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# *In vitro* digestibility and physicochemical properties of milled rice

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ABSTRACT

Rice is a staple diet as well as a major ingredient in many processed foods. The physicochemical and supra-molecular structure of eight rice varieties with amylose content from 9% to 19% were studied to elucidate the factors responsible for variation in enzymatic digestibility of raw and cooked rice. Parboiled rice had a digestion rate coefficient almost 4.5 times higher than the least digestible Low GI rice. The rate coefficient was found to be independent of helical structure and long range molecular order, possibly attributed to the effect of rice flour architecture. Strong swelling and pasting behaviour and lower gelatinisation temperature were linked with apparently higher *in vitro* digestibility but the relationship was statistically insignificant. It is concluded that the enzymatic susceptibility of rice flours are independent of supra-molecular structure and are most likely controlled by external factors not limited to particle size, presence of intact cell wall and other non-starch polymers.



## 1. Introduction

Rice (*Oryzae sativa*, L.) is the staple food for over half of the world's population. Though it is mostly consumed in cooked form, the inclusion of rice flour in a number of processed foods, such as in baby formula and gluten free foods, is commonplace. Rice starch is highly digestible; therefore can increase the post-prandial blood glucose level associated with diet-related health complications (Mohan, Radhika, Vijayalakshmi, & Sudha, 2010). The huge surge in metabolic syndrome in the Asian population is often linked with excessive consumption of rapidly digestible food sources, such as cooked rice (Hu, 2011). In order to combat this problem, consumption of minimally or un-milled rice, parboiled rice, high-amylose, high fibre and mixed grain including rice is gaining popularity. On the other hand, rice varieties with high amylose content, that are claimed to have low glycaemic response, are commercially available.

The enzymatic susceptibility of rice, rice flour or isolated rice starch has been studied extensively (Chung, Liu, Lee, & Wei, 2011; Okuda, Aramaki, Koseki, Satoh, & Hashizume, 2005; Wani, Singh, & Shah, 2012) with inconclusive findings that long range crystalline and short range molecular structures in part controls the enzymic digestibility (Chung, Lim, & Lim, 2006; Chung, Wang, Yin, & Li, 2010; Chung et al., 2011; Reed, Ai, Leutcher, & Jane, 2013; Wani et al., 2012). There is enough evidence that high amylose cereals, such as mutant maize and barley, are less susceptible to amylolytic enzymes compared to wild varieties primarily due to the interaction of amylose with longer branch chains of amylopectin strengthening the granular structure (Bird, Shrestha, Lopez-Rubio, & Gidley, 2009; Sasaki, Kohyama, Suzuki, Noel, & Ring, 2009; Shrestha et al., 2012). However, the majority of rice starches are rich in amylopectin, and are disrupted with complete or partial loss of supra-molecular and granular structure during high temperature processing, such as cooking, enhancing the enzyme susceptibility (Chung et al., 2011). Some other parameters, such as higher peak and breakdown viscosities during shear cooking, are also associated positively with both amylopectin content and enzymic susceptibility (Benmoussa, Moldenhauer, & Hamaker, 2007; Chung et al., 2011).

Apart from amylose content and molecular/supra-molecular structure; cultivars, chemical/physical modification and processing; architectural factors, such as granule/particle size and porosity; intactness of cell walls; and presence of anti-nutrients, also control the rate and extent of enzymatic hydrolysis of starches (Bird et al., 2009; Wani et al., 2012). Choi, Kim, Kang, Nam, and Friedman (2010) have demonstrated the potential value of black rice bran as an anti-inflammatory and anti-allergic food ingredient and possibly also as a therapeutic agent for the treatment and prevention of diseases associated with chronic inflammation.

Though studied extensively, most of the studies regarding the enzyme susceptibility of rice starch/flour are limited to either

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non-commercial varieties (mutants or genetically modified crops) or with few rice varieties which may not be sufficient to draw the meaningful relationship between the molecular, supra-molecular structure and enzymic susceptibility. Furthermore, very limited reports are available regarding the causal relationship between the raw and cooked rice flours. In order to address these limitations, this study demonstrated the relationship between the supra-molecular structure (short and long range molecular order), thermal and rheological properties, non-starch polysaccharides and enzymic susceptibility of both raw and cooked rice flours using a range of techniques covering wider range of length scales.

## 2. Materials and methods

### 2.1. Materials

The following enzymes and chemicals were obtained from local distributors: α-amylase (Sigma A-3176 Type VI-B from porcine pancreas), pepsin (Sigma P-6887, from gastric porcine mucosa), pancreatin (Sigma P-1750 from porcine pancreas), amyloglucosidase, (AMG 300, Novozyme), enzyme glucose reagent (TR15103, Thermoelectron), pure potato amylose (A0512, Sigma) and amylopectin (S9679, Sigma).

Eight commercial brand rice varieties were purchased from the local supermarket. Low GI brown long grain, Sushi rice Japanese style, Vita-Rice parboiled long grain and Black rice were of Sunrice brand (SunRice, Leeton, NSW, Australia), whereas Arborio and Jasmine rice were of Home Brand (Woolworths Homebrand, Bella Vista, NSW, Australia). Basmati rice was an organic product from Macro wholefoods (Bella Vista, NSW, Australia) whereas RicePlus™ was from Plus Nutrition Pty Ltd, Mordialloc, VIC, Australia. As a product, RicePlus™ is unique as it has a mixture of ingredients: Brown Rice Long Grain (25%), White Basmati (24%), BARLEYmax®, Pearl Barley, Quinoa, Red Basmati (5%), Black Rice (5%), Black Sesame Seeds.

All rice samples were ground using a B-2B Mini blender (Homemaker, Australia) into moderately fine particles. The ground rice that passed through 600 μM but was retained in 400 μM (rice grits) was used for all analytical purpose. The particle size range was selected to minimise the deviation arising from difference in particle size as reported for starch granules (Dhital, Shrestha, & Gidley, 2010) or grain fragments (Al-Rabadi, Gilbert, & Gidley, 2009) and also to mimic the size of chewed cooked rice during mastication. The sieved flours were stored in air tight glass bottles at room temperature until further use.

### 2.2. Chemical composition

The moisture content of the ground rice samples was measured by the standard air oven drying at 105 °C overnight (AOAC, 2005). The level of starch present in the ground rice was measured by acid digestion method followed by Lane and Enyon method (ASEAN Manual of Food Analysis, 2011). The apparent amylose content of the ground samples were analysed by iodine colorimetric method (Hoover & Ratnayake, 2005). All samples were analysed in triplicates.

### 2.3. Crystallinity (X-ray diffraction)

XRD measurements of samples were made with a Panalytical X'Pert Pro diffractometer. The instrument was equipped with a copper X-ray generator ($\lambda$ = 1.54 A°), programmable incident beam divergence slit and diffracted beam scatter slit, and an X'celerator high speed detector. X-ray diffraction patterns were acquired at room temperature over the $2\Theta$ range of 2–40° with a step size of 0.0330° $2\Theta$ and a count time of 400 s per step.

Crystallinity was calculated using the modified curve-fitting procedure as described by Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008). Repeat analyses indicated an accuracy of ±1% for crystallinity values.

### 2.4. Fourier transform infrared spectroscopy (FTIR)

Fourier transformed infrared (FT-IR) spectra of starch in rice flour were recorded on a FT-IR spectrometer (Nicolet 5700, Thermo Electron Corporation, Madison, WI, USA) using an attenuated total reflectance (ATR) single reflectance cell with a diamond crystal. For each spectrum, 32 scans were recorded over the range of 1200–800 $cm^{-1}$ at room temperature (about 22 °C) at a resolution of 4 $cm^{-1}$, co-added and Fourier transformed. The background spectrum was recorded on air and subtracted from the sample spectrum. The ratio of absorbance at wave numbers 1047/1022 was calculated to represent ordered short range starch structures.

### 2.5. Rice starch pasting properties (RVA)

Rapid Visco Analyser (RVA) (Newport Scientific, RVA model 4, New South Wales, Thermocline Software Version 2.2) was used to measure the rheological properties of all rice starches. Starch suspension (8%, w/w) was prepared by weighing starch (2 ± 0.1 g) into the RVA canister and making up the total weight to 25 ± 1 g with distilled-deionised water. After equilibration at 50 °C for 1 min, the starch suspension was heated at a rate of 6 °C/min to 95 °C, maintained at 95 °C for 5 min, cooled to 50 °C at a rate of 6 °C/min, and then maintained at 50 °C for 2 min. A paddle speed of 160 rpm was used throughout, except for the first 10 s when a speed of 960 rpm was used to disperse the sample. Pasting temperature (PT), peak viscosity (PV), hot paste (trough) viscosity (HPV), and cool paste (end) viscosity (CPV) were measured from the RVA software.

### 2.6. Differential scanning calorimeter (DSC)

DSC 1 (Mettler Toledo, Schwerzenbach, Switzerland) with internal coolant and nitrogen/air purge gas was used to determine gelatinisation/melting temperatures of starches in uncooked flour. DSC was calibrated for the heat flow and melting temperature using indium and zinc as standards. An empty crucible was used as a reference. Rice flour (4 ± 0.1 mg) was mixed with deionised water (12 ± 0.3 mg) in a DSC pan (low pressure 40 μl aluminium pan). The crucible was left for about 120 min for equilibrium hydration of the sample before analysis. All analyses were carried out in duplicate. The pans were held at 10 °C for 5 min and then heated to 120 °C at 5 °C/min. The onset ($T_o$), peak ($T_p$), conclusion temperatures ($T_c$) and the enthalpy of gelatinisation ($\Delta H$, J/g) were determined using the built-in software (STARe System, Mettler Toledo, Schwerzenbach, Switzerland).

### 2.7. In vitro digestion of raw starch

*In vitro* starch digestion of ground rice was carried following method described by Shrestha et al. (2010) that mimics the biochemical conditions of mouth, stomach and small intestine using porcine salivary amylase, pepsin and pancreatin and amyloglucosidase as sources of hydrolysing enzymes, respectively. The resulting oligomers from the action of the amylases were converted to glucose using fungal amyloglucosidase and quantified with GOPOD enzymic assay kit as described by Shrestha et al. (2010). The results were presented as percentage starch hydrolysis, using the conversion factor of 0.9 for anhydrous glucose to starch.