

## Effects of amylose and resistant starch on starch digestibility of rice flours and starches

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### Abstract

Amylose and resistant starch (RS) content in rice flours and starches were manipulated by adding high amylose and high RS maize starch. The maize starches were added to rice flours and rice starches at the levels of 10-50% w/w, resulting in the increase of amylose from approximately 30-56 and RS from 8 – 33 g/100 g dry sample. Physicochemical properties and starch digestibility of the mixtures, both flour and starch mixtures, were investigated. Differential scanning calorimetry (DSC) results showed that the addition of high amylose high RS maize starch at the levels in this study did not significantly ( $p > 0.05$ ) alter gelatinization temperatures. Texture analysis as exhibited by hardness and adhesiveness of the mixture gels found inconsistent results. The key benefit of adding high amylose and high RS maize starch was that it can alter starch digestion rates and consequently lower estimated glycaemic indices (GIs) in both rice flour and starch mixtures.

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### Introduction

Rice is one of the most important cereal crops and is a staple food in Asia. Starch is the major component of rice and an important part of human nutrition (Ratnayake and Jackson, 2008; Wang *et al.*, 2010; Saikia and Deka, 2011).

Starch is generally classified according to the extent and rate of digestion as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst *et al.*, 1992). RS has received much attention for both its potential health benefits (similar to soluble fibre) and functional properties. It positively influences the functioning of the digestive tract, microbial flora, the blood cholesterol level, the glycaemic index (GI) and assists in the control of diabetes (Fuentes-Zaragoza *et al.*, 2010). RS is not digested in the upper gastrointestinal tract, its microbial fermentation in the colon produces short-chain fatty acids that show beneficial to colonic health (Zhang *et al.*, 2012). At present, RS may be classified into four groups (RS1-RS4) based on their physical and chemical characteristics. RS content has been found to positively correlate with amylose content in cereal crops (Cone and Wolters, 1990; Rendleman, 2000; Evans and Thompson, 2004; Benmoussa *et al.*, 2007; Sang *et al.*, 2008).

Physicochemical and metabolic properties of rice are influenced by numerous factors. One of these factors is amylose content, which is often used to predict starch digestion rate, blood glucose and insulin responses to rice. Starchy foods that are rich in amylose content are associated with lower blood glucose levels and slower emptying of human gastrointestinal tract compared to those with low levels of amylose (Behall *et al.*, 1988, 1989; Frei *et al.*, 2003). Apart from amylose content, other starch properties such as granule size, architecture, crystalline pattern, degree of crystallinity, surface pores or channels, degree of polymerisation, and non-starch components also influence starch digestibility (Tester *et al.*, 2006; Noda *et al.*, 2008).

Rice is generally known to have a relatively high GI compared to other starchy foods. It has been reported that GIs for rice ranged from 54 - 121 (Jenkins *et al.*, 1981, 1984; Brand *et al.*, 1985; Hu *et al.*, 2004; Jaisut *et al.*, 2008). High-amylose rice varieties were reported to exhibit lower glycaemic values than low-amylose varieties (Hu *et al.*, 2004; Denardin *et al.*, 2007; Denardin *et al.*, 2012). It has been clear that amylose and RS have the influences on starch digestibility. Rice flours and starches are versatile ingredients for many products especially gluten free products. Their starch digestibility properties could

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be improved by manipulating their amylose and RS content. This paper examines the effects of amylose and RS levels on physicochemical properties and starch digestibility of rice flours and starches. The findings could help in understanding the effects of both components. In addition, they could provide the information for industries to formulate rice flours or starches with enhanced functional properties.

## Materials and Methods

### Sample preparation

Rice flours and starches were purchased locally from commercial products in Thailand. High amylose and high RS maize starch (Hi-maize™ 260) was purchased from National Starch and Chemical (Thailand) Co., Ltd. The supplier labeled a minimum of 60 g per 100 g dry sample of total dietary fibre. The maize starch was used to mix with both rice flours and starches to manipulate the amylose and RS content. The mixtures contained 10, 20, 30, 40 and 50% w/w of maize starch. All samples were sieved through 100-mesh screen prior to analysis. The physicochemical properties and starch digestibility of the mixtures (both rice flours and starches) were determined.

### Physicochemical properties

#### Starch composition

Starch composition was determined enzymatically using the Megazyme RS assay procedure (KRSTAR test kit, Megazyme International, Ireland). Briefly, 100 mg of milled sample were incubated in a shaking water bath with thermo-stable pancreatic  $\alpha$ -amylase and AMG for 16 hr at 37°C. During this incubation the non-resistant starch is solubilized and hydrolyzed to glucose by the two enzymes. The reaction was terminated by the addition of equal volume of aqueous ethanol and the RS was recovered as a pellet on centrifugation. The supernatants of this centrifugation and those of two consecutive washings were removed by decantation and stored. RS pellets were dissolved in 2 M KOH and stirred for 20 min in an ice/water bath over a magnetic stirrer. Sodium acetate buffer (1.2 M, pH 3.8) was added, the starch was quantitatively hydrolyzed to glucose with AMG. The absorbance of the released glucose was spectrophotometrically determined at 510 nm using glucose oxidase-peroxidase reagent (GOPOD) method. Glucose release of non-resistant starch was equally determined by previously pooling the supernatant and the two washings and adjusting the volume to 100 mL. Total starch was calculated as the sum of RS and non-RS. Each sample was analyzed

in duplicate.

#### Amylose content

Amylose content of the samples was determined by colorimetric measurement of the blue amylose-iodine complex (Juliano, 1971). In summary, 100 mg of sample were weighed into a 100 mL volumetric flask and mixed with 1 mL ethanol and 9 mL of 2 M NaOH. The samples were diluted and the iodine solution was added. After 10 min incubation at room temperature, the absorbance at 620 nm was analyzed with a spectrophotometer and the amylose content was calculated based on the standard curve. The samples were analyzed in triplicate.

#### Differential scanning calorimetry (DSC)

The moisture of the samples was adjusted to 70% by the addition of distilled water. A DSC (Mettler Toledo DSC 1) equipped with a refrigerated cooler was used. The hydrated samples were weighed ( $25 \pm 5$  mg) into aluminum DSC pans and hermetically sealed. The DSC analysis was run by scanning from 25-120°C, ramping at 10°C/min and an hermetically sealed empty pan was used as a reference. Nitrogen was used as a purging gas. The software used for the analysis of the resulting thermograms was Star e software (ver. 9.20, Mettler Toledo). The onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), conclusion temperature ( $T_c$ ) and transition enthalpy ( $\Delta H$ ) were determined. Each sample was analyzed in triplicate.

#### Textural properties

The samples were mixed with distilled water to prepare 30 g of paste (30% w/w) in a 50 mL cylindrical glass jar, followed by cooking at a steam bath for 30 min for gelatinization and cooling at 4°C for another 30 min. To avoid the effects of starch retrogradation, the samples were immediately measured for textural properties (Lu et al., 2011) using a Texture Analyzer (TA-XT2, Stable Micro Systems, England) equipped with a 5 mm diameter cylinder probe and compression platens. The parameters were set as follows: pretest speed 2.0 mm/s, test speed 1.0 mm/s, posttest speed 2.0 mm/s, trigger force 15 g, distance 5 mm. The resulting force-time curves were then analyzed with the Exponent software (ver. 6, Stable Micro Systems, England) for sample texture characteristics including hardness and adhesiveness. Hardness was defined as the maximum compressive force that displays substantial resistance to deformation. Adhesiveness was defined as the negative force area after the first compression, representing the work necessary to pull the compressing plunger away from the sample. At least ten measurements were conducted for each sample.

### In-vitro starch digestibility and modelling of starch digestograms

Time-course starch digestion was determined using a rapid *in vitro* digestibility assay based on glucometry (Sopade and Gidley, 2009; Mahasukhonthachat *et al.*, 2010). About 0.5 g of ground sample was treated with artificial saliva containing porcine  $\alpha$ -amylase (Sigma A3176 Type VI-B) before pepsin (Sigma P6887; pH 2.0) was added and incubated at 37°C for 30 min in a reciprocating water bath (85 rpm). The digesta was neutralized with NaOH before adjusting the pH to 6 (sodium acetate buffer) prior to the addition of pancreatin (Sigma P1750) and AMG (Novozymes AMG 300 L). The mixture was incubated for 4 hr, during which the glucose concentration in the digesta was measured with an Accu-Check® Performa® glucometer at specific periods (0, 30, 60, 90, 120, 150, 180, 210 and 240 min). Digested starch per 100 g dry starch ( $DS$ ) was calculated as in Equation (1):

$$DS = \frac{0.9 \times G_G \times 180 \times V}{W \times S[100 - M]} \quad (1)$$

where  $G_G$  = glucometer reading (mM/L),  $V$  = volume of digesta (mL), 180 = molecular weight of glucose,  $W$  = weight of sample (g),  $S$  = starch content of sample (g/100 g sample),  $M$  = moisture content of a sample (g/100 g sample), and 0.9 = stoichiometric constant for starch from glucose contents.

The digestogram (digested starch at a specific time period) of each sample was modeled using a modified first-order kinetic model, Equation (2), as described before (Mahasukhonthachat *et al.*, 2010).

$$D_t = D_0 + D_{\infty} [1 - \exp(-Kt)] \quad (2)$$

where  $D_t$  (g/100 g dry starch) is the digested starch at time  $t$ ,  $D_0$  is the digested starch at time  $t=0$ ,  $D_{\infty}$  is the digestion at infinite time ( $D_0 + D_{\infty}$ ), and  $K$  is the rate constant ( $\text{min}^{-1}$ ).

The Microsoft Excel Solver® was used to compute the parameters of the model by minimising the sum of squares of residuals (SUMSQ) and constraining  $D_{\infty} \leq 100$  g per 100 g dry starch, and  $D_0 \geq 0$  g per 100 g dry starch. In addition to the coefficient of determination ( $r^2$ ), the predictive ability of the models was assessed with the mean relative deviation modulus (MRDM) as described elsewhere (Mahasukhonthachat *et al.*, 2010).

In order to calculate the estimated GIs of the samples, the areas under the digestograms ( $AUC_{\text{exp}}$ ) were computed with Equation (3):

$$AUC_{\text{exp}} = \left[ D_{\infty} t + \frac{D_{\infty} - D_0}{K} \exp(-Kt) \right]_{t_1}^{t_2} \quad (3)$$

The hydrolysis index (HI) of each sample was

calculated by dividing the area under its digestogram by the area under the digestogram of a fresh white bread (Goñi *et al.*, 1997), which was calculated to be about 24,000 min g/100 g dry starch from 0–240 min. From Goñi *et al.* (1997), single-point measurement of starch digestion at 90 min in the samples was also used to calculate GI ( $H_{90}$ ). Hence, using the parameters of the modified first-order kinetic model for both the samples and fresh white bread, estimated GIs of the samples were also calculated, and the average GI ( $GI_{\text{AVG}}$ ) for each sample was defined as Equation (4):

$$GI_{\text{AVG}} = \frac{[(39.21 + 0.803H_{90}) + (39.51 + 0.573HI)]}{2} \quad (4)$$

### Statistical analysis

Analysis of variance (ANOVA) and test of significance were performed using SPSS® ver. 17 with confidence level of 95%. The samples were randomized for all the analyses described above.

## Results and Discussions

### Physicochemical properties

#### *Starch composition and amylose content*

Starch composition and amylose content are shown in Tables 1 and 2. Both rice flours and starches showed high starch content, ranging from 75.8–87.8 g/100 g dry sample, as expected of carbohydrate foods. The content of non-resistant starch diminished and that of resistant starch augmented, as expected, along with the addition of maize starch because of its high amylose content (Morita *et al.*, 2007; Srikaeo *et al.*, 2011). The rice starch samples contained higher amounts of RS than the rice flour samples, ranging from 7.98 to 32.90 % and from 7.78 to 31.81 % on a dry basis, respectively. The total starch content generally increased with the addition of maize starch. Amylose content of the maize starch was found to be  $76.05 \pm 2.76$  g/100 g dry sample while rice starches and flours contained  $33.77 \pm 0.33$  and  $29.72 \pm 3.85$  g/100 g dry sample respectively. The amylose content correlated strongly with the RS content and augmented linearly ( $r^2$  values of 0.98–0.99) with the addition of the maize starch. These suggested that maize starch was suitable for manipulating amylose and RS content in rice flour/starch samples.

### DSC

DSC results suggested that the flour and starch mixtures gelatinized at the temperatures ranging from 64–71°C (Tables 3 and 4). Gelatinization temperatures of rice starch mixtures were found to be generally equal to those published previously (Zhu *et al.*, 2011).

**Table 1.** Starch composition and amylose content of the rice flour mixtures (g/100 g dry sample)<sup>b,c,d</sup>

Samples <sup>a</sup>	Non-resistant starch	Resistant starch	Total starch	Amylose
Rice Flour	68.03 ± 3.05a	7.78 ± 0.35a	75.81 ± 3.40a	29.7 ± 3.8a
RF10	63.30 ± 0.29b	12.10 ± 0.51b	75.39 ± 0.23a	35.3 ± 1.9b
RF20	59.92 ± 0.33c	18.09 ± 0.59c	78.01 ± 0.25ab	39.8 ± 2.6cd
RF30	57.69 ± 0.18cd	22.05 ± 0.31d	79.74 ± 0.14bc	44.5 ± 0.7de
RF40	54.85 ± 0.17de	27.10 ± 0.29e	81.95 ± 0.13cd	48.2 ± 0.9e
RF50	52.19 ± 0.03e	31.81 ± 0.06f	84.00 ± 0.03d	56.4 ± 0.9f

<sup>a</sup>RF10 = 10%, RF20 = 20%, RF30 = 30%, RF40 = 40% and RF50 = 50% w/w addition of maize starch to rice flour samples.<sup>b</sup>Values are means ± standard deviations.<sup>c</sup>For each parameter (column), values with the same letters are not significantly different ( $P > 0.05$ ).<sup>d</sup>These apply for all tables at where they appear.**Table 2.** Starch composition and amylose content of the rice starch mixtures (g/100 g dry sample)<sup>b</sup>

Samples <sup>a</sup>	Non-resistant starch	Resistant starch	Total starch	Amylose
Rice Starch	69.73 ± 3.44a	7.98 ± 0.39a	77.7 ± 3.83a	33.8 ± 0.3a
RS10	68.73 ± 0.02ab	13.16 ± 1.04b	81.88 ± 0.92b	36.4 ± 0.6b
RS20	65.35 ± 0.13bc	17.97 ± 0.04c	83.32 ± 0.11bc	40.8 ± 0.7c
RS30	61.92 ± 1.77cd	22.88 ± 0.71d	84.79 ± 1.21bcd	44.2 ± 0.4d
RS40	58.50 ± 0.06d	27.75 ± 0.16e	86.25 ± 0.09cd	48.9 ± 0.2e
RS50	54.89 ± 0.27e	32.90 ± 0.37f	87.79 ± 0.21d	54.2 ± 1.2f

<sup>a</sup>RS10 = 10%, RS20 = 20%, RS30 = 30%, RS40 = 40% and RS50 = 50% w/w addition of maize starch to rice starch samples.<sup>b</sup>These apply for all tables at where they appear.**Table 3.** DSC parameters of the rice flour mixtures

Samples	To (°C) ns	Tp (°C) ns	Tc(°C)	ΔH (J/g dry sample) ns
Rice Flour	63.30 ± 0.10	99.67 ± 0.08	68.48 ± 0.09b	7.61 ± 0.15
RF10	64.05 ± 0.45	91.51 ± 11.46	68.73 ± 0.07a	8.73 ± 1.89
RF20	64.80 ± 1.26	77.51 ± 17.65	68.99 ± 0.21a	7.15 ± 1.18
RF30	63.64 ± 1.70	88.33 ± 16.21	69.10 ± 0.42a	6.29 ± 6.94
RF40	64.01 ± 1.27	88.05 ± 16.43	69.40 ± 0.48ab	4.01 ± 1.24
RF50	61.15 ± 4.71	88.97 ± 15.12	70.27 ± 0.54a	3.44 ± 2.80

**Table 4.** DSC parameters of the rice starch mixtures

Samples	To (°C) ns	Tp (°C)	Tc(°C)	ΔH (J/g dry sample) ns
Rice Starch	64.62 ± 0.72	91.92 ± 10.71a	67.90 ± 1.56a	7.12 ± 1.44
RS10	64.18 ± 0.02	97.65 ± 2.74a	69.67 ± 0.52ab	2.27 ± 0.78
RS20	65.45 ± 0.24	69.73 ± 1.19b	70.16 ± 0.27b	5.14 ± 0.69
RS30	65.00 ± 1.34	91.53 ± 7.55a	70.46 ± 0.80b	3.89 ± 0.20
RS40	64.30 ± 0.20	97.34 ± 2.93a	70.48 ± 0.55b	1.73 ± 0.64
RS50	64.11 ± 0.49	99.59 ± 0.00a	70.91 ± 0.89b	6.06 ± 5.08

Gelatinization temperature range ( $T_g - T_o$ ) varied from 4.2°C to 9.1°C for the rice starch mixtures and from 3.3°C to 6.8°C for the rice flour mixtures. In this study, most samples showed that the addition of high amylose high RS maize starch did not significantly ( $p > 0.05$ ) alter gelatinization temperatures (refers Tables 3-4). The energy transitions found in the mixture samples were primarily from the rice itself, not the added maize starch. Previous investigation in our laboratory found no energy transition in high amylose and high RS maize starch when assessed by DSC (Srikaeo *et al.*, 2011). In contrast, several studies suggested that increasing dietary fibre showed positive linear correlation with the gelatinization temperatures (Morita *et al.*, 2007). Also, gelatinization temperatures and enthalpies increased as amylose content increased (Chung *et al.*, 2011). This is understandable as most previous studies investigated natural flour and/or starch samples which are different from the mixtures in this study. Also, gelatinization temperatures of the

starchy samples can vary due to factors that include genetic origin, environmental conditions and age of the parent plant (Jane *et al.*, 1992; da Mota *et al.*, 2000; Moorthy, 2002; Hung and Morita, 2005).

#### Textural properties

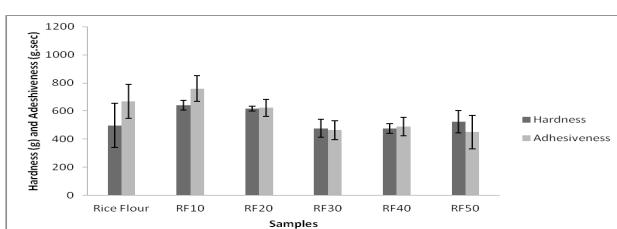
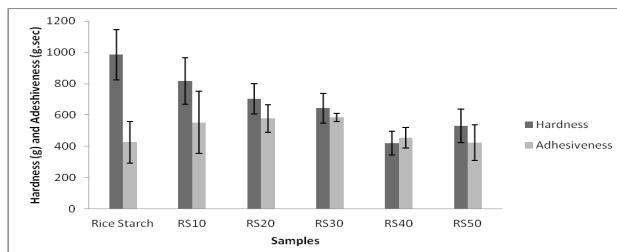
Hardness and adhesiveness of the gels prepared from the mixtures (both flours and starches) are shown in Figure 1 and Figure 2. It has been well known that rice with high amylose content provides dry and fluffy textures while low amylose rice gives moist, chewy and clingy textures after cooking. The proportion of amylose and amylopectin affected the hardness of rice starch gel (Hibi, 1998). Generally, high-amylose rice varieties give high hardness, high tensile strength, and high consistency (Lu *et al.*, 2009). Hardness usually showed a negative correlation with adhesiveness and therefore amylose (Yu *et al.*, 2009). In this study, inconsistent results were obtained in both sets of the samples. Increasing amount of maize

**Table 5.** Model parameters, hydrolysis index (HI) and glycemic index (GI) of the rice flour mixtures

Samples	$D_0$ (g/100 g dry starch)	$K \times 10^{-3}$ (min <sup>-1</sup> )	GI <sub>H90</sub>	GI <sub>HI</sub>	Average GI
Rice Flour	10.86±0.42ab	6.31±0.32c	78.96±1.35c	70.34±0.88c	74.65±1.11c
RF10	11.18±0.39b	5.69±0.14b	76.76±0.73bc	68.89±0.49bc	72.83±0.61bc
RF20	11.44±0.23b	5.25±0.05ab	75.18±0.08ab	67.82±0.06ab	71.50±0.07ab
RF30	10.52±0.73ab	5.47±0.13ab	75.60±0.14ab	68.12±0.11ab	71.86±0.12ab
RF40	9.96±0.50a	5.07±0.37ab	73.70±1.27a	66.82±0.89a	70.26±1.08a
RF50	10.36±0.21ab	4.95±0.27a	73.39±1.25a	66.60±0.87a	69.99±1.06a

**Table 6.** Model parameters, hydrolysis index (HI) and glycemic index (GI) of the rice starch mixtures

Samples	$D_0$ (g/100 g dry starch) <sup>ns</sup>	$K \times 10^{-3}$ (min <sup>-1</sup> )	GI <sub>H90</sub>	GI <sub>HI</sub>	Average GI
Rice Starch	9.44±1.98	9.39±2.15b	88.04±5.37b	75.80±3.02b	81.92±4.20b
RS10	11.05±0.18	7.65±0.24ab	83.96±0.68ab	73.50±0.41ab	78.73±0.55ab
RS20	10.74±0.13	7.51±0.93ab	82.99±3.02ab	72.88±1.86ab	77.93±2.44ab
RS30	11.00±0.26	7.48±0.41ab	83.05±1.24ab	72.94±0.76ab	77.99±1.00ab
RS40	10.70±1.57	7.76±1.30ab	83.76±3.55ab	73.34±2.14ab	78.55±2.85ab
RS50	11.76±0.97	6.33±0.24a	79.44±0.43a	70.66±0.28a	75.05±0.35a

**Figure 1.** Hardness and adhesiveness of the rice flour mixtures (RF10 = 10%, RF20 = 20%, RF30 = 30%, RF40 = 40% and RF50 = 50% w/w addition of maize starch to rice flour samples)**Figure 2.** Hardness and adhesiveness of the rice starch mixtures (RS10 = 10%, RS20 = 20%, RS30 = 30%, RS40 = 40% and RS50 = 50% w/w addition of maize starch to rice starch samples)

starch decreased the hardness values in rice starch mixtures. However, opposite results were obtained in rice flour mixtures. Hardness showed to be much higher in rice starch than those found in rice flour samples. This is probably due to the effects of other components in flour samples such as proteins (Singh *et al.*, 2011). Prolamin and glutelin were reported to either decrease or increase the gel hardness depending on the conditions (Hager *et al.*, 2012). In terms of adhesiveness, the negative force area after the first compression, both sets of the samples showed similar patterns. Increasing the levels of maize starch resulted in decreasing adhesiveness. Notably that unblended rice starch showed less adhesiveness than unblended

rice flour. Overall results suggested that the textures of the mixtures of rice flour/starch with high amylose and high RS maize starch could be difficult to predict using conventional indicators such as amylose and some instrumental texture characteristics. Special cares should be taken for industrial application and process validation is necessary.

#### In-vitro starch digestibility and modeling of starch digestograms

Tables 5 - 6 show the digestion data of rice flour and starch mixtures respectively. The modified first-order kinetic model showed suitable in describing the digestograms ( $r^2 = 0.90 - 0.99$ ; MRDM = 7 - 12%; SUMSQ = 65 - 156). In general, rice starch mixtures showed estimated GI values of 75-82 which were higher than those found in rice flour mixtures, 70-75. Low GI foods are valuable in lowering insulin response, and greater use of stored fat is expected. These, as well as the fact that RS has been studied for its potential health benefits, make high RS and low GI foods important for obesity, diabetes and its dietary management (Nugent, 2005; Sajilata *et al.*, 2006). The present study confirmed that starch digestibility could be improved by manipulating the level of amylose and RS at the end products, rice flours and starches in this case. This study used high amylose and high RS maize starch. Other sources of ingredients with similar functional properties could also be able to use. In both groups of samples, the digestion rate ( $K$ ) decreased and consequently the estimated GI values reduced with increased amount of the maize starch. That is consistent with the high fibre maize starch having higher amylose and RS, as increased amylose resulted in lower GI (Hu *et al.*, 2004; Morita *et al.*, 2007). In this study, the addition of 50% maize starch resulted in 8.39% and 6.24%

drop of estimated GIs in the rice flour and starch mixtures, respectively. In the flour samples, the estimated GIs decreased from high to medium range (<70). Comparing among all the samples, rice flour mixtures contained slightly less amylose than those of the rice starch group, although the latter showed higher digestion rate. That is explainable as *K* and GI both depend on various factors such as starch granule morphology, amylose to amylopectin ratio, molecular structure, degree of branching in terms of steric hindrance, and consequently mass transfer resistance (Fuentes-Zaragoza *et al.*, 2010; Singh *et al.*, 2010). In addition, the other constituents in the rice flour could also have impact on *K* and GI.

## Conclusion

Attempts have been made to manipulate the levels of amylose and RS in rice flours and starches by adding high amylose and high RS maize starches. Physicochemical properties including thermal, textural properties and starch digestibility were influenced by amylose and RS content. The GI values of rice flours and starches, which are generally high, can be reduced by increasing the levels of amylose and RS. Apart from producing rice flours or starches from high amylose rice varieties, it can also be done at by adding a key functional ingredient. This study used high amylose and high RS maize starches. It could also be possible to use other cereal starches which contribute to similar functional properties.

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