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Effect of pullulan on the digestible, crystalline and morphological characteristics of rice starch

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ABSTRACT

The in vitro enzymatic digestibility of rice starch (R_iS) was investigated in the presence or absence of pullulan (PUL) using the classic Englyst method and complemented by the analysis of the digestion kinetic. PUL induced a significant increase of the sum of slowly digestible starch (SDS) and resistant starch (RS) contents from 21.24% to 38.11% with a concomitant decrease of the rapidly digestible starch (RDS) content when 0.50% PUL was added. Both of the hydrolysis kinetic parameters, C_∞ and k, decreased with the increasing of PUL, indicating the deceleration of hydrolysis rate by the incorporation of PUL. As evidenced by the changes of the crystalline characteristics and morphologies of fresh starch pastes with the help of X-ray diffraction (XRD), optical microscope, and confocal laser scanning microscopy (CLSM), the inhibitory effect of PUL on gelatinization of starch and the coating effect of PUL on the surface of starch granules were hypothesized to be responsible for the reduced starch digestibility in the present work.

1. Introduction

Starch is the most important carbohydrate in a variety of diets and serves as a major energy source for humans (James & Roy, 2009). Starch digestion is crucial to metabolism, and it is no wonder that the increasing interest in the role of starch digestion in human health and its evaluation methods have evoked a wide range of research and gained considerable public awareness in the last few years (Gilbert et al., 2013; Hur, Lim, Decker, & McClements, 2011). According to the rate and extent of digestion (Englyst, Kingman, & Cummings, 1992), starch can be classified into three fractions, namely rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is defined as the fraction of starch digested in the first 20 min during the widely accepted in vitro digestion process to simulate the rapidly digested and absorbed starch in the small intestine. SDS is defined as the part of starch digested in the time quantum of 20–120 min to represent the starch which would be digested completely by intestine but with a slow rate in human body. RS is defined as the starch that survived from 120 min of the in vitro enzymatic hydrolysis to mimic the starch escaped digestion in the upper gastrointestinal tract, but RS can be fermented in the large intestine by human colonic microflora. From nutritional and healthy points of view, SDS and RS have a range of desirable health benefits to the normal population, including the stable glucose metabolism, the diabetes management, the prevention and alleviation of metabolic diseases, and the prevention of colon cancer (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Lehmann &

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Robin, 2007).

Recently, a substantial amount of studies on improvement of SDS and RS contents in starch from various starch sources have been reported using physical, chemical, and enzymatic treatments (Dupuis, Liu, & Yada, 2014; Wongsagonsup, Varavinit, & BeMiller, 2008). Among these technologies, physical combination of starch with various hydrocolloids, especially the nature polysaccharides, have been investigated to modify the digestion properties of starch with the apparent advantages of safe, eco-friendly, effective, and more convenient for the practical application. The addition of guar gum (0.5%) to cooked potatoes reduced the starch hydrolysis in vitro digestion due to the thickening effect of guar gum, which reduced the accessibility of enzymes to starch substrate (Bordoloi, Singh, & Kaur, 2012). Agar, xanthan gum, and konjac glucomannan have been found to suppress starch hydrolysis, and this inhibitory effect was dependent on the added polysaccharides concentration; both the hardening effect of hydrocolloids on the starch gel and the possible interaction between starch and hydrocolloids were hypothesized (Sasaki & Kohyama, 2011). Also, guar gum, pectin, and carboxymethyl cellulose, reduced the glucose accessibility in a simplified in-vitro intestinal models (Gouseti et al., 2014).

Pullulan (PUL) is a neutral linear polysaccharide produced by a yeast like fungus *Aureobasidium pullulans*. PUL has the starch-like structure of linkage α-D-glucan primarily consisting of maltotriose repeating units interconnected by α-(1 → 6) linkages (Singh, Saini, & Kennedy, 2008). The unique structure of PUL endows it with some useful physicochemical properties, such as non-toxicity, non-immunogenic, slow digestion, enhanced water retention capacity, relatively low viscosity, and excellent film-forming ability (Singh, Saini, & Kennedy, 2008). These special properties of PUL make it widely used in food, material, and pharmaceutical industries (Prajapati, Jani, & Khanda, 2013; Singh, Saini, & Kennedy, 2008). It's worth noting that PUL can be slowly digested and treated as dietary fiber because of its resistance to mammalian amylases, which resulting in a gradual rise in blood glucose level in humans (Wolf, Garleb, Choe, Humphrey, & Maki, 2003). Therefore, PUL may be supplemented into dietetic foods prepared for diabetics or special group who have impaired glucose tolerance (Singh, Saini, & Kennedy, 2008).

PUL was usually incorporated into starch to prepare various films with the aim to improve the mechanical and physical properties of pure starch films (Kanmani & Lim, 2013; Kim, Choi, Byul Kim, & Lim, 2014). However, only a few studies have focused on the effect of PUL on functional properties of starch itself. Based on our previous work, PUL was found to interfere with the swelling, gelatinization (Chen, Tong, Ren, & Zhu, 2014), and the subsequent retrogradation of rice starch (R_iS) (Chen, Ren, Zhang, Tong, & Rashed, 2015; Chen, Tian, Tong, Zhang, & Jin, 2017). These studies showed the potential of PUL in the starch modification. However, data are still scare on the effect of PUL on the digestion properties of starch. Thus, the objective of the present study was to investigate the effects of pullulan on the in vitro digestibility of starch. As rice has long been consumed as a staple food in many Asian countries, rice starch was selected as the research object in the present work.

These results are useful and meaningful in production of starch-based foods being rich in RS and SDS, and the application expansion of nature polysaccharides in food industry.

2. Materials and methods

2.1. Materials

Rice starch was produced by Jiangsu Baby Rice Inc. (Suqian, China). It contained 0.3% proteins, 1.2% free lipids, and 23.3% amylose. Pullulan was purchased from Hayashibara Biochemical Inc. (Shanghai, China) with 4.50% moisture content and a molecular weight of 200,000 Da. Porcine pancreas α-amylase (EC 3.2.1.1, No. 10080, 50 U/mg) and amyloglucosidase (EC 3.2.1.3, No. 10115, 70 U/mg) were purchased from Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China). All other chemicals and reagents were of analytical grade unless otherwise stated.

2.2. Preparation of starch-pullulan complex samples

Rice starch (5%, w/v)-pullulan (0, 0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5%, w/v) mixtures (R_iS/PUL) were prepared as follows and only fresh pullulan solutions were used before the experiment. Briefly, various quantities of PUL were dissolved thoroughly in 100 mL of distilled water in closed tubes, then a certain weight (5 g) of R_iS was added. The mixtures were dispersed at room temperature by magnetic stirring for 1 h. Then, the R_iS-PUL mixtures were heated at 95 °C in a water bath for 30 min with continuous stirring to obtain the complete gelatinization of R_iS. After cooling to ambient temperature, the resultant mixtures were equilibrated and stored at 4 °C for 2 h and then dried in an air oven at 40 °C for 12 h. Finally, the samples were milled to pass through a 100-mesh sieve. A blank sample without PUL was used in this study.

2.3. In vitro digestibility determination

The digestibility of samples was measured according to the procedure of Englyst et al. (1992) with some modifications. Briefly, 200 mg of prepared samples was put into the conical flask and dispersed in 15 mL sodium acetate buffer (0.2 mol/L, $CaCl_2$ 1 mM, pH 5.2). After the suspension was equilibrated at 37 °C for 5 min, five glass balls with 5 mm in diameter and 5 mL of the enzyme solution (α-amylase and amyloglucosidase mixed in a proportion of 120 U/80 U/mL) were added in the flask. Then, the samples were immersed in a shaking-water bath for hydrolysis under the condition of 37 °C at 160 rpm. Aliquots of 200 μL hydrolyzed solution were taken out at 20 and 120 min intervals and put into a plastic tube which has been treated in boiling water bath to deactivate the enzyme. Subsequently, the solution was centrifuged at 10,000 *g* for 5 min, and the glucose content in the supernatant was determined by the glucose oxidase method using the D-glucose assay kit (Megazyme, K-GLUC).

The values of different starch fractions (RDS, SDS, RS) were calculated according to the timeline of digestion using the following formulas:

$$RDS(\%) = \left[\frac{G_{20} - FG}{TS}\right] \times 0.9 \times 100 \quad (1)$$

$$SDS(\%) = \left[\frac{G_{120} - G_{20}}{TS}\right] \times 0.9 \times 100 \quad (2)$$

$$RS(\%) = \left[\frac{TG - FG}{TS}\right] \times 0.9 \times 100 - (RDS + SDS) \quad (3)$$

where G20, G120 represent the content of glucose released after 20 min and 120 min, respectively. FG is the free glucose content of R_iS which is extracted with distilled water and measured in the supernatant, while TG is the total glucose measured after the starch was thoroughly hydrolyzed into glucose. The determination of FG and TG was conducted using the glucose oxidase method as mentioned above. 0.9 is the factor conversion from glucose to starch.