LWT - Food Science and Technology 146 (2021) 111379

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Cooking of short, medium and long-grain rice in limited and excess water: Effects on microstructural characteristics and gastro-small intestinal starch digestion *in vitro*

Masatsugu Tamura[a], Chisato Kumagai[b,1], Lovedeep Kaur[c], Yukiharu Ogawa[b,**], Jaspreet Singh[c,*]

[a] *School of Agriculture, Utsunomiya University, 350, Minemachi, Utsunomiya, Tochigi, 321-8505, Japan*
[b] *Graduate School of Horticulture, Chiba University, 648, Matsudo, Matsudo, Chiba, 271-8510, Japan*
[c] *Riddet Institute and School of Food and Advanced Technology, Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand*

ARTICLE INFO

Keywords:
Starch hydrolysis
Estimated glycemic index
Tissue structure
Viscosity
Water absorption

ABSTRACT

The purpose of present study was to investigate the impact of cooking methods on starch hydrolysis of rice grain using an *in vitro* digestion model. Three varieties of short, medium and long-grain rice were cooked in two different ways: in limited water method (LWM) using a rice cooker and excess water method (EWM) using a pan. The water absorption of raw rice grain was found to differ among the different rice varieties. The moisture, crude protein, total starch and resistant starch contents of the grain were affected by the cooking method. Starch hydrolysis for medium and long-grain rice at 210 min was higher for rice cooked through LWM (75.1 and 87.5%, respectively) than those cooked using the EWM (65.8 and 64.5%, respectively). Microscopic observations of grain cooked through LWM and EWM showed that the former had bigger voids present throughout the grain and had more cell wall damage than the latter, confirming that the microstructural characteristics were responsible for better enzyme accessibility and higher starch hydrolysis. These results revealed that the starch digestibility of rice grain cooked through different methods was affected by the disruption of the tissue structure that was dependent on the cooking method.

1. Introduction

Foods consist mostly of carbohydrate, such as rice, are digested and absorbed in the small intestine, causing a rapid rise in blood glucose level. A rapid rise in glucose has been related to the onset of lifestyle-related diseases such as type 2 diabetes, hyperlipidemia and heart disease (Jenkins et al., 2002; Lehmann & Robin, 2007).

Unlike other major grains, rice is principally cooked and consumed as a whole grain. Rice, which accounts for 29% of energy, 14% of protein and 58.5% of glycemic index (GI) for Japanese adults' total intake per day (Murakami et al., 2006; Sugiyama, Tang, Wakaki, & Koyama, 2003), is available in both high and low glycemic index versions (Atkinson, Foster-Powell, & Brand-Miller, 2008). The digestion of rice starch is influenced by both endogenous and exogenous factors. Amylose content is the primary endogenous factor, which has been associated with starch digestion (Chung, Liu, Huang, Yin, & Li, 2010; Miller, Pang, & Bramall, 1992; Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013). Hu, Zhao, Duan, Linlin, and Wu (2004) and Panlasigui et al. (1991) concluded that starch digestibility was affected not only by amylose content but also other factors like interactions of starch and proteins/lipids (Ye et al., 2018) and amylopectin branch chain length distribution (Chung, Liu, Lee, & Wei, 2011). Amount and properties of components such as amylose, amylopectin, protein and lipid vary among varieties, for instance short, medium and long-grain rice. On the contrary, the exogenous factors refer to processing such as post-harvest drying (Donlao & Ogawa, 2018), polishing (Miller et al., 1992), milling (O'dea, Snow, & Nestel, 1981), soaking (Han & Lim, 2009), boiling (Tamura, Singh, Kaur, & Ogawa, 2016b), blending (Tamura, Okazaki, Kumagai, & Ogawa, 2017) and post-cooking storage conditions (Tamura, Singh, Kaur, & Ogawa, 2019).

---

* Corresponding author.
** Corresponding author.
E-mail address: J.X.Singh@massey.ac.nz (J. Singh).
[1] Present address: Fukushima Agricultural Technology Center, 116 Shimonakamichi, Takakura, Hiwadamachi, Koriyama, Fukushima, 963–0531, Japan.

https://doi.org/10.1016/j.lwt.2021.111379
Received 31 August 2020; Received in revised form 10 March 2021; Accepted 24 March 2021

Cooking method, in particular, has been known to influence starch digestion significantly. For instance, microwave cooking leads to higher starch digestibility of rice than conductive heating (Li, Han, Xu, Xiong, & Zhao, 2014). Stir-fried rice displays lower starch hydrolysis compared with steamed rice and pilaf (Reed, Ai, Leutcher, & Jane, 2013). Cooking temperature has a more pronounced effect on the *in vitro* digestibility of rice than cooking time (He, Qiu, Liao, Sui, & Corke, 2018). Cooking at low temperature leads to the reduction in equilibrium starch hydrolysis, kinetic constant and estimated glycemic index (eGI) (Guillén, Oria, & Salvador, 2018). Starch hydrolysis of non-waxy cooked rice has been reported to be not affected by pressure, soaking and water-to-rice ratio during cooking (Wang et al., 2017). However, in these studies, rice samples were prepared as a slurry or dried powder, which are different from the actual sample where the tissue structure is maintained at the time of intake or consumption. The tissue structure of rice impacts on starch digestibility more than starch gelatinization (Tamura et al., 2016b). The tissue structure of rice has also been related to kinetic constant of starch digestibility (Tamura, Singh, Kaur, & Ogawa, 2016a). In a recent study (Thuengtung, Matsushita, & Ogawa, 2019), structural damage of cooked rice grain was reported to vary between steam cooking and microwave cooking that influenced starch hydrolysis rate during *in vitro* starch digestion.

There are two common types of rice cooking methods -limited water method (fixed water-to-rice ratio, LWM) and excess water method (excess-water/minimum cooking time, EWM). Both these methods have been compared for their effects on texture, consumer preference (Maruyama, Sakamoto, & Okai, 1995), total starch (TS) and resistant starch (RS) contents (Chen, Bergman, McClung, Everette, & Tabien, 2017) of rice grain. However, there is little research done on starch digestibility in relation to the microstructural characteristics of rice grain. The purpose of the present study was to investigate the impact of different cooking methods on microstructure of rice grain and how that is related to starch hydrolysis in rice from short, medium and long-grain rice varieties.

2. Material and methods

2.1. Materials

Rice from three commercial varieties representing short (Sushi rice, Japanese style, cv. Opus, SunRice, Leeton, Australia), medium (Australian medium-grain, cv. Calrose, SunRice) and long (Basmati, Aromatic rice, cv. Basmati 198, SunRice) grain rice were purchased from the local supermarket (Palmerston North, New Zealand) and stored in a refrigerator at 4 °C before the experiment. Simulated gastric fluid (SGF) was prepared by pepsin (porcine gastric mucosa, 800–2500 units mg^{-1} protein, Sigma–Aldrich, St Louis, MO, USA) and simulated intestinal fluid (SIF) was prepared by pancreatin (hog pancreas, 4 × USP, Sigma–Aldrich), invertase (grade VII from baker's yeast, 401 U mg^{-1} solid, Sigma–Aldrich) and amyloglucosidase (E-AMGDF-100ML, 3260 U mL^{-1}, Megazyme International Ireland, Wicklow, Ireland) according to Dartois, Singh, Kaur, and Singh (2010) and Tamura et al. (2016a), respectively. For apparent viscosity analysis, three times concentrated SIF and control (SIF without any digestive enzymes) were prepared using the methods of Bordoloi, Singh, and Kaur (2012) and Tamura et al. (2016b).

2.2. Sample preparation

To obtain cooked rice samples, two types of cooking methods, LWM (Tamura et al., 2014a, 2014b) and EWM (Tamura et al., 2016b), were applied to three varieties of polished raw rice grain. For cooking of LWM, 40 g of the grain was soaked in 60 mL of Reverse Osmosis water (RO water) in a 100 mL beaker for 30 min at room temperature. The beakers with soaked grain were set in an electric rice cooker (TK-RC12, Eupa, Tokyo, Japan) with 250 mL of RO water poured around the beakers as heat medium, and was cooked for approximately 35 min, followed by steaming for 10 min in the rice cooker. Note that steaming plays a role in ripping rice and is usually done during cooking rice in Japan. For cooking using the EWM, 30 g of the grain was boiled in 500 mL of boiled RO water in a pan. Cooking time was set as the time at which the white core of rice grains disappeared when rice grains were compressed between two glass slides (Tamura et al., 2016b). Afterwards, rice cooked using both the methods were cooled down in petri dishes wrapped with a cling wrap at room temperature for 30 min. A portion of raw and cooked grains was freeze-dried (FD18LT, Cuddon Engineering, Blenheim, New Zealand) at 25 °C for 48 h after pre-freezing at −20 °C for 24 h. The freeze-dried grain was then ground and passed through a 0.5 mm mesh sieve to obtain powdered samples for analyzing their composition.

2.3. Moisture content and water absorption

The moisture content of the grain was analyzed using the standard AOAC method (AOAC, 1990). Cooked grain was dried at 105 °C for 24 h in an air dryer (Oven 8150, Labserv, Longford, Ireland) and their moisture content was calculated as the difference in their weight before and after drying.

For measuring water absorption, the raw grain weighing about 2 g were placed into commercially available polythene nets, for easy retrieval from the soaking water. The bagged and weighed grain were placed into individual glass test-tubes, containing 40 mL of RO water, and placed in a water bath at 30 and 98 °C to simulate the soaking and cooking process, respectively. The grain was soaked for 5, 10, 15, 20, 30, 60, 90, 120 and 180 min at each temperature. After soaking, the bags were removed from the water and excess water was wiped with a thin paper cloth. The bags were then reweighed to an accuracy of 0.1 mg to determine the amount of water absorbed.

To estimate the plots of moisture content during soaking of rice grain reached equilibrium moisture content, exponential model, known as the most simplified model, was used.

$$\mathrm{MR} = (\mathrm{M_t} - \mathrm{M_e})/(\mathrm{M_0} - \mathrm{M_e}) = \exp(-\mathrm{k_1 t}) \quad (1)$$

where MR is moisture ration, M_t is moisture content at any time t, M_e is equilibrium moisture content, M_0 is initial moisture content and k_1 is rate constant.

2.4. Crude protein (CP) content

Powdered samples were weighed, and nitrogen content was examined using a CN Coder (SUMIGRAPH, NC-TRINIT, Sumika Chemical Analysis Service, Tokyo, Japan). CP content was calculated by the determined nitrogen content, and a 5.95 nitrogen-protein conversion factor. DL-Aspartic acid (000–05812, Kishida chemical, Osaka, Japan) was used as the standard nitrogen material.

2.5. Apparent amylose (AA) and starch content

AA content of the rice grain was measured using the method described by Tamura et al. (2014b). Potato amylose (A0512, Sigma-Aldrich) was used as a standard material. The TS and RS contents of the powdered samples were determined using a resistant starch kit (K-RSTAR 08/11, Megazyme International Ireland).

2.6. *In vitro* gastro-small intestinal starch digestion and kinetics of starch hydrolysis

A simulated two-stage gastro-small intestinal *in vitro* digestion model (Tamura et al., 2016a, 2016b) was used in this study. The supernatant (0.5 mL) was collected to analyze the glucose content after 5 and 30 min of gastric digestion, and after 5, 10, 15, 30, 60, 90, 120 and 180 min of small intestinal digestion.

The supernatants were mixed with 3 mL of 95% ethanol, centrifuged at 1800×*g* for 10 min and incubated for 10 min at 37 °C with amyloglucosidase and invertase solutions (Dartois et al., 2010). The glucose concentration of the incubated mixture was measured using the D-glucose assay kit (GOPOD Format K-GLUK 07/11, Megazyme International Ireland). Percentage of starch hydrolysis was calculated using the following equation:

$$\%SH = (S_h / S_i) = 0.9(G_p / S_i) \tag{2}$$

where %SH is a percentage of starch hydrolysis, S_h is the mass of hydrolyzed starch, S_i is the initial mass of TS, and G_p is the mass of glucose produced. A conversion factor of 0.9, which is generally calculated from the molecular weight of starch monomer/molecular weight of glucose (162/180 = 0.9), was used (Goñi, Garcia-Alonso, & Saura-Calixto, 1997).

A first-order equation model (Goñi et al., 1997), as shown below, was applied to describe the kinetics of starch hydrolysis:

$$C = C_\infty[1 - \exp(-k_2 t)] \tag{3}$$

where C corresponds to the percentage of hydrolyzed starch at time t, C_∞ is equilibrium concentration of starch in the gastro-small intestinal digestion process and k_2 is the kinetic constant. Parameter estimation was carried out using the software, Igor Pro (ver. 4.01, Hulinks, Tokyo, Japan).

The quality of fit for the first-order equation models on the experimental data was evaluated using the coefficient of determination (R^2) (Eq. (4)) and root mean square error (RMSE) (Eq. (5)).

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(C_{exp,\ i} - C_{pre,\ i}\right)^2}{\sum_{i=1}^{N}\left(C_{exp,\ i} - C_{ave}\right)^2} \tag{4}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\left(C_{exp,\ i} - C_{pre,\ i}\right)^2}{N}} \tag{5}$$

Where, $C_{exp,\ i}$ is the *i*th experimental percentage of hydrolyzed starch, $C_{pre,\ i}$ is the *i*th predicted percentage of hydrolyzed starch, C_{ave} is the average of experimental percentage of hydrolyzed starch and N is the number of observations.

Hydrolysis index (HI) of rice samples was calculated as the area of the curves for starch hydrolysis during small intestinal digestion, using white part of sliced bread (Choujuku, Pasco Shikishima corpolation, Aichi, Japan). The eGI was calculated using the following equation (Goñi et al., 1997):

$$eGI = 39.71 + 0.549HI \tag{6}$$

2.7. *Apparent viscosity of rice pre- and post-digestion*

Rice slurries were prepared by blending (HB605, Kenwood, Havant, UK) cooked and cooled rice samples with appropriate amount of RO water for 2 min to achieve a final TS concentration of 8%. The apparent viscosity of homogenized 35 g of the slurry sample was measured using a dynamic rheometer (AR-G2, TA instruments, New Castle, DE, USA) equipped with a peltier cylinder system according to Bordoloi et al. (2012) and Tamura et al. (2016b).

2.8. *Microscopic imaging and histochemical analyses*

The sample grains were prepared for microscopic observation using a simple sectioning method (Ogawa, Glenn, Orts, & Wood, 2003; Tamura & Ogawa, 2012). Images were captured using a digital camera (DS-5M, Nikon, Tokyo, Japan) mounted on a fluorescent stereomicroscope (MZ-FLIII, Leica, Wetzlar, Germany) equipped with a 100 W mercury arc lamp (ebq 100 dc, Leica) and an ultraviolet fluorescence filter set (360/40 nm excitation filter, 420 nm barrier filter, Leica) for fluorescence observation, and with a halogen lamp for observation of the tissue morphology. The captured images were processed and analyzed by graphic software (Paintshop Pro, Corel, Ottawa, Canada). The processed image composited of both the fluorescent and transmission images captured at the same position can show both of the actual section size for the grain and cell distributions (Tamura & Ogawa, 2012). The pixel numbers of the whole cooked rice grain, cracks and voids were measured by binarizing the transmission images with ImageJ software (Schneider, Rasband, & Eliceiri, 2012). For cooked rice grains, the ratio of cracks and voids (RCV) was calculated from the number of pixels of cracks and voids in the total number of pixels of whole cooked rice grain.

2.9. *Statistical analysis*

Results were calculated as means ± standard deviations. The analyses were performed at least in triplicate for each sample. Statistical analysis was conducted using R software (R Core Team, 2014). After outliers were removed using a Smirnov–Grubbs test, Tukey's test was used in conjunction with an analysis of variance (ANOVA) and two-way analysis of variance (two-way ANOVA) to identify differences among the means, at an *a priori* significance level of $p < 0.05$. Comparisons between cooking methods were done with T-test. Pearson's product-moment correlation coefficient was used as a measure of the linear correlation between the different rice properties.

3. Results and discussion

3.1. *Cooking times and water absorption*

Soaking is needed for water to diffuse into the grain core to produce a desirable product. For EWM, cooked rice grain with no white core, were obtained by boiling short, medium and long-grain rice for 27, 25 and 20 min, respectively. Note that cooked rice grain with no white core were also obtained by cooking of LWM.

Fig. 1 shows the changes in moisture contents of three varieties of raw rice grain during soaking at (a) 30 and (b) 98 °C. Moisture content of raw rice grain varied between 13.3 and 13.7% d.b. among different varieties. During soaking at 30 °C, the moisture content of the raw rice grain increased before reaching an equilibrium. This behavior was clearly found in the long-grain rice. On the contrary, moisture contents of short and medium-grain rice attained saturation after 120 min. The highest M_e was determined for medium-grain rice (70.3% d.b.), followed by short (70.0% d.b.) and long-grain rice (43.4% d.b.), while the highest k_1 was long-grain rice (0.039 min^{-1}), followed by short (0.020 min^{-1}) and medium-grain rice (0.015 min^{-1}). During soaking at 98 °C for 30 min, the moisture content of the rice grain was increased linearly. The moisture contents of rice grain from different varieties after soaking at 98 °C were different from those after soaking at 30 °C in the order of long > short > medium at almost all times. The increase in water absorption of rice grain at 98 °C would possibly be due to increase of gelatinized starch. Kasai, Lewis, Ayabe, Hatae, and Fyfe (2007) also found differences in water diffusion and starch gelatinization during cooking between short and long-grain rice, even though their rice samples had similar amylose content. Kang, Hwang, Kim, and Choi (2006) observed that protein bodies in medium and long-grain rice are scattered throughout the endosperm cell (around the starch granules) while those in short-grain rice are concentrated near the cell wall. The authors discussed the distribution of protein bodies in medium and long-grain rice appears to prevent water from entering the starch

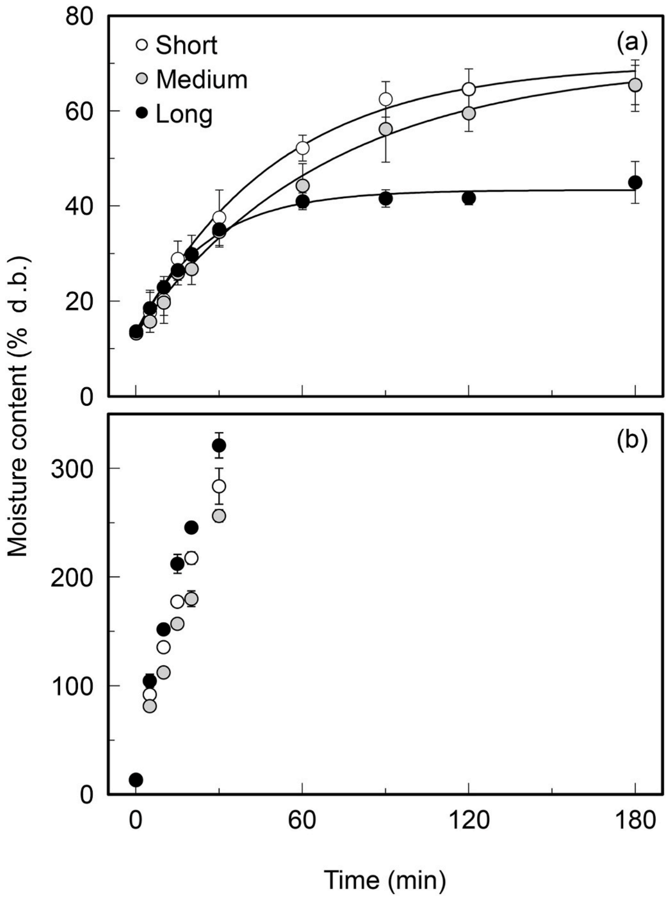

Fig. 1. Changes in moisture contents of three varieties of raw rice grain during soaking at (a) 30 and (b) 98 °C. Error bars represent standard deviation (n = 4–5). LWM: limited water method; EWM: excess water method.

granules and being absorbed. The water absorption in raw rice grain has been thought to vary with the type and crystal structure of starch and starch-protein complex (Ebata & Hirasawa, 1982; Okuno & Adachi, 1992). It was important to note that the water absorption at 30 and 98 °C did not correlate to cooking time of EWM.

3.2. *Composition*

Table 1 shows moisture, CP, AA, TS and RS contents of three varieties of raw and cooked rice from different varieties. As expected, the moisture contents of rice cooked with LWM (62.19–65.08% w.b.) were significantly lower than those cooked using EWM (70.75–74.35% w.b.). Generally, cooked rice grain prepared by LWM had lower moisture content than those cooked by EWM, because starch granules have limited water available to absorb and to gelatinize the starch, and the absorbed water evaporate in the process of steaming.

CP content of raw rice was highest for long-grain rice followed by medium and short-grain rice with significant differences, which significantly decreased upon cooking by both the methods. The decrease of CP content during cooking might be due to breaking down of protein into nitrogen-containing aroma components (Yang, Shewfelt, Lee, & Kays, 2008) and removing the germ from the grain.

Raw long-grain rice had significantly higher AA content than medium and short-grain rice. The AA content of short and long-grain rice did not change upon cooking, although that of medium-grain rice significantly decreased after cooking. TS content was the highest in raw, followed by cooking of LWM and EWM, regardless of the variety. The high standard deviation values for TS may be the reason for the sum of average CP and TS for medium-grain rice to be >100%. In previous studies using non-waxy polished rice, AA content has been reported to decrease (Rashmi & Urooj, 2003; Sagum & Arcot, 2000; Zhu et al., 2020) or change by a small extent (Guillén et al., 2018) during cooking. TS content reportedly increased (Marsono & Topping, 1993), decreased (Chung et al., 2010; Sagum & Arcot, 2000) or remained unchanged (Guillén et al., 2018; Zhu et al., 2020) during cooking. In this study, the decrease in AA and TS contents during cooking could be due to leaching of amylose and amylopectin into the cooking water, and their hydrolysis to low molecular dextrins and reducing sugars (Ikeda, 2001), and complex formation with lipids and proteins (Rashmi & Urooj, 2003). Amylose and amylopectin have been reported to selectively elute during cooking, depending on variety (Hanashiro, Ohta, Takeda, Mizukami, & Takeda, 2004). Zhu et al. (2020) have indicated that preferential leaching of amylose during cooking may not impact macroscopic characteristics of rice grains.

RS content was significantly highest for rice cooked using LWM followed by rice cooked by EWM and raw rice. According to previous reports, RS content increased (Chung et al., 2010; Marsono & Topping, 1993; Parchure & Kulkarni, 1997) or decreased (Guillén et al., 2018; Sagum & Arcot, 2000) during cooking. The changes depend on the variety, cooking method, water to rice ratio, soaking temperature and moisture content (Guillén et al., 2018; Han & Lim, 2009; Parchure & Kulkarni, 1997; Yang et al., 2006), and the method used to determine RS content (Yang et al., 2006). In this study, RS is probably formed due to the retrogradation of amylose, during cooling of rice, defined as RS3 (Tian & Sun, 2020). In LWM, amylose that leached during the cooking process would be re-absorbed in the grain during later stages of cooking due to water evaporation. Due to retrogradation of the leached amylose fraction (RS3), cooked rice grain of LWM would have higher RS content than that of EWM.

Table 1
Moisture, CP, AA, TS and RS contents of three varieties of raw and cooked rice.

		Moisture (% w.b.)	CP (% d.b.)	AA (% d.b.)	TS (% d.b.)	RS (% d.b.)
Short	Raw	13.28 ± 0.04 c, y	7.44 ± 0.03 a, z	27.95 ± 0.58 a, y	87.12 ± 8.40 a, x	0.12 ± 0.07 c, x
	LWM	65.08 ± 1.02 b, x	7.04 ± 0.01 b, z	24.87 ± 0.61 a, y	80.19 ± 6.74 ab, x	0.96 ± 0.05 a, y
	EWM	74.35 ± 0.15 a, x	6.76 ± 0.09 c, z	25.22 ± 0.17 a, y	69.71 ± 1.46 b, x	0.45 ± 0.03 b, y
Medium	Raw	13.27 ± 0.08 c, y	8.95 ± 0.15 a, y	26.74 ± 1.33 a, y	93.14 ± 4.77 a, x	0.11 ± 0.00 b, x
	LWM	62.19 ± 0.85 b, y	8.75 ± 0.02 ab, y	24.20 ± 0.89 b, y	82.92 ± 0.42 ab, x	0.80 ± 0.17 a, y
	EWM	70.75 ± 0.31 a, z	8.47 ± 0.03 b, y	24.67 ± 0.65 b, y	78.00 ± 8.43 b, x	0.26 ± 0.01 b, z
Long	Raw	13.68 ± 0.05 c, x	9.77 ± 0.14 a, x	34.99 ± 2.29 a, x	88.38 ± 4.78 a, x	0.20 ± 0.02 c, x
	LWM	63.41 ± 1.40 b, xy	8.83 ± 0.02 b, x	34.39 ± 1.41 a, x	81.74 ± 0.61 a, x	1.89 ± 0.04 a, x
	EWM	71.89 ± 0.21 a, y	8.90 ± 0.02 b, x	34.93 ± 0.66 a, x	68.20 ± 5.45 b, x	1.26 ± 0.06 b, x

Means ± standard deviation.
The sample number (n) was as follows: moisture (n = 3–4); CP (n = 4–7); AA (n = 4–7); TS (n = 3–5); RS (n = 2–6).
Different letters (a-c) within the same column and same variety indicate significant differences ($p < 0.05$).
Different letters (x-z) within the same column and same preparation indicate significant differences ($p < 0.05$).
CP: crude protein; AA: apparent amylose; TS: total starch; RS: resistant starch; LWM: limited water method; EWM: excess water method.

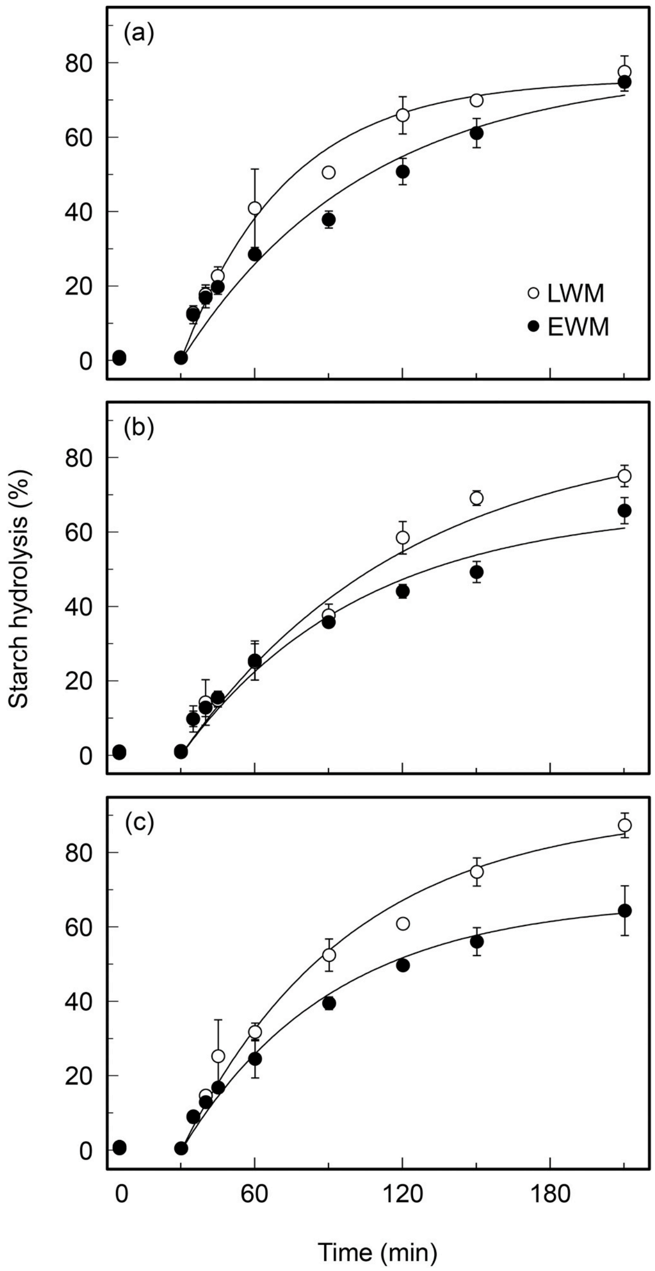

Fig. 2. Changes in the starch hydrolysis of three varieties of cooked rice grain during gastro-small intestinal starch digestion *in vitro*. (a) short, (b) medium and (c) long-grain rice. Error bars represent standard deviation ($n = 2$–4). LWM: limited water method; EWM: excess water method.

AA content of raw rice showed a correlation with k_1 ($r = 0.99$, $p < 0.05$), although TS and RS contents did not show any correlation related with M_e and k_1, respectively. AA content of raw and rice cooked by LWM or EWM had significant positive relationship with their RS contents ($r = 0.99$, $p < 0.05$).

3.3. *Starch hydrolysis during in vitro gastro-small intestinal digestion*

Fig. 2 shows changes in the starch hydrolysis of three varieties of cooked rice grain during gastro-small intestinal starch digestion *in vitro*. During simulated gastric digestion, for the first 30 min, the hydrolysis of all samples was almost 0% due to lack of amylolytic enzymes in SGF. During the following small intestinal digestion process, the starch hydrolysis increased in all sample grains. The starch hydrolysis in the middle of the small intestine digestion process was higher for rice cooked by LWM than rice cooked using EWM, for all varieties. At 210 min of simulated digestion, the starch hydrolysis for medium and long-grain rice was significantly higher for rice cooked by LWM (75.1 and 87.5%, respectively) than those cooked using EWM (65.8 and 64.5%, respectively), although the starch hydrolysis for short-grain rice was almost similar among the two cooking methods. The highest starch hydrolysis was observed for long-grain rice cooked using LWM. Rice cooked by EWM had lower starch hydrolysis values and this was true for all the varieties.

To evaluate differences in the starch digestibility among the three varieties and two cooking methods of samples, the kinetic parameters during small intestinal digestion were calculated using a non-linear fitting method to a first-order equation model (Goñi et al., 1997). Table 2 presents starch hydrolysis kinetic parameters of three varieties of cooked rice grain. LWM-cooked medium and long-grain rice showed higher C_∞ of 87.7 and 91.6%, respectively, while the EWM-cooked medium and long-grain rice had the lower C_∞ of 66.8 and 66.8%, respectively. The C_∞ of short-grain rice cooked by LWM and EWM were 75.7 and 78.4%, respectively, which did not differ significantly. The k_2 of samples was in the range between 1.1 and 2.4 min^{-1}, with significant differences among LWM-cooked short and medium-grain rice. A first-order equation model (Goñi et al., 1997) exhibited high R^2 for all samples used in the study, ranging between 0.984 and 0.996; and low RMSE (ranging between 2.188 and 4.732). Therefore, it is concluded that the first-order equation model (Goñi et al., 1997) was good to describe the starch hydrolysis of three rice varieties and two cooking methods for rice digested under small-intestinal conditions. eGI was significantly higher for short and long-grain rice cooked by LWM than others. For long-grain rice having the highest RS content, LWM-cooking had significantly higher eGI among the samples and EWM-cooking had significantly lower. Short and medium-grain rice indicated no significant difference in eGI regardless of the cooking method. It is noteworthy that C_∞, k_2 and eGI of rice grain were influenced by cooking method, variety, and both of cooking method and variety, respectively ($p < 0.05$). However, there was no clear relationship for cooking method × variety

Table 2
Starch hydrolysis kinetic parameters of three varieties of cooked rice grain.

		C_∞ (%)	$k_2 \times 10^{-2}$ (min^{-1})	R^2	RMSE	eGI
Short	LWM	75.7 ± 2.7 bc	2.4 ± 0.4 a	0.995	3.091	75.6 ± 0.7 ab
	EWM	78.4 ± 4.0 abc	1.3 ± 0.1 ab	0.984	4.732	70.0 ± 1.2 bc
Medium	LWM	87.7 ± 5.5 ab	1.1 ± 0.3 b	0.992	3.611	69.7 ± 2.7 c
	EWM	66.8 ± 2.7 c	1.4 ± 0.1 ab	0.987	3.690	65.8 ± 0.8 c
Long	LWM	91.6 ± 3.9 a	1.5 ± 0.3 ab	0.994	3.533	76.3 ± 1.8 a
	EWM	66.8 ± 7.2 c	1.5 ± 0.4 ab	0.996	2.188	67.6 ± 1.5 c

Means ± standard deviation.
The sample number (n) was as follows: C_∞ ($n = 2$–4); k_2 ($n = 2$–4); eGI ($n = 2$–4).
Different letters (a-c) in the same column indicate significant differences ($p < 0.05$).
C_∞: equilibrium starch hydrolysis; k_2: kinetic constant; R^2: coefficient of determination; RMSE: root mean square error; eGI: estimated glycemic index; LWM: limited water method; EWM: excess water method.

interaction for the C_∞, k_2 and eGI. The kinetic parameters during small intestinal digestion correlated no significantly with moisture, CP, AA, TS and RS contents of raw and cooked rice grain, respectively ($p > 0.05$). Many researchers have identified factors influencing the starch hydrolysis and kinetic parameters using slurry or dried (powder) samples. Although Chung et al. (2010) and Syahariza et al. (2013) reported amylose content is correlated with starch digestibility, Hu et al. (2004) showed amylose content is not the only factor that affects starch digestibility. Starch and protein/lipid interactions have reportedly been correlated with the C_∞ (Ye et al., 2018). A recent study carried out by Tamura, Maehara, Kumagai, Saito, and Ogawa (2019) revealed that, unlike protein rich foods such as beans and pasta, protein has no effect on starch hydrolysis during *in vitro* digestion of cooked rice grain. In this study, the same observation was recorded even when the cooking method and the rice variety was changed. The suggest that the interaction of starch digestibility with other factors such as amylose, protein, and lipids may be weaker than the relationship between the starch digestibility and structural properties of rice grain, which require further investigation.

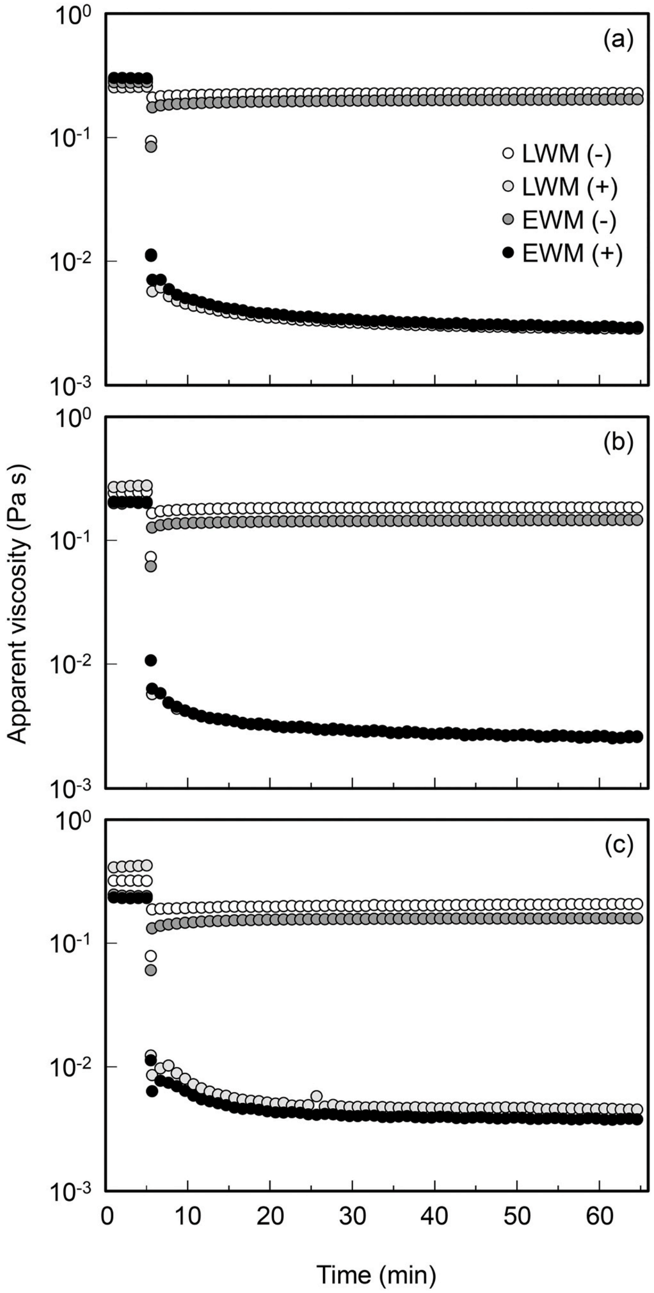

Fig. 3. Changes in the apparent viscosity of three varieties of blended cooked rice slurry by the addition of simulated intestinal fluid (SIF). (a) short, (b) medium and (c) long-grain rice. LWM (−): sample slurries cooked by LWM with control; LWM (+): sample slurries cooked by LWM with concentrated SIF; EWM (−): sample slurries cooked by EWM with control; EWM (+): sample slurries cooked by LWM with concentrated SIF. Plots represent average ($n = 3$). LWM: limited water method; EWM: excess water method; control: SIF without any added digestive enzymes.

3.4. *Apparent viscosity of rice pre- and post-digestion*

Changes in the apparent viscosity of three varieties of blended cooked rice slurry by the addition of SIF as a simulated digestive reaction is shown in Fig. 3. The viscosities of both cooked rice controls increased with time, resembling a thixotropic fluid (Rani & Bhattacharya, 1989). The apparent equilibrium viscosity for control cooked short, medium and long-grain rice was in the range of 0.15 and 0.23 Pa s, while the viscosities of all samples dropped to lower viscosity range (2.6–4.6 mPa s) after concentrated SIF was added. There were little differences observed in apparent viscosity values between varieties and between cooking methods. Previous reports have shown that changes in viscosity of the rice slurry depend on the degree of starch gelatinization (Tamura et al., 2016b). A high viscosity of the food matrix has been observed to reduce the rate of starch digestion due to less frequent of interaction between substrates and digestives enzymes (Bordoloi et al., 2012). Juliano and Goddard (1986) and Panlasigui et al. (1991) have also revealed that viscosity of rice is one of the major factors affecting the insulin response and GI. The apparent viscosity results of the present study suggested that rice slurries from the same variety cooked through different cooking methods might have similar gelatinization degree. Thus, it was implied that access between enzyme and starch in cooked rice slurry, a system with damaged and dispersed tissue structures and exposed starch granules, might not differ among varieties and cooking methods.

3.5. *Microstructure of rice grain*

Fig. 4 shows composite images of three varieties of raw and cooked rice grain. Autofluorescence of cell wall is mainly linked to the presence of phenolic compounds, which indicates histological tissue (Ogawa et al., 2003; Tamura & Ogawa, 2012). The aleurone layer at the peripheral area of the raw rice grain showed strong fluorescence and was observed with a relatively smooth surface (Fig. 4a–f), while aleurone layer of cooked rice grain showed a weak fluorescence due to damage and distortion (Fig. 4g-r). Distortion of cooked rice grain surface was caused by starch gelatinization and expansion, leading to cell disruption due to contact between boiling water and grain surface.

Coated layer was not observed on the surface of cooked rice grain except for short-grain rice (Fig. 4j). For short-grain rice, coated layer on the cooked rice grain has been reported to be formed by abrasion of cell walls from peripheral area of the grain and lamination of leached material during cooking of LWM (Tamura & Ogawa, 2012). Since medium and long-grain rice show less leached material during soaking at 90 °C than short-grain rice (Metcalf & Lund, 1985), coated layer would be not formed in these grains.

Central area of the cooked rice grain showed cracks and voids from dorsal side to ventral side (Fig. 4g–i, m-o). Furthermore, the rice grain cooked by LWM (Fig. 4g–i) indicated bigger voids and more cell wall damage than rice grain cooked by EWM (Fig. 4m–o). RCV of the short, medium and long-grain rice was significantly higher when the grains were cooked by LWM (24.56 ± 8.57, 7.42 ± 1.75 and 8.88 ± 2.99%, respectively) than the rice cooked by EWM (7.13 ± 4.26, 0.90 ± 0.72 and 1.19 ± 0.59%, respectively) ($n = 3$, $p < 0.05$), respectively. It was found that the RCV correlated with k_2 ($r = 0.85$, $p < 0.05$) and eGI ($r = 0.78$, $p > 0.05$). Sun et al. (2019) reported that hemicellulose, cellulose

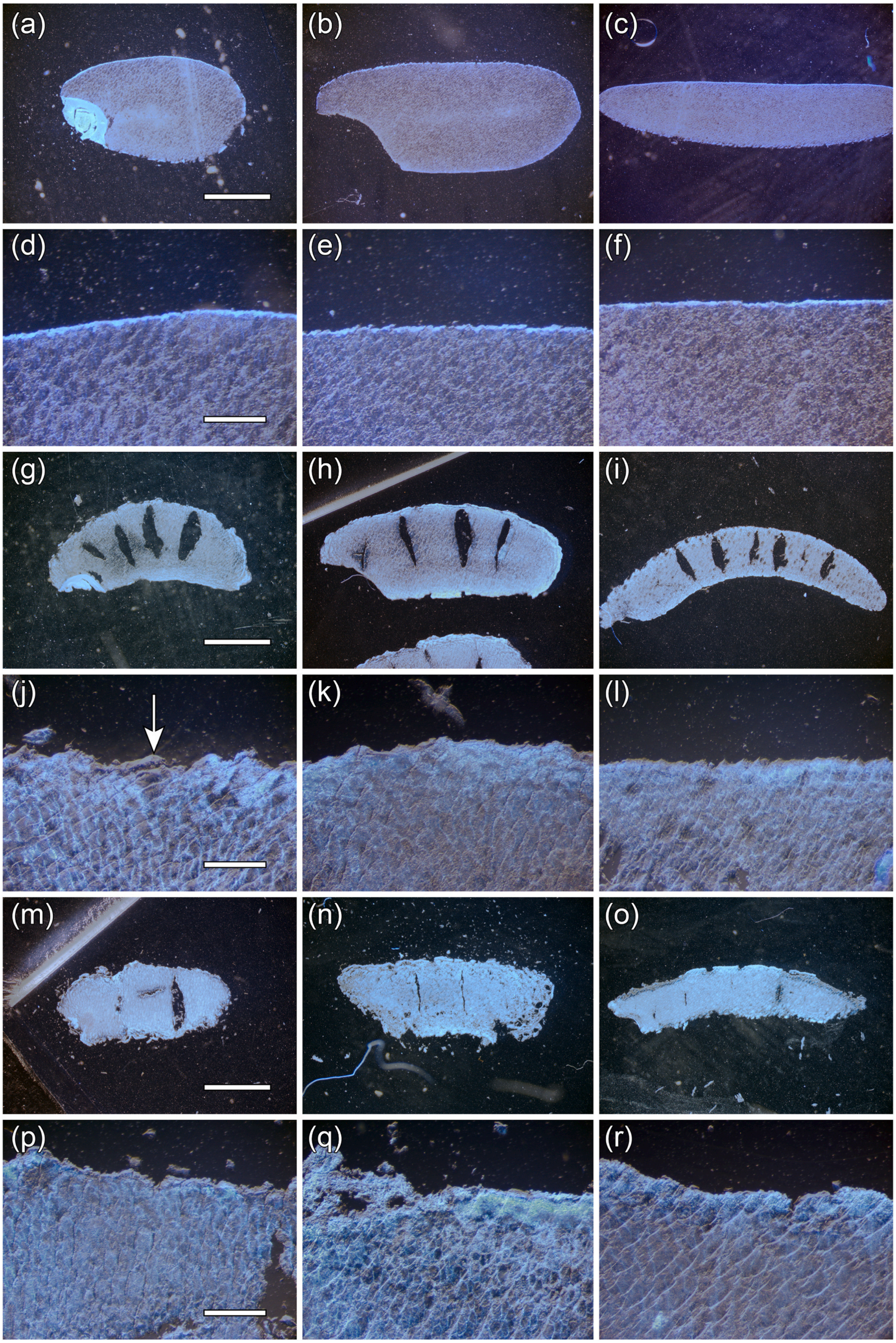

Fig. 4. Composite images of three varieties of raw and cooked rice grain. (a, d, g, j, m, p) short, (b, e, h, k, n, q) medium, (c, f, i, l, o, r) long-grain rice. (a–f) Raw, (g–l) rice cooked by LWM and (m–r) EWM. (a-c, g-i, m-o) Morphological and (d-f, j-l, p-r) dorsal side of rice grain. Arrow shows the coated layer (j). Scale bars indicate 2 mm (a-c, g-i, m-o) and 300 μm (d-f, j-l, p-r). LWM: limited water method; EWM: excess water method.

and pectin that affected the strength of the cell wall and starch digestibility were present in lesser amounts in japonica than in indica grain endosperms. Zaupa, Ganino, Dramis, and Pellegrini (2016) also suggested that the size of voids could vary among cooking methods and varieties. The voids in the grains have been reported to be generated by water evaporation through cracks in the grain tissue during steaming process in case of LWM (Ogawa et al., 2003; Tamura et al., 2014a). It is thought that majority of the cell walls of cooked rice grain are damaged with the generation of the voids, because the cracks and voids are not generated by individual cell separation at intercellular boundaries but are generated through cell wall disruption (Tamura et al., 2014a). For cereal grains, tissue structure (Tamura et al., 2016a, 2017), aleuron layer (Tamura et al., 2016a) and cell wall (Tamura, Imaizumi, Saito, Watanabe, & Okamoto, 2019) influence to resist the penetration of digestive fluid and protect the starch materials in the endosperm, defined as RS1 (Tian & Sun, 2020). Tamura et al. (2016b) and Thuengtung, Niwat, Tamura, and Ogawa (2018) have also found that structural destruction impact the starch hydrolysis rate and eGI but not equilibrium starch hydrolysis percentage. For the reasons, short-grain rice's cell wall is more likely to be damaged regardless of the cooking method, and the accessibility of digestive fluid for starch would be enhanced. The starch hydrolysis percentage additionally would be lowered for EWM-cooked rice because the method caused less RCV, i.e. starch was protected by the cell walls and accessibility of digestive fluid was restricted, compared to LWM. Thuengtung et al. (2019) also illustrated that microwave cooking caused substantial damage to the cooked rice morphological structure when compared with steam-cooking, but the extent of the disruption varied among the rice varieties. The authors further explained that the damage at the exterior layer accelerated the starch hydrolysis rate during *in vitro* gastro-small intestinal starch digestion. In the current study, it was revealed that the voids and cell wall damage in rice grains likely influenced k_2. Based on present study and previous reports, it is suggested that the cooking method has a strong influence on starch digestibility by changing the morphological and tissue structure of rice grains.

4. Conclusions

Starch hydrolysis kinetic parameters C_∞, k_2 and eGI of the rice grains were influenced by cooking method, variety, and both cooking method and variety, respectively. The moisture, CP, TS and RS contents of rice varied with the method of cooking, and these parameters were not directly correlated with starch digestibility of rice grains. The LWM caused substantial disruption to the cooked rice tissue structure when compared with EWM regardless of the rice variety. Internal structural damages might have facilitated the diffusion of SIF into the grain core and accelerated the starch hydrolysis during *in vitro* starch digestion in the former. It was suggested that starch digestibility of cooked rice grains was closely related not only to the component contents and apparent viscosity, but also to the disruption of tissue structure depending on the cooking method. Therefore, it is possible to control GI of cooked rice grain by cooking method considering the microstructural characteristics.

CRediT authorship contribution statement

Masatsugu Tamura: Writing – original draft, Validation, Data curation, Software. **Chisato Kumagai:** Investigation, Methodology, Software. **Lovedeep Kaur:** Supervision, Visualization, Methodology, Writing – review & editing. **Yukiharu Ogawa:** Conceptualization, Supervision, Visualization, Methodology, Writing – review & editing. **Jaspreet Singh:** Supervision, Visualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors have no conflict of interest to declare.

Acknowledgments

Funding from Riddet CoRE (New Zealand) and Chiba University (Japan) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111379.

References

Aoac. (1990). *Official methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists.

Atkinson, F. S., Foster-Powell, K., & Brand-Miller, J. C. (2008). International tables of glycemic index and glycemic load values: 2008. *Diabetes Care, 31*(12), 2281–2283. https://doi.org/10.2337/dc08-1239

Bordoloi, A., Singh, J., & Kaur, L. (2012). *In vitro* digestibility of starch in cooked potatoes as affected by guar gum: Microstructural and rheological characteristics. *Food Chemistry, 133*(4), 1206–1213. https://doi.org/10.1016/j.foodchem.2012.01.063

Chen, M. H., Bergman, C. J., McClung, A. M., Everette, J. D., & Tabien, R. E. (2017). Resistant starch: Variation among high amylose rice varieties and its relationship with apparent amylose content, pasting properties and cooking methods. *Food Chemistry, 234*, 180–189. https://doi.org/10.1016/j.foodchem.2017.04.170

Chung, H. J., Liu, Q., Huang, R., Yin, Y., & Li, A. (2010). Physicochemical properties and *in vitro* starch digestibility of cooked rice from commercially available cultivars in Canada. *Cereal Chemistry, 87*(4), 297–304. https://doi.org/10.1094/CCHEM-87-4-0297

Chung, H. J., Liu, Q., Lee, L., & Wei, D. (2011). Relationship between the structure, physicochemical properties and *in vitro* digestibility of rice starches with different amylose contents. *Food Hydrocolloids, 25*(5), 968–975. https://doi.org/10.1016/j.foodhyd.2010.09.011

Dartois, A., Singh, J., Kaur, L., & Singh, H. (2010). Influence of guar gum on the *in vitro* starch digestibility—rheological and microstructural characteristics. *Food Biophysics, 5*(3), 149–160. https://doi.org/10.1007/s11483-010-9155-2

Donlao, N., & Ogawa, Y. (2018). Impacts of processing conditions on digestive recovery of polyphenolic compounds and stability of the antioxidant activity of green tea infusion during *in vitro* gastrointestinal digestion. *LWT-Food Science and Technology, 89*, 648–656. https://doi.org/10.1016/j.lwt.2017.11.051

Ebata, M., & Hirasawa, K. (1982). Studies on the texture of cooked rice I. Textural parameters in relation to palatability. *Japanese Journal of Crop Science, 51*(2), 235–241. https://doi.org/10.1626/jcs.51.235

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437. https://doi.org/10.1016/S0271-5317(97)00010-9

Guillén, S., Oria, R., & Salvador, M. L. (2018). Impact of cooking temperature on *in vitro* starch digestibility of rice varieties with different amylose contents. *Polish Journal of Food and Nutrition Sciences, 68*(4), 319–325. https://doi.org/10.2478/pjfns-2018-0001

Hanashiro, I., Ohta, K., Takeda, C., Mizukami, H., & Takeda, Y. (2004). Leaching of amylose and amylopectin during cooking of rice grains and their effect on adhesiveness of cooked rice. *Journal of Applied Glycoscience, 51*(4), 349–354. https://doi.org/10.5458/jag.51.349

Han, J. A., & Lim, S. T. (2009). Effect of presoaking on textural, thermal, and digestive properties of cooked brown rice. *Cereal Chemistry, 86*(1), 100–105. https://doi.org/10.1094/CCHEM-86-1-0100

He, M., Qiu, C., Liao, Z., Sui, Z., & Corke, H. (2018). Impact of cooking conditions on the properties of rice: Combined temperature and cooking time. *International Journal of Biological Macromolecules, 117*, 87–94. https://doi.org/10.1016/j.ijbiomac.2018.05.139

Hu, P., Zhao, H., Duan, Z., Linlin, Z., & Wu, D. (2004). Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science, 40*(3), 231–237. https://doi.org/10.1016/j.jcs.2004.06.001

Ikeda, H. (2001). Relationship between the saccharides extracted from rice grains during cooking and the sensory taste of cooked rice. *Journal of Home Economics of Japan, 52*(5), 401–409. https://doi.org/10.11428/jhej1987.52.401

Jenkins, D. J., Kendall, C. W., Augustin, L. S., Franceschi, S., Hamidi, M., Marchie, A., et al. (2002). Glycemic index: Overview of implications in health and disease. *American Journal of Clinical Nutrition, 76*(1), 266S–273S. https://doi.org/10.1093/ajcn/76.1.266S

Juliano, B. O., & Goddard, M. S. (1986). Cause of varietal difference in insulin and glucose responses to ingested rice. *Plant Foods for Human Nutrition, 36*(1), 35–41. https://doi.org/10.1007/BF01091751

Kang, H. J., Hwang, I. K., Kim, K. S., & Choi, H. C. (2006). Comparison of the physicochemical properties and ultrastructure of japonica and indica rice grains.

Journal of Agricultural and Food Chemistry, 54(13), 4833–4838. https://doi.org/10.1021/jf060221+

Kasai, M., Lewis, A. R., Ayabe, S., Hatae, K., & Fyfe, C. A. (2007). Quantitative NMR imaging study of the cooking of Japonica and Indica rice. *Food Research International, 40*(8), 1020–1029. https://doi.org/10.1016/j.foodres.2007.05.010

Lehmann, U., & Robin, F. (2007). Slowly digestible starch–its structure and health implications: A review. *Trends in Food Science & Technology, 18*(7), 346–355. https://doi.org/10.1016/j.tifs.2007.02.009

Li, J., Han, W., Xu, J., Xiong, S., & Zhao, S. (2014). Comparison of morphological changes and *in vitro* starch digestibility of rice cooked by microwave and conductive heating. *Starch-Stärke, 66*(5–6), 549–557. https://doi.org/10.1002/star.201300208

Marsono, Y., & Topping, D. L. (1993). Complex carbohydrates in australian rice products—influence of microwave cooking and food processing. *LWT-Food Science and Technology, 26*(4), 364–370. https://doi.org/10.1006/fstl.1993.1072

Maruyama, E., Sakamoto, K., & Okai, K. (1995). Cooking and eating qualities of white rice (Part II): The cooking properties of japonica rice and indica rice. *Journal of Cookery Science of Japan, 28*(4), 224–230. https://doi.org/10.11402/cookeryscience1995.28.4_224

Metcalf, S. L., & Lund, D. B. (1985). Factors affecting water uptake in milled rice. *Journal of Food Science, 50*(6), 1676–1679. https://doi.org/10.1111/j.1365-2621.1985.tb10563.x

Miller, J. B., Pang, E., & Bramall, L. (1992). Rice: A high or low glycemic index food? *American Journal of Clinical Nutrition, 56*(6), 1034–1036. https://doi.org/10.1093/ajcn/56.6.1034

Murakami, K., Sasaki, S., Takahashi, Y., Okubo, H., Hosoi, Y., Horiguchi, H., et al. (2006). Dietary glycemic index and load in relation to metabolic risk factors in Japanese female farmers with traditional dietary habits. *American Journal of Clinical Nutrition, 83*(5), 1161–1169. https://doi.org/10.1093/ajcn/83.5.1161

O'dea, K., Snow, P., & Nestel, P. (1981). Rate of starch hydrolysis *in vitro* as a predictor of metabolic responses to complex carbohydrate *in vivo*. *American Journal of Clinical Nutrition, 34*(10), 1991–1993. https://doi.org/10.1093/ajcn/34.10.1991

Ogawa, Y., Glenn, G. M., Orts, W. J., & Wood, D. F. (2003). Histological structures of cooked rice grain. *Journal of Agricultural and Food Chemistry, 51*(24), 7019–7023. https://doi.org/10.1021/jf034758o

Okuno, M., & Adachi, K. (1992). Relationship between physical properties and water absorption characteristics of milled rice. *Japanese Journal of Crop Science, 61*(2), 244–250. https://doi.org/10.1626/jcs.61.244

Panlasigui, L. N., Thompson, L. U., Juliano, B. O., Perez, C. M., Yiu, S. H., & Greenberg, G. R. (1991). Rice varieties with similar amylose content differ in starch digestibility and glycemic response in humans. *American Journal of Clinical Nutrition, 54*(5), 871–877. https://doi.org/10.1093/ajcn/54.5.871

Parchure, A. A., & Kulkarni, P. R. (1997). Effect of food processing treatments on generation of resistant starch. *International Journal of Food Sciences & Nutrition, 48*(4), 257–260. https://doi.org/10.3109/09637489709028570

R Core Team. (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org.

Rani, M. S., & Bhattacharya, K. R. (1989). Rheology of rice-flour pastes: Effect of variety, concentration, and temperature and time of cooking. *Journal of Texture Studies, 20*(2), 127–137. https://doi.org/10.1111/j.1745-4603.1989.tb00427.x

Rashmi, S., & Urooj, A. (2003). Effect of processing on nutritionally important starch fractions in rice varietie. *International Journal of Food Sciences and Nutrition, 54*(1), 27–36. https://doi.org/10.1080/096374803161976

Reed, M. O., Ai, Y., Leutcher, J. L., & Jane, J. L. (2013). Effects of cooking methods and starch structures on starch hydrolysis rates of rice. *Journal of Food Science, 78*(7), H1076–H1081. https://doi.org/10.1111/1750-3841.12165

Sagum, R., & Arcot, J. (2000). Effect of domestic processing methods on the starch, non-starch polysaccharides and *in vitro* starch and protein digestibility of three varieties of rice with varying levels of amylose. *Food Chemistry, 70*(1), 107–111. https://doi.org/10.1016/S0308-8146(00)00041-8

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Sugiyama, M., Tang, A. C., Wakaki, Y., & Koyama, W. (2003). Glycemic index of single and mixed meal foods among common Japanese foods with white rice as a reference food. *European Journal of Clinical Nutrition, 57*(6), 743–752. https://doi.org/10.1038/sj.ejcn.1601606

Sun, J., Fu, J., Wang, Y., Ye, H., Wu, D., & Shu, X. (2019). Endogenous rice endosperm hemicellulose slows *in vitro* starch digestibility. *International Journal of Food Science and Technology, 54*(3), 734–743. https://doi.org/10.1111/ijfs.13988

Syahariza, Z. A., Sar, S., Hasjim, J., Tizzotti, M. J., & Gilbert, R. G. (2013). The importance of amylose and amylopectin fine structures for starch digestibility in cooked rice grains. *Food Chemistry, 136*(2), 742–749. https://doi.org/10.1016/j.foodchem.2012.08.053

Tamura, M., Imaizumi, R., Saito, T., Watanabe, T., & Okamoto, T. (2019). Studies of the texture, functional components and *in vitro* starch digestibility of rolled barley. *Food Chemistry, 274*, 672–678. https://doi.org/10.1016/j.foodchem.2018.09.016

Tamura, M., Maehara, N., Kumagai, C., Saito, H., & Ogawa, Y. (2019). Changes in starch digestibility and tissue structure of cooked rice grain under different *in vitro* simulated gastric digestive conditions. *Journal of the Japanese Society for Food Science and Technology, 66*(5), 170–178. https://doi.org/10.3136/nskkk.66.170

Tamura, M., Nagai, T., Hidaka, Y., Noda, T., Yokoe, M., & Ogawa, Y. (2014a). Changes in histological tissue structure and textural characteristics of rice grain during cooking process. *Food Structure, 1*(2), 164–170. https://doi.org/10.1016/j.foostr.2013.10.003

Tamura, M., Nagai, T., Hidaka, Y., Noda, T., Yokoe, M., & Ogawa, Y. (2014b). Changes in nonwaxy japonica rice grain textural-related properties during cooking. *Journal of Food Quality, 37*(3), 177–184. https://doi.org/10.1111/jfq.12085

Tamura, M., & Ogawa, Y. (2012). Visualization of the coated layer at the surface of rice grain cooked with varying amounts of cooking water. *Journal of Cereal Science, 56*(2), 404–409. https://doi.org/10.1016/j.jcs.2012.06.002

Tamura, M., Okazaki, Y., Kumagai, C., & Ogawa, Y. (2017). The importance of an oral digestion step in evaluating simulated *in vitro* digestibility of starch from cooked rice grain. *Food Research International, 94*, 6–12. https://doi.org/10.1016/j.foodres.2017.01.019

Tamura, M., Singh, J., Kaur, L., & Ogawa, Y. (2016a). Impact of structural characteristics on starch digestibility of cooked rice. *Food Chemistry, 191*, 91–97. https://doi.org/10.1016/j.foodchem.2015.04.019

Tamura, M., Singh, J., Kaur, L., & Ogawa, Y. (2016b). Impact of the degree of cooking on starch digestibility of rice – an *in vitro* study. *Food Chemistry, 191*, 98–104. https://doi.org/10.1016/j.foodchem.2015.03.127

Tamura, M., Singh, J., Kaur, L., & Ogawa, Y. (2019). Effect of post-cooking storage on texture and *in vitro* starch digestion of japonica rice. *Journal of Food Process Engineering, 42*(2), Article e12985. https://doi.org/10.1111/jfpe.12985

Thuengtung, S., Matsushita, Y., & Ogawa, Y. (2019). Comparison between microwave-cooking and steam-cooking on starch properties and *in vitro* starch digestibility of cooked pigmented rice. *Journal of Food Process Engineering, 42*(6), Article e13150. https://doi.org/10.1111/jfpe.13150

Thuengtung, S., Niwat, C., Tamura, M., & Ogawa, Y. (2018). *In vitro* examination of starch digestibility and changes in antioxidant activities of selected cooked pigmented rice. *Food Bioscience, 23*, 129–136. https://doi.org/10.1016/j.fbio.2017.12.014

Tian, S., & Sun, Y. (2020). Influencing factor of resistant starch formation and application in cereal products: A review. *International Journal of Biological Macromolecules, 149*, 424–431. https://doi.org/10.1016/j.ijbiomac.2020.01.264

Wang, S., Li, P., Zhang, T., Yu, J., Wang, S., & Copeland, L. (2017). *In vitro* starch digestibility of rice flour is not affected by method of cooking. *LWT-Food Science and Technology, 84*, 536–543. https://doi.org/10.1016/j.lwt.2017.06.023

Yang, D. S., Shewfelt, R. L., Lee, K. S., & Kays, S. J. (2008). Comparison of odor-active compounds from six distinctly different rice flavor types. *Journal of Agricultural and Food Chemistry, 56*(8), 2780–2787. https://doi.org/10.1021/jf072685t

Yang, C. Z., Shu, X. L., Zhang, L. L., Wang, X. Y., Zhao, H., Ma, C. X., et al. (2006). Starch properties of mutant rice high in resistant starch. *Journal of Agricultural and Food Chemistry, 54*(2), 523–528. https://doi.org/10.1021/jf0524123

Ye, J., Hu, X., Luo, S., McClements, D. J., Liang, L., & Liu, C. (2018). Effect of endogenous proteins and lipids on starch digestibility in rice flour. *Food Research International, 106*, 404–409. https://doi.org/10.1016/j.foodres.2018.01.008

Zaupa, M., Ganino, T., Dramis, L., & Pellegrini, N. (2016). Anatomical study of the effect of cooking on differently pigmented rice varieties. *Food Structure, 7*, 6–12. https://doi.org/10.1016/j.foostr.2015.12.001

Zhu, L., Bi, S., Wu, G., Gong, B., Zhang, H., Wang, L., et al. (2020). Study of the migration and molecular structure of starch and protein in rice kernel during heating. *International Journal of Biological Macromolecules, 147*, 1116–1124. https://doi.org/10.1016/j.ijbiomac.2019.10.080