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Understanding the mechanism of starch digestion mitigation by rice protein and its enzymatic hydrolysates

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ABSTRACT

Starch digestibility strongly depends on the food composition and microstructure formed during food processing. Identifying the interplay among food ingredients is vital to design starch-based foods with low digestibility. In this work, the effects of native and enzymatic (pepsin and pancreatin) hydrolyzed rice proteins on structural features, enzyme activity and digestibility of cooked rice starch were systematically investigated. All protein and its hydrolysates showed potent abilities in mitigating starch digestion. Native and pepsin hydrolyzed proteins increased starch retrogradation extent and thus increased ordered and aggregated structures of cooked starch. Pepsin-pancreatin hydrolyzed proteins displayed anti-retrogradation activity and decreased starch ordered structures, however, increased V-type inclusion complexes and displayed a potent mixed-type (competitive and non-competitive) inhibitory activity against α-amylase. Based on these findings, it can be concluded that native and pepsin hydrolyzed proteins decreased starch digestibility via increasing ordered structures of cooked starch, while pepsin-pancreatin hydrolyzed proteins mitigated starch digestion by the synergistic effects of V-type structures enhancement and mixed-type suppression activity against α-amylase. The data is of significant to formulate low glycemic health-promoting food products via native or proteolytic proteins complexation.

1. Introduction

Rice (*Oryza sativa L.*) is the most important agricultural cereal in south Asia and consumed as the staple food for half of the world's population (Muthayya, Sugimoto, Montgomery, & Maberly, 2014). It has been increasingly used for preparing rice foods such as rice noodle, rice dumpling and glutinous rice cakes (mochi) in East and Southeast Asia (Amagliani, O'Regan, Kelly, & O'Mahony, 2016; Wandee et al., 2015), due to its desirable essential amino acids, lipids and carbohydrate required for human health (Zhou, Robards, Helliwell, & Blanchard, 2002). However, the rapidly digestible nature of rice starch was negatively impact on human health with decreased glucose tolerance, which is a major cause of diabetics, obesity and other metabolic complications (Lehmann & Robin, 2007; Zhang & Hamaker, 2009).

Long-term consumption of foods with low rapidly digestible starch (RDS) and high slowly digestible starch (SDS) or resistant starch (RS) is of great benefits for human health (Lehmann & Robin, 2007). SDS digested slowly in small intestine provides sustained glucose release with a low initial glycemia and subsequently a slow and prolonged release behaviors; RS escapes digestion from small intestine and produces short chain fatty acid to improve colonic health (Zhang & Hamaker, 2009). Rice rapid digestion behavior is harmful to human health (Brand-Miller, 2007; Brand-Miller, Dickinson, Barclay, & Celermajer, 2007), due to its relatively high RDS content and glycemic response compared to other starchy foods (Jenkins, Wolever, Jenkins, Josse, & Wong, 1984). Over the last decades, rice starch digestibility was mitigated by recrystallization of debranched starch (Guraya, James, & Champagne, 2001; Zhang, Li, Chen, & Situ, 2016), heat-moisture treatment (Wang et al., 2018; Zavareze, Storck, Suita de Castro, Schirmer, & Guerra Dias, 2010) and annealing treatment (Pham Van, Huynh Thi, & Nguyen Thi Lan, 2016). In addition, endogenous or exogenous ingredients such as lipids and phenolic compounds were also complexed to decrease starch digestion behaviors (Dupuis, Liu, & Yada, 2014). Phenolic compounds mitigate starch digestion mainly by suppressing activity upon α-amylase (Miao et al., 2014a, 2014b), and lipids complexation with starch are likely to form V-amylose crystalline units to decrease starch susceptibility to enzymes (Dupuis et al., 2014). Improving starch ordered structures and complexation with exogenous/endogenous ingredients have been ideal ways to control starch digestion behaviors.

Protein or its enzymatic hydrolysates are key food-derived ingredients complexed with starches to regulate the digestibility of starch-based foods systems (López-Barón, Gu, Vasanthan, & Hoover,

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2017; López-Barón et al., 2018). The correlated mechanisms behind starch digestion mitigation by complexation with native proteins or its enzymatic hyrolysates have been studied in recent years. In barely flour, water-soluble protein fractions reduced the enzyme activity of α-amylase and water-insoluble proteins increased steric hindrance for enzymes accessibility via binding with starch granules, leading to reduced starch hydrolysis (Yu et al., 2018). Besides, denatured and/or enzymatically hydrolyzed proteins were indicated to interact with starch and form a layer of coating on the surface of starch during cooking, which restricted starch hydration and enzymes accessibility (López-Barón et al., 2017). Most previous studies concerned the catalytic activity of enzymes directly influenced by proteins or its hydrolysates. However, the effect of interactions between starch and native or modified proteins on multi-scale structures of cooked starch and its effects on enzymatic catalysis have been little examined. According to (López-Barón et al., 2018), the interaction, especially hydrogen bonding, has been confirmed within the binary systems of hydrolyzed pea protein and starch, which seems that hydrolyzed proteins would influence hierarchical structures of cooked starches. As a consequence, starch digestibility is likely to be altered due to the changes of starch hierarchical structures which induced by the interplay located in the binary systems of starch molecules and proteins or its hydrolysates. Hence, it is urgent to understanding the effects of proteins and its enzymatic hydrolysates on cooked starch microstructures and its correlated relationship to starch digestibility.

In this study, rice protein, the most important protein in our daily diet, was hydrolyzed with pepsin and pancreatin to obtain different protein hydrolysates. The effects of native rice protein and its hydrolysates on cooked starch structural features were measured by Small Angle X-ray Scattering, X-ray Diffraction and ^{13}C CP/MAS Nuclear Magnetic Resonance Spectroscopy. In addition, the inhibitory activity of rice protein and its hydrolysates against α-amylase was carried out to systematically investigate the mechanism of protein and its hydrolysates on starch digestion behaviors. The results are expected to reveal the digestion mechanisms of cooked rice starch which complexation with rice protein and its enzymatic hydrolysates.

2. Materials and methods

2.1. Materials

Rice starch and rice protein powder were purchased from Jinnong biotechnology Co., Ltd. (Jiangxi, China). Pepsin, α-amylase, pancreatin and amyloglucosidase were purchased from Sigma-Aldrich Co. LLC (Santa Clara, USA). A glucose oxidase/peroxidase (GOPOD) used to determine glucose content was obtained from Megazyme International Ireland (Bray Business Park, Bray, Co. Wicklow, Ireland). Other reagents were all analytical grade. Rice protein powder (GABIOTEIN-A80) prepared through complex of alkali and enzymatic extraction method were purchased from Jinnong biotechnology Co., Ltd. (Jiangxi, China).

2.2. Preparation of rice protein hydrolysates

Rice protein powder was hydrolyzed by pepsin and pancreatin. Briefly, 5.2 g of rice protein powder were dissolved in 100 mL of HCl-acidified water (pH 1.2) which containing pepsin from porcine gastric mucosa (2000 U/mL). After 60 min of pepsic digestion, the action was stopped by adjusting the pH to 7.0 with sodium hydroxide solution (1 M), then snap freezing in liquid nitrogen and dried by a lyophilizer. Dual enzymatic hydrolyzed proteins were obtained by additional pancreatin (100 U trypsin activity/mL) hydrolyzation in phosphate buffer (pH 7.0, 0.1 M). Subsequently, the reactions were withdrawn after 60 and 120 min enzymatic digestion, and the samples were imminently froze in liquid nitrogen. All enzymatic digestions were performed in a water bath (37 °C) with 150 rpm stirring. Hydrolyzed rice proteins were freeze-dried and kept at −20 °C until analysis. Rice protein hydrolyzed by pepsin and pepsin-pancreatin (60 and 120 min) were referred to RP, RPP60 and RPP120, respectively. Characteristic of rice protein or its hydrolysates was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to previous study (Agboola, Ng, & Mills, 2005).

2.3. In vitro digestibility of starch

In vitro starch digestibility was measured based on the *Englyst* method (Englyst & Cummings, 1985) with slight modification. 12 g porcine pancreatin (1.4×10^4 USP, Sigma Aldrich) was completely suspended in deionized water (80 mL) and followed by centrifugation at 3000*g* for 15 min to obtain working solution A. Afterwards, amyloglucosidase (3.15 mL, 3260 units) was mixed with 3.85 mL of deionized water to obtained working solution B. Then, the fresh enzyme working solution was prepared by mixing 54 mL of solution A and 6 mL of solution B.

Starch (1 g, dry basis) and 0.08 g of native or hydrolyzed rice proteins were completely dispersed in 20 mL 0.1 M acetate buffer (pH 5.2). It was cooked at 95 °C for 30 min and then cooled down at 37 °C for 20 min. Subsequently, 5 mL of enzyme working solution and 5 glass balls were added and incubated at 37 °C with 190 rpm stirring in a water bath. 0.5 mL of hydrolysate at different times (20 and 120 min) was removed and mixed with 20 mL of 66% ethanol to denature the enzymes. The samples were centrifuged at 4000 g for 5 min and the hydrolyzed glucose content was measured using a GOPOD reagent by measuring the absorbance at 510 nm. The glucose content after 20 and 120 min hydrolysis were labeled as G20 and G120, respectively, by which starch fractions were classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) based on the hydrolysis rate using the following formulas:

$$RDS = G20 \times 0.9$$

$$SDS = (G120 - G20) \times 0.9$$

$$RS = TS - RDS - SDS$$

Rice starch complexed with native protein, RP, RPP60 and RPP120 were referred to rice-Pro, rice-RP, rice-RPP60 and rice-RPP120, respectively.

In order to reveal the structural features of starch pastes which used for digestibility evaluation, a starch suspension (5%, w/w) with or without protein/protein hydrolysates complexation was cooked at 95 °C for 30 min and held at 37 °C for another 20 min, then snap frozen in liquid nitrogen. The starch pastes were freeze-dried, ground and passed through a 150 μm sieve. Freeze-dried pastes of rice, rice-Pro, rice-RP, rice-RPP60 and rice-RPP120 were labeled as rice-D, rice-Pro-D, rice-RP-D, rice-RPP60-D and rice-RPP120-D, respectively.

2.4. Pasting properties

Rice starch (1.0 g, dry basis), with or without protein and protein hydrolysates (0.08 g, dry basis), was weighted and added with deionized water to make a total weight of 20 g. The suspensions were homogeneously dispersed before pasting test. Each sample was heated from 30 to 95 °C at 7.5 °C/min, held at 95 °C for 30 min, cooled from 95 °C to 37 °C at 7.5 °C/min and then held at 37 °C for another 20 min. The paddle speed was kept at 210 rpm. Each test was carried out in triplicates.

2.5. Determination of the leached amylose amount

The leached amylose content from starch granule during hydrothermal treatment was determined using iodine colorimetric reaction. Briefly, the native or starch-protein/protein hydrolysates mixed systems were gelatinized according to section 2.3 (cooked at 95 °C for 30 min