STARCH DIGESTIBILITY OF HYDROTHERMALLY TREATED GERMINATED BROWN RICE TO REGULATE BLOOD GLUCOSE LEVEL

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ABSTRACT

Different degree of germination could increase damaged starch level hence effect on degree of polymerization of starch. Heat moisture treatment (HMT) of germinated paddy rice with appropriated chain length could improve crystalline perfection of starch. Slowly digestible starch (SDS) is digested slowly but completely in human’s small intestine. It could stabilize glucose metabolism, diabetes management, mental performance, and satiety. Paddy rice (Pathumthani 1 and Riceberry cultivars) was germinated at 30 °C for 24, 36, 48, 60 h to initiate damage starch content by amylolytic enzyme. Pasting properties and gelatinization properties were found to exceedingly decrease. After germination, rapidly digestible starch (RDS) was increase substantially (60.9-66.4%) comparing to normal cooked rice (39.4-42.3%). However, the slowly digestible starch (SDS) and resistant starch (RS) of the cooked germinate rice were rapidly decreased. In order to benefit both blood glucose regulation and GABA content of germinated rice, Heat moisture treatment (HMT) was applied after paddy’s germination at 90°C for 1h. Result showed that RDS of HMT germinated rice was lowering to 40.2-40.8%. Its SDS and RS increased to 42.1-43.6% and 7.6-7.7% respectively comparing to germinate rice (24.9-26.9 and 2.5-2.8 for SDS and RS respectively).

Key words: germination, damaged starch, slowly digestible starch, hydrothermal treatment

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INTRODUCTION

During germination process, there are considerable changes in physico-chemical properties and bioactive compound in grain. These changes are synthesis of hydrolytic enzymes, improvement of protein and carbohydrate digestibility, reduction of flatulence caused by oligosaccharide, denaturing of amylase inhibitors and other antinutritional factors. As a result, the overall nutritional quality enhances (Frias, 1997). The amounts of most active components such as dietary fiber, gamma aminobutyric acid, gamma oryzanol, ferulic acid and most free amino acids were depending on degree of germination. The numerous health benefits obtained for germinated brown rice include antihyperlipidemia, antihypertension, psychosomatic health effect and the reduction in some chronic disease such as Alzheimer's disease, cardiovascular disease and diabetes (Wu et al., 2013). Moreover, germination could improve the organoleptic qualities such as softening of texture and increasing in flavor components (Wu et al., 2011).

Heat moisture treatment (HMT) is a physical modification that could change the physicochemical properties of starch without destroying its granule structure. The moisture content, temperature and heating time are critical parameters that must be controlled (Chung, Liu, & Hoover, 2009). HMT is carried out under restricted moisture content (10-30%) and temperature (90-120°C) which supported the increase of gelatinization transition temperature, a decrease in granular swelling, amyllose leaching and enzyme susceptibility but promote thermal stability. These starches are applied as food ingredients to increase slowly digestible starch. HMT process is claimed to increase SDS level in sweet potato starch (Shin et al. 2005)

Slowly digestible starch (SDS) and resistant starch (RS) is classified starch which depended on the rate of glucose released during digestive starch. SDS is the part of starch was digested glucose to between 20 and 120 min in vitro enzyme hydrolysis which dominated in cereal product. RS is starch which cannot hydrolysis after 120 min of in vitro enzyme hydrolysis. The potentially predominant health benefits of SDS are networked a stable metabolism. SDS has a medium to low glycemic index which effect to decrease the glycemic load of food product. Product was processed to increase SDS have positive effect to preventing diabetes and cardiovascular disease. Hence this regulates the rate of glucose in the blood.

The objective of this study was to evaluate the influence of damaged starch from germination follow HMT was used as procedure to modification. HMT was demeaned at a higher than glass transition temperature but was below gelatinization temperature. Germination together with HMT proposed to increase starch resistance that could pass through the digestive system. To some degree of damaged starch produced a higher more rapidly digestible (RDS) that cannot resist
digestive tract. HMT process has intended a suitable amount of RDS, SDS, RS. that can regulate digestive system in human.

**MATERIALS AND METHOD**

1. Germination process

Paddy grains (200 g) of Pathumthani1 cultivars (Rice Department, Pathumthani, Thailand) and Riceberry cultivars (Rice science center, Kasetsart University Kamphaengsaen campus, Thailand) were thoroughly rinsed with water and soaked with 0.1% sodium hydrochloride for 15 min. Then it was transferred into a flask containing 1000 mL of water for 12 h at 30°C. After that the water was removed and the grains were washed again with water. The steeped paddy rice grains were subjected to germination in an incubator (40°C) for different period (24, 36, 48, 60 h), water was sprayed for 15 min in every 4 h to control moisture content (80-95%) of rice grains.

2. Heat moisture treatment process

The germinated brown rice (GBR) grains obtained in section 1 were weighed in glass containers and dried at 40°C until moisture content reached 25%. The containers were sealed, kept for 1 h at ambient temperature, and then put in an oven at 90°C for 1 h. The treated samples were subsequently dried in an oven at 40°C until 12% moisture content was obtained.

3. Damaged Starch

Ungerminated and (24, 36, 48, 60 h) germinated flour on Pattumthani 1 and Riceberry cultivar. In each treatment was determined damaged starch content using by Spectrophotometric method. Briefly, 100 mg of starch was weighed into centrifuge tubes. 1 ml of pre-equilibrated (at 40°C for 5 min) fungal –amylase solution (50 U/ml) was added. The tubes were vortexed for 5 s and incubated at 40°C for exactly 10 min. Sulphuric acid (8 mL) (0.2% v/v) was then added and the tube was centrifuged at 1000 × g for 5 min. Supernatant (0.1 mL) was transferred to the bottom of a new test and treated with amyloglucosidase (0.1 mL) (2 U) and incubated at 40 °C for 10 min. Then glucose oxidase /peroxidase (4 mL) was added to each tube, the mixture was incubated for 20 min at 40 °C and the absorbance was read at 510 nm against a blank reagent.

4. **In vitro** starch digestibility

After germination the number of ungerminated (0h), germinated (24, 36, 48,60h) and germinated heat moisture treatment grains (24h HMT, 36h HMT, 48h HMT, 60h HMT) in each treatment were prepare for experiments. A Sample (4g) was weighed into a beaker, 5 mL of distilled water was added and using an electric rice cooker (Tatung, Taiwan) for 30 min. The cooked rice sample was cooled to ambient for 15 min and passed through blender. The digestibility of samples was determined according to the method of Englyst et al (1992), with modifications. Porcine pancreatic alpha-amylase (4.0 g, activity 8 USP/g) was dispersed in water (30 mL) by magnetic
stirring for 10 min and was centrifuged at 1500x g for 10 min. The supernatant was collected and amylglucosidase (2.8 mL; activity 5000-8000 unit/mL) was added in the solution. The enzyme solution was freshly prepared for each digestion analysis. The minced rice sample (1.0 g, db) was mixed with sodium acetate buffer (20 mL) (0.1M, pH5.2). The enzyme solution (10 mL) was added to each tube altogether with seven glass beads (10 mm diameter), incubated in a shaking water bath (37°C, 170 rpm). Aliquots (5 mL) were taken at intervals, and then mixed with 20 mL absolute ethanol and centrifuged at 1500x g for 10 min. The amount of glucose in the supernatant was determined using the GOPOD. Starch was classified according to its digestibility as rapidly digestible starch (RDS) which was digested within 20 min, slowly digestible starch was hydrolysed between 20 and 120 min, and those resistance starch (RS) which was not digested at 120 min.

5. The in-vivo blood glucose response
The sample contains of

The animal study was carried out at the Bio-rat company (Taichung, Taiwan). Male ICR mice (6 weeks of age) were housed in PC–cage in temperature controlled room (23±2 °C) with a standard 12 h light/dark cycle. Blood glucose readings were determined from the tail of mice using a glucose readings (Accu-Chek, Performa, USA). Mice were divided into six groups (5 mice each). The ungermination (NGBR), 24h germination (24hGBR), 24h germinated heat moisture treatment (24hHGBR) and on Pattumthani 1 and Riceberry cultivars were determined. Samples of cooked brown rice were blended with distilled water (100 mg/mL). The suspensions were orally administered at dose 20 mL/kg mice. Blood glucose was assayed at 0, 30, 60, 90 and 120 min after feeding.

6. Statistical analysis
Data is reported as the average value of triplicate measurements. Difference analysis between means of data was carried out using ANOVA and Duncan’s multiple range tests at p≤ 0.05 (SPSS program version 19.0 for Windows)

RESULT AND DISCUSSION

1. Damaged starch
During germination process bioactive compounds, starch, protein and lipid were changed by enzyme such alpha amylase, beta amylase, alpha glucosidase and debranching enzyme were induced. Damaged starch was determined by measuring glucose content. So 60 h germination has the highest damaged starch compare with control (0 h germination). The damaged starch content was gradually upswing form 7.12% to 14.37% and 5.74% to 14.21% on Pattumthani 1 cultivars and Riceberry cultivars respectively (Figure 1). Because starch was the main reserve compound in grains. Germination process has effect to contained less starch. This may result from the fact that sprouting process triggered the production of enzyme. Those enzymes degrade starch granules and become
damaged starch (Noda et al, 2004). The oligosaccharides and reducing sugar were increased by starch hydrolysis (Xu et al, 2011). Therefore damaged starch content was influenced by cultivars, steeping time and its interaction (Rathavati & Ravi, 1991; Saman et al, 2008).

Figure 1 Damaged starch form germinated brown rice (A) Pathumthani 1 Cultivars and (B) Riceberry Cultivars at various germination time 24 h, 36 h, 48 h, 60 h.

2. In vitro starch digestibility of cooked germinated brown rice grain

Starch digestibility was measured rate of starch which digestive in human digestion model (in vitro). That was determining as rapidly digestible starch (RDS; digested within 20 min), slowly digestible (SDS; digested between 20 and 120 min) and resistance starch (RS; undigested after 120 min). RDS, SDS, RS of germinated brown rice and germinated heat moisture treatment are exhibited in Figure 2. RDS content of both cultivars were significantly increased as a result during germination induced amylolytic enzyme degrade starch and alter reducing sugar content. This effect has impact to proportion of SDS and RS were decrease. But after HMT, the hydro thermal treatment usually raise the extents of perfection of already existing crystalline structure, and formation of physical cross link in amorphous regions. This amorphous area was more rapidly degraded by alpha amylase than the crystalline. However the structural changed induce by thermal process which impact to rigidify starch granules and to digestive enzyme molecules to be less susceptible to the digestive enzyme. The reduced digestibility was confirmed by increased in SDS and decrease in RDS. Slowly digested carbohydrates are generally recognized to be favorable for dietary management.
3. Postprandial blood glucose levels in mice

The blood glucose level was determined by monitoring glucose content at 0, 30, 60, 90 and 120 min after take by mice. The 24 h germination (24h GBR) of both cultivars exhibited a more than higher blood glucose response, particularly at 30 min, and gradually decreased. The 24 h germinated heat moisture treatment (24h HGBR) of both cultivars were regulated blood glucose response constantly after 30 min Blood glucose level responded well with germination and heat moisture treatment better than control. The slower blood glucose release indicates HMT has highly effect on the reduction of the postprandial blood glucose levels. Slowly digestible starch was increased greatly by HMT. A further reduction in the sugar content of rice grain will result in a more remarkable effect on blood glucose levels (Figure 3).
Figure 3 Blood glucose levels after oral administration in mice (A) Pathumthani 1 Cultivars (B) Rice berry Cultivars. (NGBR : non-germinated brown rice, 24h GBR : 24h germinated brown rice, 24h HGBR : heat moisture treatment germinated brown rice). The values are expressed as mean + SD (n = 5). Asterisk indicated a significant difference in the glucose levels is the corresponding control at p<0.05

COUNCLUSION
The result indicated that significant changes properties of starch digestion (in vitro and in vivo) of brown rice flour were found during the process of germination. The structure of starch was degraded by amylotic enzyme. That has effect to increase RDS which is starch fraction that cause a sudden increase in blood glucose after ingestion. However heat moisture treatment modification it can help increase the fraction of slow digestible starch. That can help to control blood glucose level.

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