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Formulating low glycaemic index rice flour to be used as a functional ingredient

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ABSTRACT

Amylose and resistant starch (RS) content in rice flour were manipulated. The experiment was conducted using a full factorial design. Rice flour with average amylose content of 20 and RS content of 0.5 g/100 g dry sample was fortified with pure amylose from potato and high RS modified starch to reach the final amylose content of 30, 40 and 50 and RS content of 2, 4 and 6 g/100 g dry sample. The fortified rice flours were examined for their gelatinisation properties, in-vitro enzymatic starch digestion and gel textural properties. It was found that amylose and RS significantly affect all the fortified rice flour properties ($p < 0.05$). High amylose and RS improved starch digestion properties, reducing the rate of starch digestion and lowering the glycaemic index (GI) values. Amylose had a more pronounced effect on the fortified rice starch properties than RS. In this study, the fortified rice flour which contained amylose and RS of approximately 74 and 9 g/100 g dry sample respectively was used to produce rice noodles. The noodles exhibited low GI values ($GI < 55$). However, amylose and RS affected the textures of rice noodles providing low tensile strength and break distance (extensibility).

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1. Introduction

Rice is grown worldwide and provides food for more than half of the world's population, especially those living in populous countries in Asia. Polished or white rice is the predominant type of rice consumed worldwide. It can also come in the form of rice flour and starch.

However, white rice is generally known to have a relatively high glycaemic index (GI) compared to other starchy foods. It has been reported that GIs of rice ranged from 54 to 121 (Hu et al., 2004).

In a meta-analysis which included seven prospective cohort studies in Asian and Western populations, it was found that high white rice consumption is associated with a significantly increased risk of type 2 diabetes, especially in Asian (Chinese and Japanese) populations (Hu et al., 2012). However, a later study (Soriguer et al., 2013) showed different results for a population from Southern

Spain. They found that people who ate rice more frequently were less likely to develop type 2 diabetes mellitus. This is understandable because, apart from the large range of GI values of rice, ethnic issues also pose major influences (Brand-Miller et al., 2009). For example, glycaemic load or amount of rice consumed, cooking methods and other ingredients in rice diets of Asian and European countries are different. A recent study has reported that glycaemic responses following ingestion of glucose and several rice varieties are appreciably greater in Chinese compared with Europeans (Kataoka et al., 2013).

In addition, amylose content plays an important role in controlling the starch digestion rate. Hence, it 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 (Frei et al., 2003). Several investigators have reported that high amylose rice exhibited lower GI values than low amylose varieties (Denardin et al., 2007; Hu et al., 2004).

Apart from amylose, resistant starch (RS) has recently received much attention for both its health benefits and functional properties. It positively influences the functioning of the digestive tract, microbial flora, prebiotic properties, the blood cholesterol level, the

Abbreviations: D_0 , initial digested starch; GI, glycaemic index; GT, gelatinisation temperature; ΔH , gelatinisation enthalpy; K , starch digestion rate constant; RS, resistant starch.

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GI and assists in the control of diabetes (Fuentes-Zaragoza et al., 2010). The degree of starch hydrolysis was found to highly correlate with RS (Shu et al., 2009).

The recent transition in nutrition with particularly the decreased physical activity levels and much improved security and variety of food has led to increased prevalence of obesity and insulin resistance in Asian countries (Popkin, 2001). Although rice has been a staple food in Asian populations for many years, this transition may render Asian populations more susceptible to the adverse effects of high intakes of white rice (Hu et al., 2012). The development of rice and rice products which exhibit lower GI values should benefit the populations. The Waxy gene has been identified as the major genetic determinant of GI in rice (Fitzgerald et al., 2011). Attempts are continuing to develop low GI rice varieties. A high amylose transgenic rice line has been developed by antisense RNA inhibition of the starch branching enzymes. The milled rice flour from the transgenic rice line gave amylose content up to 49.2% and RS up to 14.9% (Zhu et al., 2012). From a food technology perspective, the formulation of rice flour to exhibit lower GI values should be a promising approach. Rice flour can be used for various rice products. This study aims to investigate the effects of amylose and RS on glycaemic response of rice flour and consequently manipulate the levels of amylose and RS content to formulate low GI rice flour.

2. Material and methods

2.1. Materials

Commercially available rice flour in Thailand was used. The average amylose and RS contents of the flour were approximately 20 and 0.5 g/100 g dry sample respectively. Amylose content of the flour was adjusted by adding high purity amylose from potato (A0512, Sigma–Aldrich Singapore). RS content was adjusted by adding ActiStar[®], a resistant starch material produced by enzymic modification of tapioca starch (Megazyme International Ireland). The manufacturer labelled RS content of 52.9 g/100 g dry sample.

2.2. Experimental and statistical analysis

Statistical analysis was performed using Minitab ver. 16 (Minitab Inc., USA). Full factorial design (two factors and each at three levels with duplication) was used. The levels for the first factor (amylose) were set to 30, 40 and 50 g/100 g dry sample, while the levels for the second factor (RS) were set to 2, 4 and 6 g/100 g dry sample. Seven responses including gelatinisation temperature (GT), gelatinisation enthalpy (ΔH), initial digested starch (D_0), starch digestion rate constant (K), GI, hardness and adhesiveness were determined.

2.3. Amylose content

Amylose content of the samples was determined by colourimetric measurement of the blue amylose-iodine complex (Juliano, 1971). In summary, 100 mg of sample was 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 analysed with a spectrophotometer and the amylose content was calculated based on the standard curve. The samples were analysed in triplicate.

2.4. RS content

RS was determined enzymatically using the Megazyme RS assay procedure (KRSTAR test kit, Megazyme International, Ireland).

Briefly, 100 mg of milled sample was incubated in a shaking water bath with thermo-stable pancreatic α -amylase and AMG for 16 h at 37 °C. During this incubation, the non-resistant starch is solubilised and hydrolysed to glucose by the two enzymes. The reaction was terminated by the addition of an equal volume of aqueous ethanol and the RS was recovered as a pellet on centrifugation. 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 and the starch was quantitatively hydrolysed to glucose with AMG. The absorbance of the released glucose was spectrophotometrically determined at 510 nm using the glucose oxidase–peroxidase reagent (GOPOD) method. Each sample was analysed in triplicate.

2.5. Differential scanning calorimetry (DSC) gelatinisation properties

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 ± 5 mg) into aluminium DSC pans (120 μ L) and hermetically sealed. The DSC analysis was run by scanning from 25 to 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[®] software (ver. 9.20, Mettler Toledo). Transitional peak was evaluated for GT and ΔH . Each sample was analysed in triplicate.

2.6. 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 (Mahasukhonthachai et al., 2010). About 0.5 g of ground sample was treated with artificial saliva containing porcine α -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 neutralised 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 h, during which the glucose concentration in the digesta was measured with an Accu-Check[®] Performa[®] glucometer (Roche, Germany) 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 Eq. (1):

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

where G_C = glucometer reading (m M/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 modelled using a modified first-order kinetic model, Eq. (2), as described before (Mahasukhonthachai et al., 2010).

$$D_t = D_0 + D_{\infty-0}(1 - \exp[-Kt]) \quad (2)$$

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_{∞} is the digestion at infinite time ($D_0 + D_{\infty-0}$), and K is the rate constant (min^{-1}). $D_{\infty-0}$ was estimated from $t = 0$ –240 min.

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 (Mahasukhonthachai et al., 2010).

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

$$AUC_{exp} = \left[D_{\infty}t + \frac{D_{\infty}-0}{K} \exp(-Kt) \right]_{t_1}^{t_2} \quad (3)$$

Estimated GI values were determined using the method proposed by Goñi et al. (1997) with some modifications (Mahasukhonthachai et al., 2010). 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, which was calculated in our laboratory to be about 14,000 min g/100 g dry sample (from 0 to 240 min). Using the parameters of the modified first-order kinetic model for both the samples and fresh white bread, average GI (GI_{AVG}) for each sample was calculated using Eq. (4):

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

For convenient interpretation, this study selected three digestion parameters as the responses, initial digested starch (D_0), starch digestion rate constant (K) and average GI.

2.7. Flour gel textural properties

Textures of the rice flour gel were evaluated using the method from Lu et al. (2011) with some modifications. The samples were mixed with distilled water to prepare 30% w/w paste. The paste was filled into a 16 × 120 mm plastic test tube. The sample tubes were cooked using a steam bath for 30 min until starch gelatinisation and cooling at 4 °C for another 30 min. The cooked sample gel was removed from the test tube and cut at a 20 mm length. This provided the sample gel (16 × 20 mm) ready for immediate texture measurement using a TA-XT₂ Texture Analyser (Stable Micro System, 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 10 g, distance 5 mm. The resulting force–time curves were then analysed with the Exponent software (ver. 6, Stable Micro Systems, England) for sample texture characteristics including hardness and adhesiveness. At least ten measurements were conducted for each sample.

2.8. Food product application (rice noodles)

The rice flour with fortified amylose and RS content at the selected level was used to produce rice noodles. The control sample was produced from normal rice flour without any modification. The water was added to the flour to give the slurry at the same specific gravity (1.15 Baume). The same amount of slurry was poured into the nonstick trays to give the final rice sheet thickness at approximately 1 mm. The slurry was steamed for 10 min in the steamer at 95 °C to obtain cooked rice sheets. The cooked rice sheets were tempered at room temperature for 3 h before being cut to 7 mm width and dried at 50 °C until the final moisture content was about 10–13 g/100 g dry basis. The noodle samples were analysed for in-vitro starch digestion assay (described above) and textural properties.

The texture quality of the cooked noodles was determined by measuring the tensile strength and break distance (extensibility) using a TA-XT₂ Texture Analyser (Stable Micro System, England) (Inglett et al., 2005). Briefly, dried noodles were soaked in water for 15 min to rehydrate and cooked in boiling water for 3 min. Five strands of cooked noodle were fixed to the arms of the tensile grips. Force (tensile strength) at the break point was measured at a speed of 1.0 mm/s. Tensile strength and break distance were recorded using the Exponent software (ver. 6, Stable Micro Systems, England).

3. Results

3.1. Effects of amylose and RS content

Table 1 shows the average values for all the responses determined. It was found that both amylose and RS significantly affected all the responses as indicated by the p values (<0.05). However, the interaction of the factors (amylose*RS) did not show much significant effect. It only affected some of the responses (D_0 and hardness). Main effects plots for all the responses are shown in Fig. 1. Graphical outputs confirmed the numerical results, indicating both amylose and RS affected all the studied responses. Amylose provided more pronounced effects than those of RS as observed by the strong slope lines in the main effects plots. GI and hardness correlate positively with the amylose and RS content. In contrast, D_0 , K , GI and adhesiveness correlate negatively with the factors. Special attention was made for ΔH as it correlates negatively with amylose but positively with RS content.

To visually define the effects of both amylose and RS on the response values, the contour plots are shown in Fig. 2. Considering the in-vitro starch digestion parameters, it can be clearly seen that the higher the amylose and RS content, the more desirable the starch digestion parameters (low D_0 , K and GI values).

Although high amylose and RS content contributed to the desired starch digestion parameters, they also affected the textural properties of the rice flour gels. When amylose and RS content of the flour increased, the hardness increased while adhesiveness decreased. 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.

Based on the GI, a regression equation was established; $GI = -0.32\text{Amylose} - 2.36\text{RS} + 105$ ($r^2 = 0.96$). Note that the interaction effect (amylose*RS) was omitted as it was not significant ($p \geq 0.05$). Aiming the GI value at 55, the rice flour was formulated to contain approximately 74 g/100 g dry sample of amylose and 9 g/100 g dry sample of RS. This flour was used to produce rice noodles. The product properties were determined in comparison with the control sample (normal rice flour).

It should be noted that Table 1 represents numerical results and statistical evaluation of various properties of rice flour mixed with different levels of amylose and RS. This represents the rice flour properties as the base ingredient. In terms of metabolic properties of rice, however, they are influenced by numerous factors. Amylose/amylopectin ratio and other starch properties such as granule size, architecture, crystalline pattern, degree of crystallinity, surface pores or channels, degree of polymerisation, and nonstarch components also influence starch digestibility. In addition, processing techniques affect starch digestibility of the finished rice products.

3.2. Food product application (rice noodles)

Table 2 shows the starch digestion parameters of the rice noodles produced from the fortified rice flour in comparison with the control

Table 1

Average values and *p* values of the responses (gelatinisation temperature (GT), gelatinisation enthalpy (ΔH), initial digested starch (D_0), starch digestion rate constant (*K*), glycaemic index (GI), hardness and adhesiveness).

Factors		Responses						
Amylose (g/100 g dry sample)		GT (°C)	ΔH (J/g dry sample)	D_0 (g/100 g dry sample)	$K \times 10^{-3}$ (min ⁻¹)	GI	Hardness (g)	Adhesiveness (g sec)
30		71.0	4.70	15.5	10.0	86	429	593
40		75.3	3.37	12.3	8.67	83	490	496
50		78.5	2.88	9.17	5.83	71	602	376
RS (g/100 g dry sample)								
2		72.8	3.63	15.2	10.2	89	476	512
4		74.3	3.52	12.2	8.17	81	497	485
6		77.7	3.80	9.67	6.17	71	549	468
Amylose	RS							
30	2	68.5	4.85	17.5	11.5	97	430	620
30	4	71.5	4.45	15.5	10.0	86	426	585
30	6	73.0	4.80	13.5	8.50	78	432	574
40	2	72.5	3.15	15.5	10.5	91	474	534
40	4	75.5	3.30	11.5	8.50	84	490	484
40	6	78.0	3.65	10.0	7.00	75	505	472
50	2	77.5	2.90	12.5	8.51	82	524	382
50	4	76.0	2.80	9.50	6.02	73	573	385
50	6	82.0	2.95	5.50	3.01	61	710	359
<i>p</i> values (amylose)		0.004	<0.0005	<0.0005	0.001	<0.0005	<0.0005	<0.0005
<i>p</i> values (RS)		0.036	0.032	<0.0005	0.002	<0.0005	0.019	0.015
<i>p</i> values (amylose*RS)		0.647	0.114	0.012	0.659	0.546	0.040	0.472

sample. Fig. 3 illustrated the textural properties (tensile strength and break distance) of the rice noodles. It was clear that the fortified rice flour produced rice noodles with low GI value (<55). However, as expected, it induced the changes in textural properties. The tensile strength and break distance of the rice noodles were lower than those of the control sample. The noodles with low tensile strength and break distance would provide low extensibility. This affected cooking and eating qualities of rice noodles.

4. Discussion

From the results, it is clear that amylose and RS affect the properties of rice flours as determined by various parameters in this study.

In terms of gelatinisation properties, compared with normal cereal starches, high amylose cereal starches have higher GT and lower gelatinisation enthalpies, lower swelling powers, and higher resistances to acid hydrolysis and enzyme digestion (Jiang et al., 2010). The findings in this study agree with the gelatinisation properties of high amylose rice reported previously (Qin et al., 2012). GT is influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains, and not to the proportion of the crystalline region, which corresponds to the amylose/amylopectin ratio. The difference in GT may be attributed to the difference in amylose content, size, form and distribution of starch granules, as well as the internal arrangement of starch fractions within the granule (Miao et al., 2011). Gelatinisation enthalpy is due mainly to the disruption of the double helix rather than the long-range disruption of crystallinity (Cooke and Gidley, 1992). Previous studies suggested that increasing dietary fibre or RS showed a positive linear correlation with the GT (Morita et al., 2007). Also, as amylose content increased, GT increased while enthalpies decreased (Chung et al., 2011).

The key benefit of formulating normal rice flour to contain high amylose and RS content in this study was that it can improve starch digestibility. Generally, rice and rice products are high in GI. The production of low GI rice products would be challenging for industry. Rice is a choice for people who suffer from coeliac disease, formulating rice products with low GI properties would provide extra benefits especially rice flour which can be used as the base ingredient for many food products.

Clinical trials have suggested that low GI diets resulted in modest improvements in overall blood glucose control, reduced insulin secretion and lowered blood lipid concentrations. 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 (Sajilata et al., 2006). The present study confirmed that starch digestibility could be improved by manipulating the level of amylose and RS content in the ingredients. Starchy foods that are rich in amylose content are associated with lower blood glucose levels and slower emptying of the human gastrointestinal tract compared to those with low levels of amylose (Zhu et al., 2012). Amylose content was reported to have an obvious impact on GI values and RS content. The contents of RS were increased with increasing amylose as found in several food systems (Hu et al., 2004; Morita et al., 2007).

In this study, rice noodles produced from the fortified rice flour which contained approximately 74 g/100 g dry sample of amylose and 9 g/100 g dry sample of RS was lower than 55 (low GI foods) (see Table 2). In Table 2, the estimated GI value of noodles produced from the fortified rice flour was 54.35 ± 0.72 , compared with 99.03 ± 1.20 of the control sample. In this phenomenon, the proportion of rice flour would be less than 50 g/100 g to be able to reduce the GI to the expectation. This paper studied the mechanistic effects of amylose and RS. It is highlighted in this paper that amylose and RS can lower the GI of rice flour and amylose has a more pronounced effect than RS. This information would be beneficial for further investigation on finding ways to increase amylose and RS content in rice. Research on this aspect is currently being conducted. They could start from the breeding of new rice varieties that exhibit high amylose content to the application of modification techniques during processing and storage of the rice and rice products.

High amylose rice provides a slow starch digestion rate but it affects the textures. High amylose and RS content contributed to the higher hardness and lower adhesiveness in rice gels (see Table 1).

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 (Lu et al., 2009). Hardness usually showed a negative correlation with adhesiveness and therefore

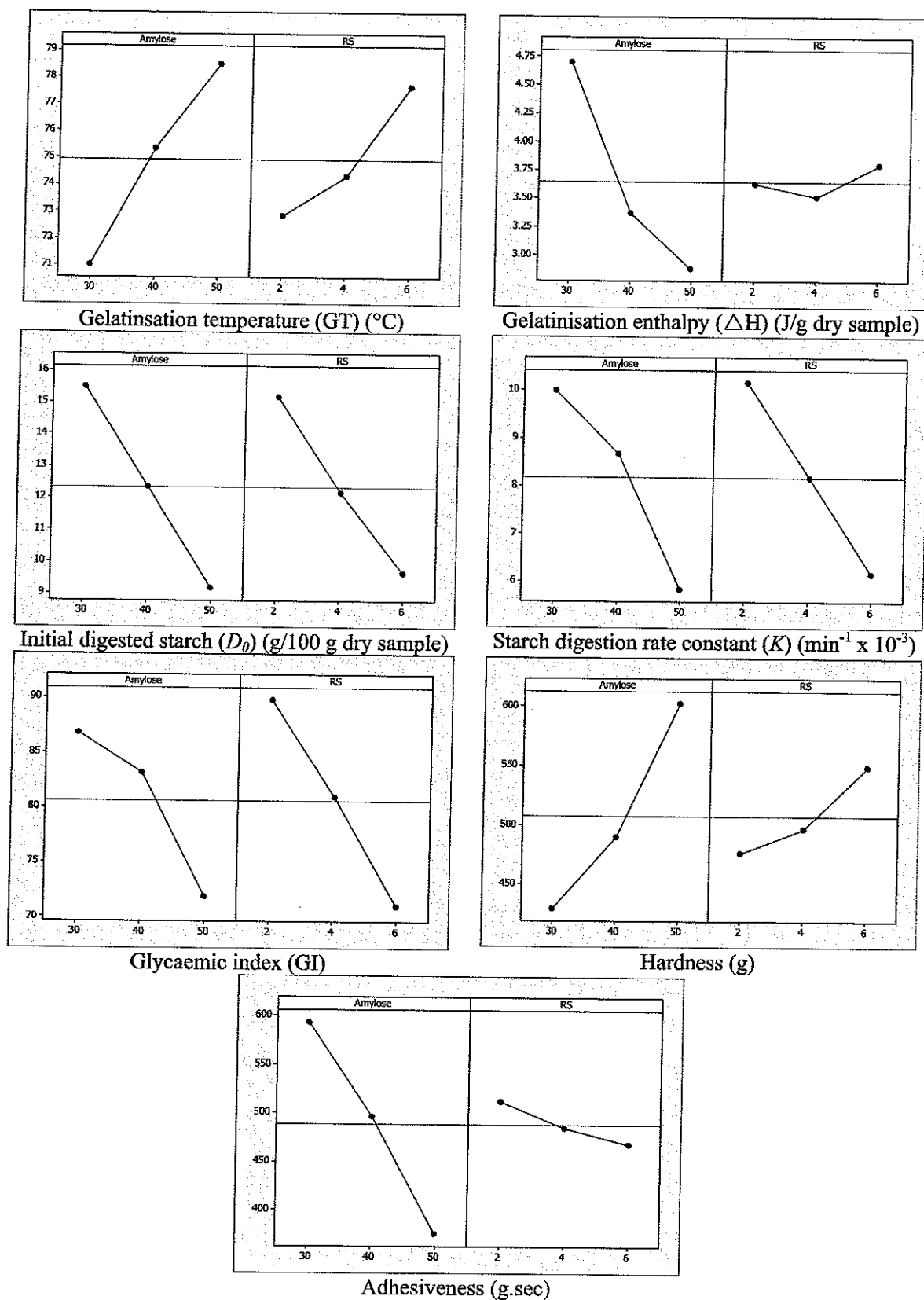


Fig. 1. Main effects plots for all the responses.

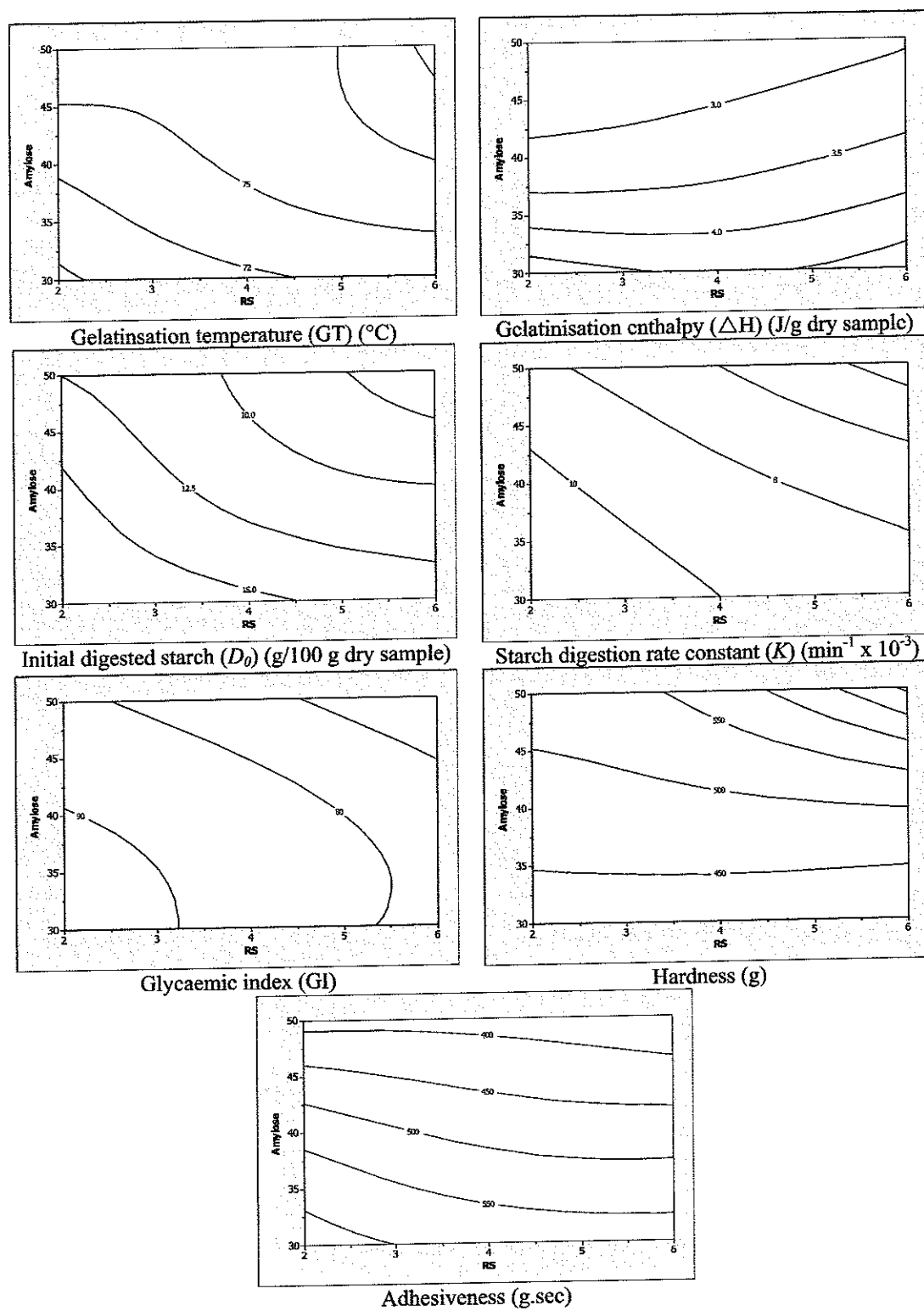


Fig. 2. Contour plots for all the responses.

Table 2
Model parameters and in-vitro glycaemic index (GI) of the rice noodles^{a,b}.

Rice noodles	D_0 (g/100 g dry starch)	D_{∞} (g/100 g dry starch)	$K \times 10^{-3}$ (min ⁻¹)	Estimated GI_{H90}	Estimated GI_{HI}	Average GI
Control	4.41 ± 1.08	100 ± 0.78	11.30 ± 0.42	89.07 ± 1.70	107.20 ± 1.51	99.03 ± 1.20
Fortified rice flour	1.86 ± 0.53	98.7 ± 0.45	5.11 ± 0.06	53.51 ± 0.40	55.43 ± 0.45	54.35 ± 0.72

^a All parameters are significantly different ($p < 0.05$).

^b Model quality parameters, $r^2 = 0.90$ – 0.99 ; MRDM = 7–12%; SUMSQ = 65–156.

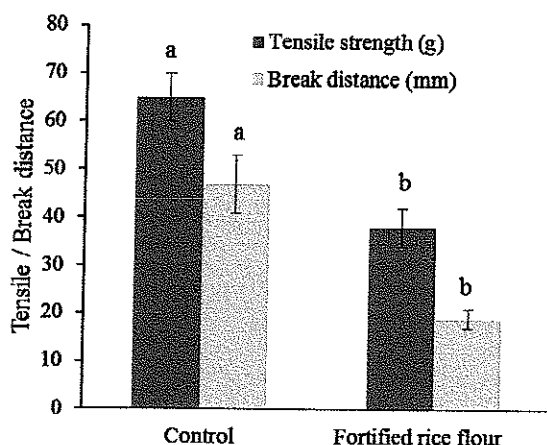


Fig. 3. Tensile strength and break distance of the control and fortified rice flour noodles. Bar shows mean and standard deviation values. For each parameter, bars with the same letters are not significantly different ($p > 0.05$).

amylose (Yu et al., 2009). Hence, rice noodles made from fortified rice flour gave high hardness and fluffy textures. Consequently, their tensile strength and break distance (extensibility) values were low (see Fig. 3). Consumer preferences influence desired food texture. In Thailand, high tensile strength and high extensibility rice noodles are preferred. However, this may be different in other countries. Moreover, other components in the ingredient mixtures e.g. proteins from rice flour could also influence the textures of the products (Hager et al., 2012). In commercial practice, chemically modified starch (such as by cross-linking or acetylation) together with various hydrocolloids are widely used in the rice noodle industry to improve the textures (Cham and Suwannaporn, 2010). In addition, hydrothermal treatment of rice flour could enhance the cooking and textural qualities of rice noodles (Hormdok and Noomhorm, 2007).

5. Conclusions

Higher consumption of white rice may associate with an increased risk of type 2 diabetes due to its high glycaemic response. Amylose and RS play an important role in lowering the glycaemic response in rice. Rice flour which was formulated to contain high amylose and RS content contributed to better starch digestion properties. This paper demonstrated that normal rice flour which was fortified with a certain amount of amylose and RS can be used to produce rice noodles with low GI values. However, care should be taken as high amylose and RS affected the textures of rice flour and consequently rice noodles.

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