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Digestibility and structural changes of waxy rice starch during the fermentation process for waxy rice vinasse

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ABSTRACT

During the fermentation process of waxy rice vinasse, the waxy rice starch granules were collected and their digestibility and structural changes were investigated, and the fermentation time–starch structure–starch digestibility relationships were revealed. It was found that the waxy rice starch digestibility from the fermented waxy rice was changed significantly during the fermentation. The fermentation reduced rapidly digestible starch (RDS) and resistant starch (RS) content but increased the slowly digestible starch (SDS) content in the cooked waxy rice starch. The SDS obtained from the fermentation process ranged between 24.53 and 43.85%, which is about 3.6–6.4 times over that of the cooked waxy rice starch. After fermentation, the Mw of waxy rice starch decreased. The A type crystallinity and molecular helices were increased during the early stage of the fermentation and then decreased as the fermentation time increased, compared with that of the cooked waxy rice starch, suggesting that the higher A type crystallinity and more molecular helices induced slow digestion of the fermented waxy rice starch. But when the fermentation time increased further, the slightly thicker semi-crystalline repeat structure, a thicker crystalline lamellar structure and the higher starch molecular aggregation orders contributed to the higher SDS contents in the fermented waxy rice starches. By controlling the extent of waxy rice fermentation, the waxy rice starch digestibility could be reduced which is benefited for improving the nutrition of the waxy rice vinasse with low glycemic response.

1. Introduction

Starch is the main energy-providing carbohydrate in foods for daily human life (K. N. Englyst, Englyst, Hudson, Cole, & Cummings, 1999). Based on the digestion rate, starch has been classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (H. N. Englyst, Kingman, & Cummings, 1992). RDS can be rapidly digested and absorbed in the duodenum as well as proximal regions of the small intestine, which instantly elevates blood glucose. SDS is digested slowly in small intestine with a moderate glycemic rise and prolonged energy supply. RS is not hydrolyzed in the upper gastrointestinal tract but can be degraded by colon microorganisms (G. Zhang & Hamaker, 2009).

Metabolic syndrome, such as obesity, glucose intolerance, hypertension and dyslipidemia, are associated with abnormal energy metabolism (Kaplan, 1989; McCullough, 2011). Glucose is an important metabolic fuel and a signaling molecule that stimulates insulin secretion. A high concentration of glucose in cells can lead to free radical O_2 production in the mitochondria, which damages cell membranes and DNA (Brownlee, 2005). Compared to RDS consumption, a slow and prolonged postprandial release of glucose and corresponding low insulin level were observed after SDS intake (Eelderink et al., 2012; Seal et al., 2003; Vinoy et al., 2013). Good control over glycemia and few diabetics-related complications were observed after diabetic patients consumed SDS (Wachters-Hagedoorn et al., 2006). Potential beneficial effects from starchy food intake, especially foods containing slow digestible and resistant starches, have become apparent and are associated with a reduced risk of cardiovascular and other chronic diseases (Aller, Abete, Astrup, Alfredo Martinez, & van Baak, 2011; Blaak et al., 2012; Ludwig, 2002).

Previous studies have shown that the structural characteristics of starches exhibit a major impact on digestibility (Han & BeMiller, 2007; Lehmann & Robin, 2007). Compared to B-type starches, A-type starches are slowly digested due to the channels that connect

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the central hilum region to the surface of the starch granules. When starch granules are incubated with enzymes, the enzymes migrate inside the starch granules through the channels and initiate hydrolysis. More recently, a side-by-side digestion mechanism was proposed that generally accepts an inside-out digestion pattern and better explains the digestion process (Huber & BeMiller, 1997; G. Zhang, Ao, & Hamaker, 2006a; Zhang & Hamaker, 2009). To increase α-1,6 linkages, β-amylase, transglucosidase and maltogenic α-amylase were used to modify starch molecular structures. Enzymatic modification is a useful method for changing the starch molecular structure for enhanced SDS (Li et al., 2014; Miao et al., 2014).Both the increase in starch branch density and crystalline structure in the treated starch likely contributed to slow digestion (Ao et al., 2007). Sixty mutated maize flour samples were tested and the data showed that the relationship between SDS content and weight ratio of the amylopectin short chains (DP $\leq$ 13) to long chains (DP $\geq$ 13) exhibit a parabolic correlation (G. Zhang, Ao, & Hamaker, 2008). Amylopectin with more either short chains or long chains can produce relatively high levels of SDS. Many researches have also revealed that the starch crystalline structure, branch density, chain length and amylopectin content can affect the slowly digestibility of starch.

Meanwhile, food processing, including thermal processing, extrusion cooking, autoclaving etc., can alter starch structures and also can influence the starch digestibility (Dreher, Dreher, & Berry, 1984; Singh, Dartois, & Kaur, 2010). The digestibility of starch depends on the starch microstructure formed during food processing. Thus, it is more important that to create starchy health-promoting foods through controlling starch digestibility depends on not only the structures of native starches but also the changes in their structures resulted from food processing (Ubbink & Mezzenga, 2006).

Rice starch or waxy rice starch is an important cereal in Asian areas and exhibits an A-type crystalline structure. Due to the diverse growing areas, conditions and processing techniques, the structural characteristics of rice starch differ, which effects its digestibility and human health upon consumption. Waxy rice vinasse is one of Asian traditional foods produced through waxy rice cooking and fermentation by rice leaven, which contains different types of nutrients, such as amino acids, polypeptides, oligosaccharides and polyphenols. In certain areas, it is used as an energy-providing functional food for elderly, children or patients who are recovering. Certain starches remain after fermentation during the manufacture process of waxy rice vinasse. As a starchy food, the digestibility of the residual starch is important for energy supply and health benefits.

Relying on fungal species or yeast in rice leaven, the cooked rice were fermented, and the rice starches were enzymatically hydrolyzed by amylase and glucoamylase secreted from the microorganisms during the fermentation, which could affect the starch aggregation structures as well as chain structures and change the starch digestibility. In this study, waxy rice starches were purified and collected from waxy rice vinasse during fermentation. The starch digestibility, weight-average molecular molar mass, crystalline structure, lamellar structure and granular morphology were analyzed. We focused on the change in waxy rice starch digestibility and its structural changes during fermentation. The results of this study may demonstrate the health benefits of this vinasse products based on the starch digestibility.

2. Materials and methods

2.1. Materials

Waxy rice was obtained from the Shantou Special Economic Zone Golden Resources Grain Co., Ltd (Shantou, Guangdong Province, China). The rice leaven was purchased from Angel Yeast Co., Ltd. (Yichang, Hubei Province, China). A glucose oxidase/peroxidase (GOPOD) kit was supplied by Megazyme International Ireland (Bray Business Park, Bray, Co. Wicklow, Ireland). Pancreatin and Amyloglucosidase were purchased from Sigma–Aldrich Co. LLC (Santa Clara, USA). The remaining chemical reagents were analytical grade.

2.2. Preparation of waxy rice vinasse and starch extraction

About waxy rice (1000 g, dry weight) samples were added to 4000 mL of water at 25 °C and soaked for 12 h, drained off the water and cooked the sample at 121 °C and 0.05 MPa for 20 min. The cooked waxy rice samples were cooled to 30 °C immediately using cold air followed by adding rice leaven and fermentation at 30 °C. Waxy rice samples were collected from waxy rice vinasse at the predetermined time points regularly during waxy rice fermentation, dried at 45 °C, ground and sieved within 0.15 mm.

About 30 g of the sample powders obtained above were added to 100 mL 4 g/L NaOH, stirred for 4 h at 25 °C and centrifuged at 322 g for 20 min. The precipitates were washed with 100 mL distilled water and centrifuged; this process was repeated twice. The precipitates were resuspended into water and the pH was adjust to 7.0 using 1 M HCl, and the samples were centrifuged. The precipitates were air dried at 45 °C for 48 h and then ground and sieved within 0.15 mm.

2.3. In-vitro starch digestibility

In-vitro starch digestibility was analyzed using a slightly modified Englyst procedure (H. N. Englyst & Cummings, 1985). Preparation of the enzyme solution: 2.8 mL amyloglucosidase (40 units) were diluted into 8 mL distilled water. Pancreatin (3.0 g, 3×10^3 USP) was added into each of four centrifuge tubes and suspended each portion in 20 mL water. The samples were magnetically stirred for 10 min and centrifuged for 10 min at 573 g. 13.5 mL supernatant were collected from each tube, which was mixed with 6 mL diluted amyloglucosidase. The solution should be prepared immediately before use.

Starch (1.5 g) sample was placed into 50 mL polypropylene screw-cap tubes, pipetted 20 mL 0.1 M acetate buffer (pH 5.2), added three glass balls (diameter at 1.5 cm) and equilibrated at 2 g and 37 °C in a water bath for 10 min. Next, 5 mL enzyme solution as mentioned above was added and timed the analysis from this point. After 20 min, 0.5 mL of the hydrolysate was removed, added to 20 mL 66% ethanol and mixed well. After an additional 100 min, a second 0.5 mL sample was removed in a similar manner. The portions collected after 20 min and 120 min were designated G20 and G120, respectively. After centrifuging G20 and G120 (7269 g, 2 min), the glucose concentration of the supernatant was measured using a GOPOD kit. The values for the different levels of starch fractions (RDS, SDS and RS) were calculated using the following formulas:

$$\mathrm{RDS} = \mathrm{G20} \times 0.9,$$
$$\mathrm{SDS} = (\mathrm{G120} - \mathrm{G20}) \times 0.9,$$
$$\mathrm{RS} = \mathrm{TS} - \mathrm{RDS} - \mathrm{SDS}.$$

2.4. Weight-average molecular molar mass analysis

The weight-average molecular molar mass (*Mw*), mean square radius of gyration (*Rg*) and the molecular molar mass (*M*)