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In vitro digestibility and *in vivo* glucose response of native and physically modified rice starches varying amylose contents

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ABSTRACT

The native and physically modified rice starches with varying amylose contents were subjected to investigate the *in vitro* digestibility and the *in vivo* glucose tolerance in mice. The amylose and resistant starch (RS) contents of five native rice starches ranged in 4.7–30.6% and 6.3–11.8%, respectively. The RS contents of rice starches increased to 18.5–23.9% after heat-moisture treatment (HMT) and to 19.5–26.9% after annealing treatment (ANN). The heat-moisture and annealing treatments significantly reduced glycemic index (GI) values of the rice starches. GI values of the native, heat-moisture treated and annealed rice starches ranged in 68.9–100, 61.2–88.9 and 21.2–43.9, respectively. There was no correlation between amylose contents and the RS contents or GI values, while a strong negative correlation between RS contents and GI values was found ($R^2 = -0.747$, $P < 0.01$).

1. Introduction

Rice (*Oryza sativa* L.) is one of the oldest food crops in the world. Nowadays, rice is the most important grain in the Asian countries where it is consumed as a main source of human nutrition and calories. Rice starch, approximately 90% of whole rice grain in dry weight, plays an important role in cooked rice quality and nutrition based on its physicochemical properties and digestibility. A better understanding on the digestibility of rice starch has recently gained much attention not only for calories supply but also for the dietary management of diet-related health complications, particularly obesity, type 2 diabetes, and colorectal cancers (Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013).

The glycemic index (GI) was proposed as an *in vivo* method of ranking foods on the basis of the incremental blood glucose responses they produce for a given amount of carbohydrate (Jenkins et al., 1981), whereas an *in vitro* digestibility technique using controlled enzymatic hydrolysis to characterize starchy foods into different fractions in terms that reflect the rate of glucose release and its absorption in the gastrointestinal tract such as rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). The GI must be carried out *in vivo* by clinical trials which are difficult and costly tests in human. Therefore, the *in vitro* digestion methods could be useful in the estimation of the GI because of a high correlation between two methods (Goni, Garcia-Alonso, & Saura-Calixto, 1997; Granfeldt, Björck, Drews, & Tovar, 1992). Shin et al. (2007) reported that the RDS, SDS and RS in the uncooked raw rice starch were 25.4%, 51.3% and 23.3%, respectively. However, the RDS fraction significantly increased (74.9%) and the SDS and RS fractions decreased markedly (12.0% and 13.0%, respectively) after cooking. The GI values of three cooked rices (glutinous, jasmine and broken rices) were 94, 109 and 86, respectively (glucose GI = 100) (Chan et al., 2001). Thus, the starch in the cooked rice was easily digested by human enzymatic system and could not be used as low-carbohydrate foods.

The amounts of SDS and RS in rice starch can be improved by physical modification such as annealing (ANN) and heat-moisture treatment (HMT). The HMT increased the RS contents of all rice starches, in which the high-amylose starch had a higher resistant starch content as compared to the medium- and low-amylose starches (Zavarezel et al., 2012). The rice noodles containing starch which were pre-gelatinized and retrograded gave the lower GI values (40–61) than did the cooked rices (86–109) (Chan et al., 2001). Thus, differences in starch digestibility and the glycemic index are reported to relate to the source and nature of starches (Aarathi, Urooj, & Puttaraj, 2003). In addition, several studies have demonstrated that the digestibility of rice starch (both isolated starch and that in the grains) is associated with amylose content (Chung, Liu, Wang, Yin, & Li, 2010; Frei, Siddhuraju, & Becker, 2003; Zhu, Liu, Wilson, Gu, & Shi, 2011) and molecular structural

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characteristics of rice starch, including fine structures of the distributions of branch (chain) lengths in both amylose and amylopectin (Syahariza et al., 2013). However, little information reported on the *in vitro* digestibility and the glycemic index of native and physically modified rice starches are found in the literature (Chan et al., 2001; Frei et al., 2003). Therefore, the objective of this study is to investigate the *in vitro* digestibility and glycemic index values of native, heat-moisture treated and annealed rice starches of varying amylose contents and a relationship between starch fractions (RDS, SDS and RS) and their GI, evaluated by measuring glucose responses in mice.

2. Materials and methods

2.1. Materials

Mature grains of five rice cultivars (*Oryza sativa* L.) with different amylose contents were purchased from local provinces in Vietnam. The nonsticky, short-grained varieties, Ham Trau rice (OM 576) and 504 rice (IR 50404) were grown in Can Tho Province, Vietnam. The sticky, long-grained varieties, 64 rice (IR 64) and Huong Lai rice (Jasmine 85) were grown in Long An Province, Vietnam. The sticky, short-grained variety, Nep Cai Hoa Vang rice (Glutinous rice) was harvested in Hai Duong province, Vietnam. The cultivars, "Ham Trau", "64" and "504", contained high-, intermediate- and normal-amylose contents (HAR, IAR and NR), respectively, and the "Huong Lai" and "Nep Cai Hoa Vang" cultivars were low-amylose and waxy rices (LAR and WR, respectively). The grains were ground into fine flour passing through a sieve of 0.105 mm in aperture size.

Alpha-amylase from *Aspergillus oryzae* (~30 U/mg) and amyloglucosidase from *Aspergillus niger* (⩾300 U/ml) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Other chemicals were purchased from Merck Co. (Darmstadt, Germany).

2.2. Rice starch isolation

Rice starch was isolated based on the method of Sodhi and Singh (2003) with a slight modification. Rice flour (50 g) was soaked in 0.2% sodium hydroxide solution (400 ml) at 4 °C overnight to remove proteins. The supernatant was discarded and alkaline extraction was repeated twice. Resultant starches were washed thoroughly in clean water to remove the contaminant substances and passed through the sieves (0.232 and 0.105 mm in aperture size). Finally, the starch sediment was recovered by centrifugation and dried in an oven at 40 °C to 10–11% moisture.

After isolation, rice starches were analyzed for their composition and purity. Protein, lipid and ash contents were determined based on the standard AACCI Approved Methods 46–10, 30–10 and 08–01, respectively (AACC International, 2000). Total starch was calculated as follows: total starch (% in dry basis, db) = 100% − protein content (%, db) − lipid content (%, db) − ash (%, db).

Amylose content of starch was determined according to the method previously descried by Hung and Morita (2005). Amylose content was calculated by the equation: AM (%) = 110.78 × BV − 24.481, R^2 = 0.9995, in which BV is blue value of starches measured at 620 nm.

X-ray diffraction analysis was performed using an X-ray diffractometer (Rigaku Co., Ltd., Rint-2000 type, Tokyo, Japan) operated at 40 kV and 80 mA. Diffractograms were obtained from 2° 2θ to 35° 2θ with a scanning speed of 8°/min and scanning step of 0.02° (Hung, Maeda, Miskelly, Tsumori, & Morita, 2008).

2.3. Physical modification of rice starches

The heat-moisture treatment of rice starches was done according to the method of Gunaratne and Hoover (2002) with a slight modification. The isolated starch (100 g, db) was weighed into a glass container and then a measured volume of distilled water was added to raise the starch moisture content to 30%. The sealed flasks were equilibrated at room temperature for 24 h before heating in a forced air oven at 110 °C for 8 h. After cooling, the heat-moisture treated starch sample was dried at 40 °C for 24 h to a moisture content of 9–10%.

The isolated rice starches were also treated by annealing method as previously reported by Jacobs, Eerlingen, Clauwaert, and Delcour (1995) with a slight modification. Starch (100 g, db) was mixed with distilled water at a ratio of 1:2 (w/w). The sealed container was then heated in a water bath at 45 °C for 24 h. After incubation, the starch sample was dried at 40 °C for 24 h to a moisture content of 9–10%.

2.4. In vitro starch digestibility assay

In vitro starch digestibility assay based on the method of Englyst et al. (1992) with a moderate modification was used to measure percentages of starch fractions including rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) of native and physically modified rice starches. Starch (0.3 g, db) was mixed with 20 ml of sodium acetate buffer (pH 6.0) and then boiled for 30 min in a water bath. After equilibrating at 37 °C for 15 min, an enzyme solution (5 ml) of α-amylase (1400 U/ml) and amyloglucosidase (13 AGU/ml) was added and the slurry was incubated with shaking at 37 °C. After 20 min and 120 min, the hydrolysate (0.5 ml) was removed and determined for total glucose concentrations (G_{20} and G_{120}, respectively) using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Reber, & Smith, 1956). The remained residue was intensively hydrolyzed with 7 M KOH and then with amyloglucosidase (50 AGU/ml). The final hydrolysate was then determined for total glucose concentration (TG). The values obtained for G_{20}, G_{120} and TG were used to calculate for RDS, SDS and RS as follows:

$$RDS = G_{20} \times 0.9$$

$$SDS = (G_{120} - G_{20}) \times 0.9$$

$$RS = (TG - G_{120}) \times 0.9$$

2.5. In vivo glucose tolerance test in mice

Glucose tolerance test in mice was carried out according to the method of Shin et al. (2007) with a slight modification. Forty-five 25-gram white mice (*Mus musculusdomesticus*) of the Swiss line were supplied by the Pasteur Institute in Ho Chi Minh City and individually housed in a light-controlled (12 h dark, 12 h light) under fully nutritional condition at the laboratory of the University based on the instruction of the Pasteur Institute in Ho Chi Minh City for at least 1 week before testing. The mice, divided into 15 groups of three mice, were fasted for 16 h, and then given 500 mL gelatinized starch solution (7.5%, w/v) or glucose (7.5%) via an oral Zonde needle. Blood samples were taken from the tail vein of each mouse at 0, 30, 60, 90, 120, and 180 min. Blood serum glucose levels were measured with an Accu-Chek Active Glucose System (Roche Ltd., Basel, Switzerland) using the glucose oxidase reaction and colorimetric determination. All procedures used in this study were approved by the Institutional Animal Care and Use Committee at the International University, Vietnam National University in HoChiMinh City.

Glycemic index value was calculated according to the formula reported by the Wolever and Jenkins (1986) as follows. The area under the blood glucose curve includes the area above the fasting level only. The incremental area under the blood glucose response

curve is the sum of the areas of the triangles and rectangles, calculated geometrically (Wolever & Jenkins, 1986).

$$GI = \frac{\text{Area under the curve for starch}}{\text{Area under the curve for glucose}}$$

2.6. Statistical analysis

All tests were performed at least in duplicate. Analysis of variance (ANOVA) was performed using Duncan's multiple-range test to compare treatment means at $P < 0.05$ using SPSS software (SPSS Inc., USA). Correlation coefficients were also done using SPSS program (SPSS Inc., USA).

3. Results and discussion

3.1. Rice starch characteristics

Starch purity and characteristics of the native rice starches used in this study are shown in Table 1. Total starch contents of the isolated starches were in a range of 99.0–99.4%, indicating that almost protein, lipid and ash in rice flours were removed and the isolated starches had high purity after isolation. The rice starches with different amylose contents including high-amylose rice (HAR), intermediate amylose rice (IAR), normal rice (NR), low amylose rice (LAR) and waxy rice (WR) contained 30.6%, 26.7%, 24.3%, 21.7% and 4.7% amylose, respectively. All rice starches exhibited the *A*-type crystalline structure with the major peaks at around d-spacings 5.8 Å (line 3b), 5.2 and 4.8 Å (line 4a, 4b) and 3.8 Å (line 6a) as classified by Zobel (1988) (Fig. 1). However, the WR and LAR had no 5a peak because of no amylose–lipid complex existed in these low-amylose starches. Although the HAR and IAR, which had high amylose contents, had lower degrees of crystallinity than the lower-amylose rice starches (NR, LAR and WR). However, the degrees of crystallinity of NR, LAR and WR were not significantly different. The degrees of crystallinity of the rice starches were in order of HAR < IAR < NR = LAR = WR) (Table 1). These results are consistent with previous studies reported that the amylose contents of rice starch ranged from 1.7% to 55.4% depending on rice varieties and rice starch had the typical *A*-type crystalline structure (Chung et al., 2010; Frei et al., 2003; Zhu et al., 2011). The differences in amylose contents and degree of crystallinity may result in different physicochemical properties and digestibility of rice starches even though they had the same crystalline structure.

Table 1
Purity and characteristics of rice starches.[a,b,*]

Sample	Total starch (%, db)	Amylose content (%)	Degree of crystallinity (%)
HAR	99.0 ± 0.1a	30.6 ± 0.01e	30.8 ± 0.02a
IAR	99.3 ± 0.1a	26.7 ± 0.03d	31.7 ± 0.02b
NR	99.2 ± 0.1a	24.3 ± 0.03c	33.2 ± 0.01c
LAR	99.4 ± 0.0a	21.7 ± 0.02b	33.4 ± 0.03c
WR	99.4 ± 0.1a	4.7 ± 0.04a	33.8 ± 0.04c

[a] HAR, high-amylose rice; IAR, Intermediate-amylose rice; NR, normal rice; LAR, low-amylose rice; WR, waxy rice.
[b] Data are the mean ± SD, $n = 3$.
[*] Data by the same letter in the same column are not significantly different ($P < 0.05$).

3.2. Digestive starch fractions (RDS, SDS, RS)

The starch digestive fractions including rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) of the native and physically treated rice starches in their gelatinized state are shown in Table 2. Amounts of RDS of the gelatinized rice starches were in a range of 77.0–90.0%, where the HAR contained the highest amount of RDS and the WR had the lowest one. Thus, the gelatinized rice starches with higher amylose contents were easily hydrolyzed by the amylases than those, which had lower amylose contents and higher degrees of crystallinity. The amount of SDS of NR was the highest, followed by those of WR, IAR, LAR and HAR. RS contents of HAR, IAR and NR were not significantly different, while the LAR and WR exhibited significantly higher amounts of RS than did the HAR, IAR and NR. The RS content of the native waxy rice starch in this study is consistent with the result of Chung, Lim, and Lim (2006) and Zhu et al. (2011), who reported that the waxy rice starch (Remyline AX-DR) contained 9.3% of RS and the cultivar 'Yang-fu-nuo' (Chinese waxy rice) contained 12.76% of RS. Zhu et al. (2011) also reported that the SDS content of rice starch decreased as amylose content increased and RS content of the waxy rice starch was higher than that of the low-amylose rice starch. Thus, the differences in amounts of SDS and RS of the gelatinized rice starches were not only dependent on their amylose contents but also due to the fine structures of amylose and amylopectin, chain-length distributions and degrees of crystallinity.

After heat-moisture and annealing treatments, the amounts of RDS decreased as compared to those of the native starches. The treated starches from the lower amylose rice starches also had lower amounts of RDS than those of the higher amylose rice starches. The heat-moisture treatments have been used to increase both SDS and RS contents in starches without the disruption of granular structure (Zavarezel & Dias, 2011). In this study, the RS contents in the HAR, IAR and NR significantly increased after heat-moisture treatment, whereas the SDS contents did not change in the HAR

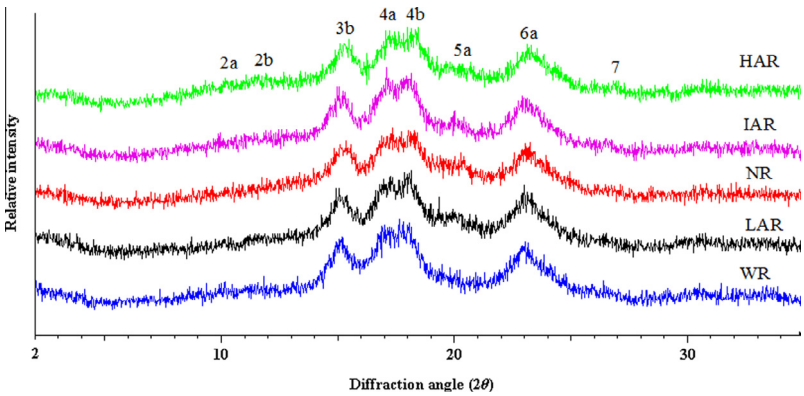

Fig. 1. X-ray diffraction patterns of rice starches. Abbreviations are the same as in Table 1.

Table 2
RDS, SDS and RS (%, w/w) of native and physically modified rice starches.[a,*]

Sample	Native			HMT			ANN		
	RDS	SDS	RS	RDS	SDS	RS	RDS	SDS	RS
HAR	90.0d	3.7a	6.3a	73.2e	4.4a	22.4b	69.8d	10.7a	19.5a
IAR	82.9c	10.9b	6.2a	66.3d	11.1b	22.6b	69.2d	10.2a	20.6b
NR	79.5b	14.0d	6.5a	65.3c	10.8b	23.9c	58.4b	19.0d	22.6c
LAR	77.7a	10.5b	11.8c	63.8b	17.5c	18.7a	63.3c	12.3b	24.4d
WR	77.0a	12.8c	10.2b	57.5a	24.0d	18.5a	56.6a	16.5c	26.9e

[a] HAR, high-amylose rice; IAR, intermediate-amylose rice; NR, normal rice; LAR, low-amylose rice; WR, waxy rice; HMT, heat-moisture treatment; ANN, annealing treatment; RDS, rapid digestible starch; SDS, slowly digestible starch; RS, resistant starch.
[*] Data by the same letter in the same column are not significantly different ($P < 0.05$).

and IAR or decreased in NR. Amounts of both SDS and RS in the LAR and WR were found to significantly increase after heat-moisture treatment as compared to those in the native starches. However, RS contents in the heat-moisture treated LAR and WR were significantly lower than those of the heat-moisture treated HAR, IAR and NR, while their SDS contents were significantly higher. Zavarezel et al. (2012) also reported that the HMT increased the resistant starch contents of all of the rice starches and the heat-moisture treated high-amylose starch had a higher resistant starch content as compared to the heat-moisture treated medium- or low-amylose starch at the same treatment condition. The increase in amounts of SDS and RS (by 2.5–4.7% and 7.7–11.2%, respectively) and the decrease in amounts of RDS (by 10.2–15.1%) in corn, pea and lentil starches after heat-moisture treatment as compared to gelatinized unmodified starches were also observed by Chung, Liu, and Hoover (2009). Thus, the changes in the structure of the lamellae in waxy and high-amylose rice starches were different after heat-moisture treatment. The interaction between starch chains after heat-moisture treatment in the high-amylose starch was more stable than that in the waxy starch, resulting in the higher amount of RS of the high-amylose starch compared to the waxy starch. In addition, the amylose–lipid complex contributed to the increased RS in the high- and intermediate-amylose rices as compared to the waxy and low-amylose rice starches. Annealing treatment also improved both SDS and RS of rice starches. Amounts of SDS and RS in the annealed rice starches were significantly higher than those of the native rice starches in the same variety except the amount of SDS in the annealed IAR, which was no change as compared to the native starch. The RS contents of the annealed rice starches increased with reducing amylose contents, while the rice starches with higher amylose content (HAR and IAR) also contained lower amounts of SDS as compared to the rice starches having lower amylose content (NR, LAR and WR). The results also show that the annealed high-amylose starches contained higher SDS but lower RS contents as compared to the heat-moisture treated starches, whereas the RS contents of the lower amylose starches treated by ANN were significantly higher than those treated by HMT. The increase in thermo-stable SDS and RS might be explained that some interactions formed during annealing or heat-moisture treatment may have survived after gelatinization, thereby partly restricting accessibility of starch chains to the hydrolyzing enzymes (Chung et al., 2009).

3.3. In vivo glucose response and glycemic index (GI) of rice starches

Blood glucose concentrations in mice after an intake of glucose, raw and physically modified rice starches are shown in Fig. 2. The released blood glucose after the intake of an amount of glucose (reference) reached a maximum level after 30 min of ingestion and then rapidly reduced to the initial level after 180 min. Ingestion of native and physically modified rice starches significantly lowered the level of blood glucose released as compared to the direct glucose ingestion. The blood glucose concentrations also reached a maximum level after ingestions of the native HAR and IAR for 30 min and then dramatically reduced for a longer digestion. The maximum level of blood glucose was released at 60 min after the intakes of native NR, whereas the blood glucose concentrations, released after ingestion of native LAR and WR for 30–60 min, were not significantly different. The longer ingestion time of the native NR, LAR and WR might be due to the fine structures of amylose and amylopectin molecules of these starches which had higher degrees of crystallinity and amounts of SDS and RS as compared to the native HAR and IAR. The annealed rice starches of HAR, IAR and NR (ANN-HAR, ANN-IAR and ANN-NR) showed the lowest blood glucose levels, followed by the heat-moisture treated starches (HMT-HAR, HMT-IAR and HMT-NR) and then native starches (N-HAR, N-IAR and N-NR). These results were due to the high amounts of SDS and RS in the annealed starches than those in the heat-moisture treated starches and native starches. The annealed starches of LAR and WR also released low blood glucose concentration as compared to the heat-moisture treated or native starches, whereas there were not significant difference in the blood glucose levels released between heat-moisture treated starches and native starches of LAR and WR. Thus, the different results of blood glucose released after the intakes of rice starches varying amylose contents were positively related to total amounts of SDS and RS. The high amount of SDS and RS lasted the digestion time and blood glucose-releasing duration. Shin et al. (2007) also reported that the much lower blood glucose level after the intake of acid-treated rice starch compared to the native starch was presumably due to the content of SDS and RS fractions.

The GI of native and physically modified rice starches was calculated based on the area under the blood glucose response above the glucose baseline according to the concept of the glycemic index (Jenkins et al., 1981) and shown in Table 3. The GIs of the native rice starches were in a range of 93.2–100 except for the WR which had the significantly lower GI value (68.9). The low GI value of native waxy starch compared to other native rice starches in this study might be due to the remained crystalline structure of the gelatinized waxy starch which resisted to the enzymatic hydrolysis while the disruption of starch structure of the gelatinized normal or high-amylose starches increased its susceptibility to enzymatic degradation (Holm, Lundquist, Bjorck, Eliasson, & Asp, 1988). Both heat-moisture and annealing treatments reduced the GI values of rice starches, in which the annealed rice starches had the lowest GI values, followed by the heat-moisture treated rice starches. Among the heat-moisture treated rice starches, the GI values increased in the order of HAR = NR < WR < IAR < LAR, whereas the increase in GI values of the annealed rice starched followed the order of IAR < WR < HAR < LAR < NR. These results suggest that the compartmentalization of amylose–amylose, amylopectin–amylopectin and amylose–amylopectin chains during

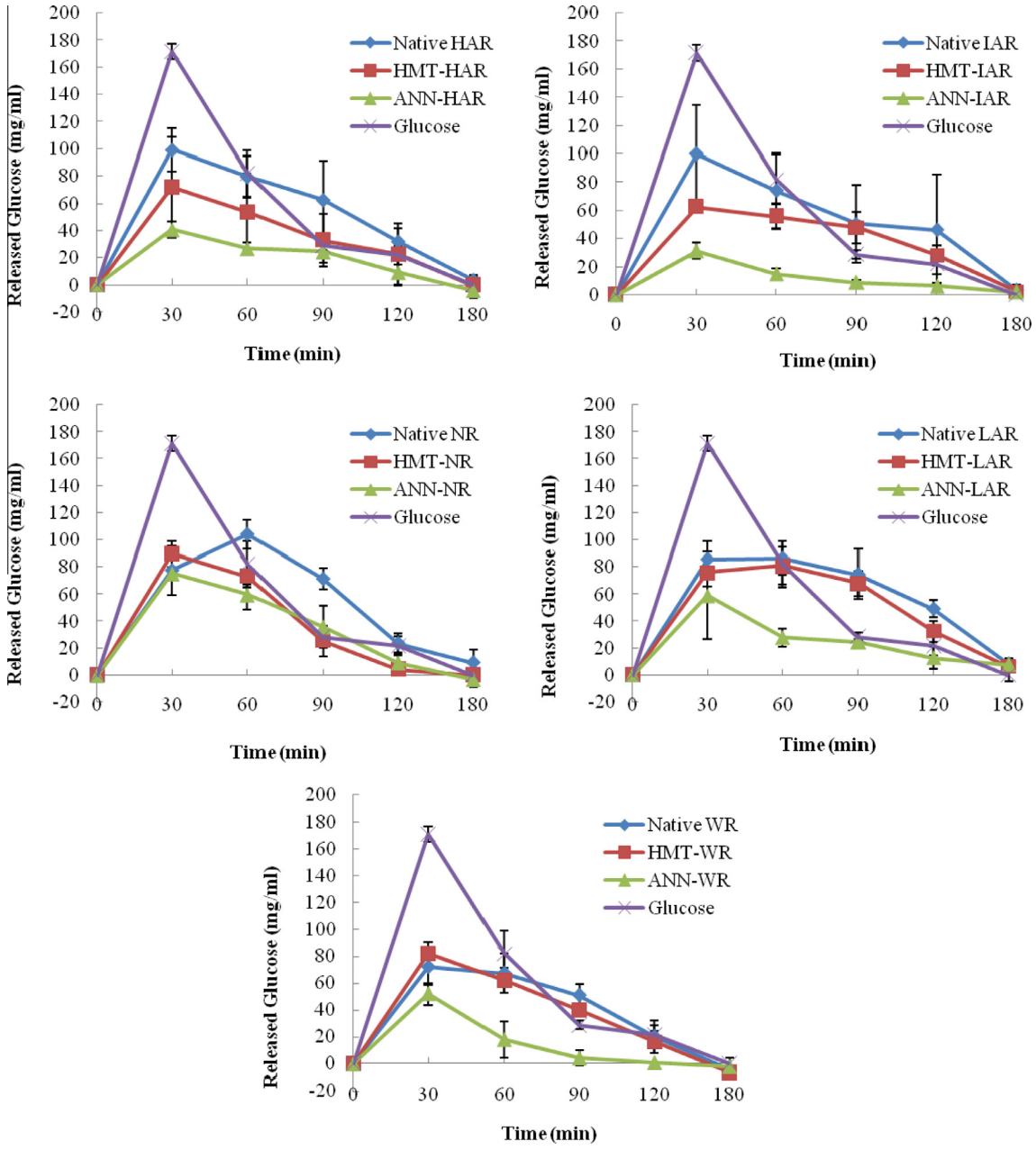

Fig. 2. Mean blood glucose concentration in mice after intake of glucose, native and physically modified rice starches. HAR, high-amylose rice; IAR, intermediate-amylose rice; NR, normal rice; LAR, low-amylose rice; WR, waxy rice; HMT, heat-moisture treatment; ANN, annealing treatment. Data are the mean ± SD ($n = 3$).

Table 3
Glycemic index (%) of glucose, native and physically modified rice starches.[a,*]

Sample	Native	HMT	ANN
Glucose	100c	–	–
HAR	93.2b	61.2a	32.5c
IAR	94.6b	67.1c	21.2a
NR	94.4b	61.9a	58.0e
LAR	100c	88.9d	43.9d
WR	68.9a	64.6b	23.9b

[a] HAR, high-amylose rice; IAR, intermediate-amylose rice; NR, normal rice; LAR, low-amylose rice; WR, waxy rice; HMT, heat-moisture treatment; ANN, annealing treatment.
[*] Data by the same letter in the same column are not significantly different ($P < 0.05$).

Table 4
Correlation coefficients among amylose contents, starch digestive fractions and glycemic index of rice starches.[a]

	AM	RDS	SDS	RS
RDS	0.430			
SDS	−0.658**	−0.705**		
RS	−0.113	−0.859**	0.243	
GI	0.165	0.607*	−0.115	−0.747**

*,**Correlation is significant at the 0.05 and 0.01 levels, respectively.
[a] AM, amylose content; RDS, rapid digestible starch; SDS, slowly digestible starch; RS, resistant starch; GI, glycemic index.

heat-moisture or annealing treatment of various rice starches were different and responsible for starch chain accessibility to hydrolyzing enzymes, therefore decreasing GI (Hoover & Vasanthan, 1994; Chung et al., 2009). The arrangements of starches after heat-moisture or annealing treatment related to GI values

were not affected by amylose contents of the starches. The lower GI values of heat-moisture treated or annealed starches as compared to the native starches were also found on corn, pea and lentil starches (Chung et al., 2009), suggested that heat-moisture treated and annealed starches could be used as food ingredients for low-available-calorie foods.

3.4. Correlations among amylose content, starch digestive fractions and GI values

The correlations among amylose content, starch digestive fractions and GI values were analyzed using Pearson correlation analysis and are given in Table 4. The amylose contents of rice starches were negatively correlated with amounts of SDS only, whereas RDS, RS and GI values did not correlate with amylose contents. Among the starch digestive fractions, RDS values were highly negatively correlated with SDS and RS values, whereas no correlation between SDS and RS values was found. The GI values of rice starches were highly positively correlated with RDS values, whereas the GI values were highly negatively correlated with RS and were not correlated with SDS values. As a result, the amylose contents of rice starches did not affect the *in vitro* and *in vivo* digestibilities of the gelatinized starches even though the high-amylose rice variants have been reported to exhibit lower metabolic responses and GI values (Hua, Zhao, Duan, Linlin, & Wu, 2004). The heat-moisture and annealing treatments (HMT and ANN) significantly increased eGI values in all granular starches. However, the HMT and ANN decreased eGI values of all gelatinized starches as reported by Chung et al. (2009). Similar results were found in this study when GI was tested using the *in vivo* digestibility method in mice. Thus, the interactions of amylose–amylose, amylopectin–amylopectin and amylose–amylopectin chains during heat-moisture or annealing treatment formed an inherent structure to the accessibility of hydrolyzing enzymes and are responsible for GI values. The well correlation between RDS and RS values and GI values indicates that the *in vitro* digestibility method can be used as a replacement for the GI measurement, which is a time-consuming and expensive process.

4. Conclusion

The rice starches with varying amylose contents were subjected to this study to investigate the change in starch digestive fractions and glucose response after heat-moisture and annealing treatments (HMT and ANN). The results indicate that total amounts of SDS and RS of the gelatinized rice starches decreased with increasing in amylose contents. HMT resulted in the increase in RS content, while ANN induced the increase in both SDS and RS contents. The RS contents of the rice starches with higher amylose contents significantly increased after HMT as compared to those of the rice starches with lower amylose contents. In contrast, the ANN significantly improved the RS contents in the rice starches with lower amylose contents. Both heat-moisture and annealing treatments reduced the GI values of rice starches, in which the annealed rice starches had the lowest GI values, followed by the heat-moisture treated rice starches. The change in RS contents and GI values of physically modified starches were not correlated with amylose contents suggesting that the interactions of amylose–amylose, amylopectin–amylopectin and amylose–amylopectin chains during heat-moisture or annealing treatment formed an inherent structure to the accessibility of hydrolyzing enzymes and are responsible for GI values. The results also show that the well correlation between GI values and the RDS and RS contents of rice starches.

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