



# 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 (Soriquer 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;  $\Delta H$ , 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<sup>R</sup>, 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 ( $\Delta H$ ), 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  $\alpha$ -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 ( $25 \pm 5$  mg) into aluminium DSC pans (120  $\mu$ 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<sup>e</sup> software (ver. 9.20, Mettler Toledo). Transitional peak was evaluated for GT and  $\Delta H$ . 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 (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 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<sup>®</sup> Performa<sup>®</sup> 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):

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

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

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