triangle).

determined as the gel hardness.

Large scale rice-gel preparation. Three hundred grams of rice grain samples (HA1, HA2, or Yume) were measured in a rice-cooker bowl and a total of 900 g of water was added and soaked for 1 h. Rice was cooked with a rice cooker (ECJ-ES35, Sanyo Electric Co., Ltd., Osaka, Japan) using the "rice porridge mode". The cooked rice was cooled to 35 °C and transferred to a blender (Blixer-5 Plus, FMI Corp., Tokyo, Japan) and sheared at 1,500 rpm for 3 min. The rice gel was packed into a plastic petri dish with 86-mm diameter and 13-mm height and kept at 5 °C for 1, 3, or 5 days. The measurement at 1 h (0 day) was omitted because the gel was not set to obtain any reading. The hardness of the rice gel in the petri dish was measured using the rheometer. The center of a gel was uniaxially compressed with a 10-mm cylindrical plunger at 1 mm/s to 77 % penetration, and the gel hardness of the gel was determined as above.

RESULTS AND DISCUSSION

Protocol for new rice-gel preparation.

To 4 g of polished rice grain in a canister for RVA, 5.5-fold amount of water was added to make the cooked rice soft enough for the paddle to turn properly and shear the cooked grain. Too much or too less water resulted in insufficient shearing (data not shown). A rice cooking program in a canister for RVA was designed according to the profile of temperature shift in a commercial rice cooker at the porridge mode (Fig. 1), as referred to by Shibata *et al.* (2012).[8] The designed program for RVA and actual temperature shifts during rice cooking with 3- and 5-fold water were also shown in the figure, suggesting that temperature shift was reproduced well in the small cooking system. A total replacement of rice cooker with RVA is not practical since important factors like heat transfer patterns, water absorption patterns and patterns of water loss cannot be reproduced. Meanwhile, one of the critical points in the process for new rice-gel preparation should be the use of rice grain instead of rice flour for cooking. Rice grains absorb water from restricted paths in the grain, the central line serving as a channel for the migration of water into the grain.[14] Then the water penetrates along the amyloplast into the compound structure, and finally into single starch granules by capillary action.[15] The progression of gelatinization of starch in the grain during cooking depends on the soaking condition of the grain[16] and location of starch in the grain.[17] Whereas flour does not have such ordered structure, the water absorption condition would be different which affect the gelatinization condition during cooking.

During the cooking process, the paddle was kept inserted in the canister without rotation, and the grain remains its granulous form after cooking (data not shown).

In 1957, Batcher *et al.* reported a model experiment for cooking rice grain,[18] and numerous evaluation methods have been proposed, including a process by Nagato and Kishi (1966)[19] and Juliano *et al.* (1981).[20] More recently, Okadome *et al*. (1999) adopted a cooking system with 10-g grain with 16-mL water in an aluminum cup to cook in a commercial electric rice cooker.[21] Sasaki *et al*. (2018) used 16- to 27-g grain with water in a 100-mL homogenizer cup to heat at 98 °C to cook in a water bath.[22] Our system with RVA in this study would offer one of the smallest, automatic systems to cook rice grain.

Then, the program automatically starts the paddle to rotate for mashing the cooked rice grain in the canister. In the reference procedure for shearing with cooked rice corresponding to 200-g polished rice grain,[23] the cooked rice was transferred to a food processor equipped with an 11.25-cm cutting knife for shearing for 3 min at 3,000 rpm. Meanwhile, the maximum rotation speed of RVA is 2,000 rpm, and the paddle is 1.5-cm propeller-type shape; the maximum centrifugal acceleration (67 × G) is much lower than that of the reference (1,132 × G). We, therefore, extended the rotation time to 30 min for preparation of affordably homogenous paste of mashed rice grain. A stepwise raise of the rotation speed of the paddle was adopted at the initial stage of shearing to avoid the scattering of the sample to the side of the canister and above the paddle. An example of the whole RVA method for 4 g of rice grain is shown in Table 1, which is used for high-speed shearing at 80 °C and for analysis of initial network formation.

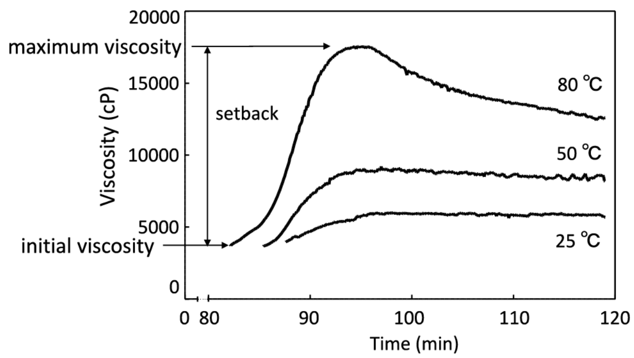

Fig. 3. Viscosity profile of cooled samples after high speed shearing at different temperatures.

Properties of initial network formation in new rice-gel.

The rapid cooling of the paste after high-speed shearing starts starch network formation to harden the matrix to organize a gel structure; this phenomenon is regarded as among the causes of retrogradation[1] and mainly attributed to amylose-amylose network formation.[24] The characteristics during the network formation at the initial stage of gelation of the cooled paste was continuously evaluated in the canister by using the same paddle as was used for shearing of the rice grain. This profile can be basically interpreted as the final holding stage in a typical RVA measurement.[1] Figure 3 shows the effect of shearing temperature on the viscosity profile of the cooled paste samples of Momiroman. In the tested condition, shearing at higher temperature gives higher maximum viscosity, implying that amylose-amylose network formed during low temperature shearing is weaker compared to that sheared at the higher temperature. The viscosity dropped as the rotation continued for 30 min implying that the network was being broken down during the final holding stage.

Shearing step would significantly affect the characteristics of formed gel, where various phenomena including amylose-amylose network formation and breakage, bubble production, and water loss would happen. Kokawa *et al.* (2017) observed a difference in the number- and the sizes of bubbles between new rice-gel sheared at high temperature and that at low temperature.[23] In this step as well as the cooking process, water loss should be taken into account because there is a gap between the ridge of the canister and the rid of the plastic paddle; the amount of water loss significantly increases when shearing condition at a high temperature is adopted. The average percentage of water loss after shearing at 80, 50, and 25 °C were 45, 29, and 26 %, respectively (data not shown). When the effect of shearing at different conditions is to be precisely compared, the total weight of each sample in the canister should be measured to calculate the water content of the formed paste or gel for further analysis, and water content at the formed paste or gel should be controlled based on preliminary experiments to estimate the water loss for each condition.

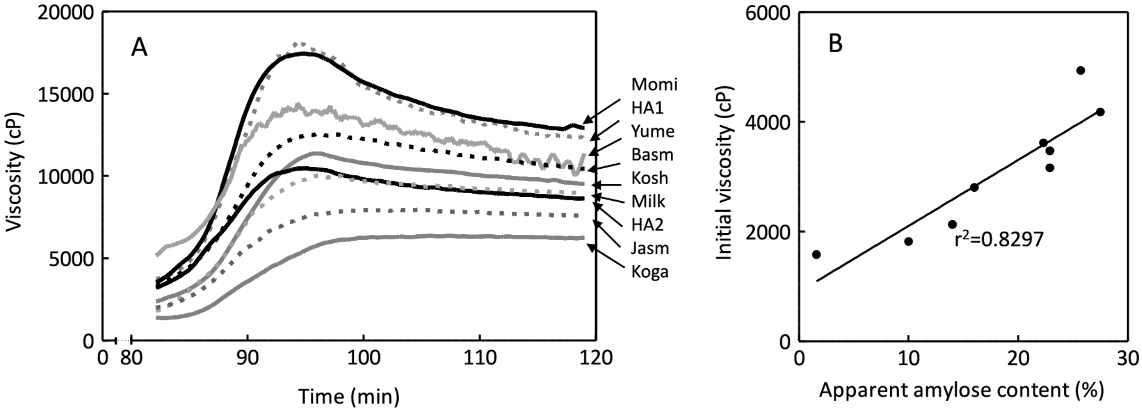

Fig. 4. Viscosity of various samples.
(A) Viscosity profile after high speed shearing at 80 °C. Average of two measurements. Names of rice varieties used are listed in the Materials section. (B) Correlation between apparent amylose content and initial viscosity.

Table 2. Structural characteristics of the rice samples.

Rice starch samples	Apparent amylose content (%)	Amylose content (%) by ConA method	Chain length distribution of amylopectin (area %)		
			DP 6–12	DP 13–24	DP 25–36
HA1	27.5	22.0	24.9	52.7	10.1
HA2	22.9	13.1	17.8	46.9	14.7
Yume	25.7	17.9	30.3	48.7	11.0
Momi	22.9	19.8	31.8	46.8	10.2
Kosh	16.0	15.4	28.7	47.6	11.5
Milk	10.0	8.7	29.0	47.3	11.7
Koga	1.6	1.5	29.0	49.8	11.3
Basm	22.3	22.4	25.7	50.9	10.4
Jasm	14.0	13.7	29.4	46.0	11.0

DP, degree of polymerization,

Next, we evaluated the viscosities of cooled paste samples made by shearing at 80 °C from various cultivars with different amylose-amylopectin structures (Table 2). The initial viscosity (a viscosity value immediately after high-speed shear treatment as shown in Fig. 3) of each cultivar was plotted as the function of apparent amylose content (Fig. 4B). A positive correlation (r^2 = 0.8297) was obtained between them, supporting that amylose-amylose interaction should be crucial in the initial stage of gelation. The correlation between the ConA amylose content and the value of initial viscosity was lower (r^2 = 0.5717), suggesting that certain amylopectin structures like super-long chains would participate at the beginning of the network formation.[25] Meanwhile, correlation between the viscosity increase (the setback) and apparent amylose content was lower than ConA amylose content (r^2=0.5435 and 0.6010, respectively) indicating more involvement of true amylose in the network formation.

Change of hardness of new rice-gel during storage.

Figure 5A shows the hardness of new rice-gel samples prepared from three cultivars: Yume, HA1, and HA2, after 0, 1, 3, and 5 days of storage at 4 °C, using a small-scale process established in this study. The compressive force required to penetrate 20 % before fracture shifted only slightly during storage of Yume, whereas the values dramatically increased in the cases of HA1 and HA2. The hardness of gel on 0 day was positively related to the amylose content. HA1 has a higher amylose content of 22.0 % by ConA method than the others (Table 2), suggesting a large amount of true amylose would contribute to the rapid formation of gel hardness at the first day of preservation. Although it was reported that amylose gel network is formed within 48 h of gelation,[24] the hardness of HA1 gel further increases after 3 and 5 days of preservation suggesting the involvement of amylopectin chains of DP 13–24.

HA2 has much lower true amylose content (13.1 %), whereas the content of apparent amylose is much higher (22.9 %). This gap implies that while hardness of the gel on day 0 is low due to low amylose content, long amylopectin chains expressed as DP 25–36 in Table 2, would interact with amylose network during preservation[26] resulting in even faster increase in the hardness compared to effect of DP 13–24.

Starch from Yume is known to possess a significant amount of super long chain[27] and has the similar gap of amylose contents by the two methods. Meanwhile, in Fig. 5A, the shift of gel hardness of Yume after 3 and 5 days of preservation appears to be very low compared to the others. It suggests that although the contribution of super long chain in Yume to the hardness of the gel on day 0 is high, contribution to the shift of gel hardness after 3 and 5 days of preservation would be limited. Shibata *et al.* (2012) found that new rice-gel samples prepared from Yume and Momi exhibit a stable rheological property during storage.[8] Shi and Seib (1992) reported that the mole fraction of short unit chains (of degrees of polymerization from 6 to 9) in amylopectin inversely correlates with retrogradation; the amylopectin structure may affect the stability of the gel during storage as both cultivars have relatively high proportions of the short chain (DP 6–12 in Table 2).[28]

The same samples (300 g) were used for a larger scale preparation using the laboratory-scale food processor equipped with an 11.25-cm cutting knife (Fig. 5B).[23] The trends of profiles during storage appears to be similar in all the three cultivars, whereas the individual values of com-

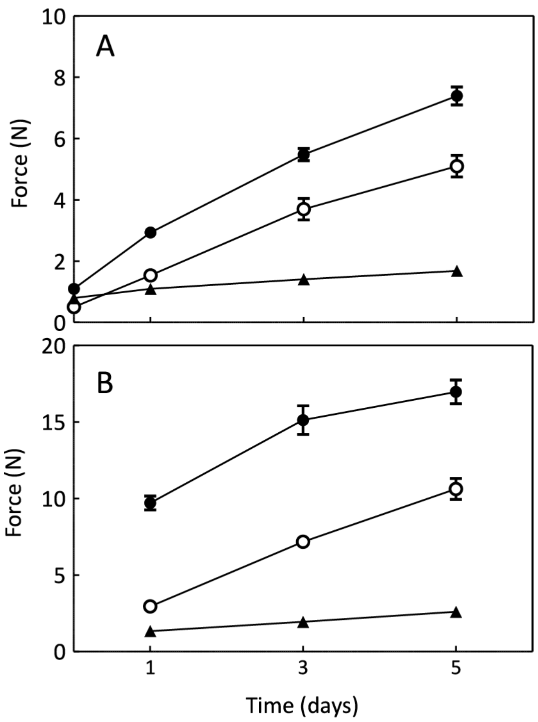

Fig. 5. Comparison of the hardness of rice-gels prepared from three rice varieties.

H2 (closed circle), H1 (open circle), Yume (closed triangle); prepared with RVA by shearing at 80 °C (A) or with a Blixer 5-Plus blender (B). Error bars represent SD (*n*=3).

pressive forces in the small-scale preparations were about a half of the larger-scale preparations. As mentioned above, the limitation in the upper speed of paddle rotation in the small-scale system would affect the actual gel hardness. Meanwhile, the small-scale system is advantageous over the food processor in terms of precise control of rotation velocity and temperature during the shearing step.

CONCLUSION

Herein we showed a model process for new rice-gel preparation and evaluation in a small-scale with 4 g of polished rice grain. Although some parameters during RVA manipulation such as rice-cooking temperature, rotation speed of the paddle, and water loss may have limitations of ranges for simulating commercial-scale processing, comparison of viscosity measurements using this small, one-pot model would make it possible to rapidly compile data on process development and optimization for new rice-gel preparation by modification of formulation, rice-cooking conditions, and shearing conditions before cooling for gelation. Also, this system would facilitate the screening of additives which affects the hardness of the new rice gel. The selection and optimization of grain are also crucial because unique amylopectin structure, as well as that of amylose, are suggested to affect important gel properties relating the structure and stability during storage. Currently, development of new rice varieties and cultivars with unique gelation properties is carried out using genomic information to modify the starch structure;[29] with various promotion activities for widening the usage of domestic rice grain,[2)27] the potential need for small-scale, high-throughput evaluation systems for rice grain cooking would be increasing. We believe that our new protocol can be used by various researchers with their own modifications to evaluate their focusing basic or applied phenomena for processing novel foods made from rice grain.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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