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Rapid Communication

Parboiling reduced the crystallinity and *in vitro* digestibility of non-waxy short grain rice

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ABSTRACT

The impact of parboiling on starch digestibility of cooked rice was examined through an *in vitro* digestion model. Results indicated that the equilibrium starch hydrolysis of polished rice was the highest (86.55%), followed by that of parboiled-polished (83.94%), brown (80.59%) and parboiled rice (76.95%). X-ray diffraction analysis indicated that A-type crystals were predominant in brown rice and polished rice, while A-, B- and V-type crystalline structures coexisted in parboiled rice and parboiled-polished rice. Thin and compact layers were observed on the surfaces of parboiled rice and were considered to be physical barriers that reduce the starch digestibility. The study demonstrates that parboiling could change the crystallinity and reduce the starch digestion of rice significantly.

1. Introduction

As an important and stable starch food for nearly one half of the global population, rice (*Oryza sativa* L.) has been widely planted around the world and contributes about 21% energy for humans (Oli, Ward, Adhikari & Torley, 2014). Normally, rice is consumed after polishing. However, polished rice is considered a high glycaemic index (GI) food, as it is digested rapidly. Studies have confirmed that long-term overconsumption of polished rice is closely associated with hyperglycaemia or type II diabetes (Van Hung, Chau & Phi, 2016). Thus, ways to slow the digestion rate of rice have attracted intense attention. Examples include various cooking methods (Reed, Ai, Leutcher & Jane, 2013), improving the amylose content by transgenic engineering or retaining the physical barriers against enzymes via alternative pre-processing techniques (Alsaffar, 2011; Ordonio & Matsuoka, 2016). In particular, the pre-processing strategies have gained considerable interest, providing an option to reduce the starch digestion while simultaneously retaining the nutrients in rice.

Parboiling, which is the main step in the pre-processing of rice grain, has been widely used in Asian countries, such as India, Pakistan and Bangladesh and is also gaining popularity in Europe (Leethanapanich, Mauromoustakos & Wang, 2016). According to literature reviews, about 20% of the world's rice is parboiled (Bhattacharya, 2013; Buggenhout, Brijs, Celus & Delcour, 2013). Parboiling was considered an effective pre-processing technique to increase the storage stability of rice, with minimal changes in nutritional quality (Paiva et al., 2016). During parboiling, the starch is gelatinised by the thermal treatment, and the subsequent dehydration causes starch retrogradation, irreversibly modifying the microstructure of the biopolymer (Hapsari, Kim & Eun, 2016). Thus, the digestibility of parboiled rice might differ from the polished rice grains. However, to the best of our knowledge, few studies have focused on the relationships between the digestibility of parboiled rice and its microstructure, despite numerous studies comparing the differences in nutrition, microstructure and starch properties between the parboiled and polished rice.

To expand our understanding of the impact of parboiling on rice microstructure, crystallinity and digestibility, the present study focuses on the microstructure changes and *in vitro* digestibility of brown, parboiled, polished and parboiled-polished rice. Additionally, a first-order reaction equation was also introduced, to calculate the kinetics of starch hydrolysis. The work provides some new insight into the microstructure and crystalline structure of parboiled rice, as well as its digestibility. The results will help other researchers and industries better understand the impacts of parboiling on rice.

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2. Materials and methods

2.1. Materials

A non-waxy, short grain brown rice (*O. sativa* L. cv. Koshihikari) was purchased from a local rice shop in Matsudo, Chiba, Japan, in 2016, and stored at 4 °C in a refrigerator for later use. Pepsin (porcine gastric mucosa, 800–2500 U mg^{-1} protein), pancreatin (hog pancreas, 4 × USP), and invertase (Invertase, grade VII from baker's yeast, 401 U mg^{-1} solid) were purchased from Sigma-Aldrich Ltd. (St Louis, USA). Amyloglucosidase (3260 U mL^{-1}), the total starch assay kit and the glucose assay kit were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Other chemicals and reagents were analytical grade and purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

2.2. Preparation of parboiled and polished rice

The parboiled rice and polished rice were produced according to previous research (Paiva et al., 2016), with some modification. Briefly, the polished rice was obtained by polishing the brown rice with a rice polishing machine (BT-AF05, Zojirushi, Japan). The degree of polishing was 10% of the original brown grain weight. Thus, 90% remained as a white rice grain. For parboiling, 1 L of distilled water was added to the beakers containing 250 g brown rice and maintained in a water bath at 60 °C for 4 h. Then, the hydrated rough rice was autoclaved at 108 °C for 10 min (Lab Autoclave, Sanyo, Japan) before it was left to stand at room temperature overnight. Finally, the rough rice was oven-dried (WFO-400, Eyela, Japan) at 38 °C, until the moisture content was below 13%. For the parboiled-polished rice, the dried parboiled rice (100 g) was also polished (BT-AF05, Zojirushi).

2.3. Cooking time and texture analysis

In order to cook the rice fully, 100 g rice in a glass beaker was mixed with 300 mL distilled water and heated in boiling water. Cooked rice grains were compressed between two glass slides and were considered as fully cooked when the white core disappeared and then the cooking time was recorded (Tamura, Singh, Kaur & Ogawa, 2016a). After cooling to room temperature, the textural properties of fully cooked rice were analysed (Creep Meter RE2-3305S texture analyser, Yamaden, Japan) at room temperature (25 °C), following the manufacturer's protocols. Briefly, a 200 N load cell was fitted, and a cylindrical flat-end punch (ϕ = 17 mm) was performed at a speed of 4.5 mm s^{-1}. In total, 15 samples were measured for each determination, and the recorded values were used to assess the hardness, cohesiveness and adhesiveness.

2.4. X-ray diffraction analysis

X-ray measurements were made with an X-ray diffractometer (MiniFlex600, Rigaku, Japan). To prepare the samples, uncooked rice grain was ground with a grinder (SC-01, Sansho, Japan) and passed through a 200-mesh sieve. X-ray diffraction patterns were acquired at room temperature under the following conditions: 40 kV and 20 mA for the Cu-Kα radiation source at a wavelength of 0.15418 nm, and a scanning rate of 2° min^{-1} in the scattering range (2θ) of 5–40°. Diffraction data were analysed with Jade software (version 6.5; Material Date, Inc., Livermore, California, USA). The crystallinity (Xc) of ground rice samples (brown, polished, parboiled and parboiled-polished rice grain) was calculated as the ratio of $Ac/(Ac + Aa)$, where Ac and Aa are the areas of crystalline and amorphous phases, respectively (Tian et al., 2016).

2.5. In vitro digestion of parboiled rice

Attributing to its texture and small size, most tissues of cooked rice could remain during mouth chewing and, consequently, the starch hydrolysis could mainly occur in the small intestine (Tamura, Okazaki, Kumagai & Ogawa, 2017; Woolnough, Bird, Monro & Brennan, 2010). Thus, the present study applied a simulated gastro-small intestinal *in vitro* digestion model, as described by Tamura et al. (2016a), with some modifications. Briefly, rice containing 6.8 g total starch (measured with a total starch assay kit) was accurately weighed and fully cooked. Next, the cooked rice was mixed with distilled water to a total weight of 170 g. Afterwards, the mixture was transferred to a glass reactor and incubated at 37 ± 0.5 °C in a thermostatic water bath. To begin the simulated gastric process, the pH of the mixture was adjusted to 1.20 ± 0.05 with HCl solution, following the addition of 19 mL digestive juices (containing 91.20 mg pepsin), and stirring continuously with a magnetic bar at the bottom of the reactor. Thirty minutes later, the pH was increased to 6.80 by adding NaOH solution, and the simulated intestine digestion was started by adding 23 mL artificial intestinal fluid (containing 6.9 mg invertase, 92 mg pancreatin and 1.84 mL amyloglucosidase). The pH was maintained at 6.80 ± 0.05 for 120 min.

Simulated digestion supernatants (0.5 mL) were collected at various time points throughout the simulated gastric (0, 15, 30 min) and small intestinal (5, 10, 15, 30, 60, 90, 120 min) digestion processes and immediately diluted in 2.5 mL of 95% ethanol, to inactivate the enzymes. Then, the mixed solutions were centrifuged (2000*g*/10 min). Finally, 0.1 mL of the supernatant was incubated with 0.5 mL amyloglucosidase/invertase solution (37.50 mg invertase and 1 mL amyloglucosidase dissolved in 100 mL potassium acetate) at 37 °C for 10 min, to convert all the oligo- and disaccharides produced during hydrolysis to glucose.

2.6. Glucose measurement and kinetics of starch digestibility

The glucose concentrations of the incubated mixtures mentioned in section 2.5 were measured using a glucose analysis kit, according to the manufacturer's instructions. The results were expressed as the percentage of starch hydrolysis, SH (%) = 0.9 × Gp/Si, where SH is the percentage of starch hydrolysis (total), Gp is the amount of glucose produced (g), and Si is the initial amount of starch (g). The conversion factor of 0.9 represents the ratio of the molecular weight of the starch monomer to the molecular weight of glucose (162/180 = 0.9). The kinetics of starch hydrolysis was modelled as a first-order reaction. The first-order equation $C = C_\infty(1-e^{-kt})$ was applied (Goñi, Garcia-Alonso & Saura-Calixto, 1997), where C is the percentage of hydrolysed starch at time t; C_∞ is the equilibrium concentration of hydrolysed starch in the simulated gastro-small intestinal digestion process, and k is the kinetic constant.

2.7. Microscopy

To investigate microstructure changes on the surfaces of intact rice during *in vitro* digestion, the cooked rice was digested using the procedure described in section 2.6. The digested rice was collected after simulated gastric digestion for 30 min (G30), and after 5 (I5) and 120 min (I120) of simulated small intestinal digestion, from the digestion reactors. The collected samples were immediately soaked in liquid nitrogen, and freeze-dried. Then, the lyophilised samples were coated with gold (JFC-1100, Joel, Japan) and observed with a scanning electron microscope (SU1510, Hitachi, Japan).

2.8. Statistical analysis

All the experiments were carried out in triplicate, and the results reported as mean value ± standard deviation. One-way analysis of variance (ANOVA) was performed to determine the significance of variables, using an SPSS Program (version 20.0; SPSS, IBM, USA). The significant difference between means was determined by Duncan's test