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Antioxidant activity and glycemic index of resistant starch from black glutinous rice

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***Abstract*---**Type 2 diabetes mellitus (T2DM) is a global public health manifested by hyperglycemia due to insulin resistance. Rice is the main staple food for the world's population but has a high glycemia index (GI) value. Therefore, this research was aimed to produce resistance starch (RS) from black glutinous rice with different methods – autoclave and annealing, freeze-thawing, and acid methanol methods. The RS content, total phenolic compound content, antioxidant activity, and estimated GI (pGI) were investigated. Results showed that the autoclave and annealing method yielded highest RS content of 27.33±8.56%, followed by freeze-thawing, 15-day acid methanol, and 30-min acid methanol methods. The RS from the autoclave and annealing method also had highest total phenolic content (61.55±1.19 mg gallic acid/g sample) and antioxidant activity by 2,2-Diphenyil-picrylhydrazyl (DPPH, IC50 of 7.40±0.24 mg/ml) and ferric reducing antioxidant power (FRAP, 75.47±0.51 mg tocopherol equivalent/g extract). Moreover, The RS from the autoclave and annealing method also had the lowest pGI (57.32±0.74). In conclusion, the autoclave and annealing process gave the highest yield of RS with the highest total phenolic content and antioxidant activity; and with the lowest GI compared to the freeze-thawing and acid methanol methods. These finding signifies a possibility to use this production method to produce RS as food ingredient in preventing hyperglycemia and T2DM.

***Keywords*---**black glutinous rice, resistant starch, glycemic index, antioxidant, phenolic compounds.

Introduction

Diabetes mellitus (DM) is a chronic non-communicable disease (NCD) characterized by elevated levels of blood glucose, which in long term leads to damage of cardiovascular, nervous, and renal systems. Type 1 DM (T1DM) is caused by impaired insulin secretion, while type 2 DM (T2DM) is due to resistance to peripheral actions of insulin (Goyal & Jialal, 2022). Based on data from the International Diabetes Federation (IDF), DM is a global public health burden as its prevalence is expected to rise to from 415 million in 2015 to 600 million by 2040 (Zheng et al., 2018). DM is a multifactorial disease predisposed by non-modifiable factors (age, gender, ethnicity, and genetics) and modifiable factors (obesity, physical activity, and diets) (Galicia-Garcia et al., 2020).

Dietary starches are primary sources of energy for human beings. For nutritional purposes, food starches may be classified as either glycemic or resistant. Glycemic starch (GS) can be hydrolyzed to glucose by enzymes in the digestive tract. Resistant starch (RS) is not hydrolyzed even after 120 minutes of incubation (Sharma et al., 2008). There are 5 different types of RS based on their digestibility in small intestine (Sajilata et al., 2006; Sharma et al., 2008). First, RS1 is physically inaccessible starch with slow rate of complete digestion (whole or partially milled grains, seeds, and legumes). Secondly, RS2 forms starch granules with very slow rate of small-scale digestion (raw potato and banana). Next, RS3 is retrograded starch solely resistant to digestion by pancreatic amylases (breads and cooked and cooled potato). RS4 is chemically modified starches due to cross-linking with chemical reagents (breads and cakes made from modified starches). Lastly, RS5 amylose–lipid complexed starch produced in the lab by adding lipids to starch and heat-processed in order to obtain the starch-lipid complexes (Panyoo & Emmambux, 2017).

With its beneficial physicochemical properties of gel formation, viscosity, swelling capacity, and water-binding capacity, RS is useful in functional food production (Sharma et al., 2008). Particularly, the gel formation property can increase dietary fiber content, thus lowering the GI of the starch products, slowing digestion, and reducing abdominal fat (Keenan et al., 2013; Yu & Shin, 2015). According to Similä and colleagues, starches can be classified as low GI (≤ 55), medium GI (56-69), and high GI (≥ 70) (Similä et al., 2011). Some types of RS can improve digestive functions of gastrointestinal tract, modulate probiotic functions of enteric microflora, and lower blood cholesterol level (Fuentes-Zaragoza et al., 2010). Moreover, it has been reported that the RS also reduced fasting insulin, fasting glucose, low-density lipoprotein cholesterol (LDL-c) concentration, and hemoglobin A1c (HbA1c) in overweight adult with T2DM (Wang et al., 2019). For this reason, the RS-containing food has been widely used in T2DM-controlling recipes.

Among 5 types of RSs, the RS3 represents the most resistant fraction in the heat-processed foods and relatively easy to produce for household use purpose. It is mainly the retrograded amylose formed during cooling of gelatinized starch. There are 3 methods in producing RS3 – autoclave and annealing, freeze-thawing, and acid methanol methods. This study was aimed to compare bioactive compounds,

antioxidant activity, and pGI of RS produced from black glutinous rice (*Oryza sativa* L.) with different methods.

Materials and Method

Rice samples

Thai black glutinous rice (*Oryza sativa var. glutinosa*) was purchased from a local farm market in Roi-Et province, Thailand. The rice samples were harvested between 240-280 days after planting. The grinded rice samples were filtered with 0.25-mm sieve and stored in aluminum foil sheets at 4°C until use. Humidity was measured with the MB23 Moisture Analyzer (Ohaus®). Unbound water vapor pressures were measured with the WA-60A Water Activity Meter.

Resistant starch processing methods

The autoclave and annealing process consisted of autoclaving black glutinous rice starch at temperature of 121°C for 1 h, incubating 60°C for 48 h, and storing at -20°C for 22 h. For the freeze-thawing method, the black glutinous rice starch was boiled for 30 min, stored at -20°C for 22 h, and thawed at 30°C for 90 min with 3 repetitions. For the acid methanol method, 100 ml methanol and 1 ml 0.36% concentrated HCl were added to the black glutinous rice starch, followed by shaking in 25°C water bath for either 30 min or 15 days, and dried in 40°C drying oven.

Measurement of resistant starch yields

Percentage of RS yields was analyzed with the Association of Official Agricultural Chemists (AOAC) official method 2002.02 assay for RS content analysis. In principle, the non-RS was hydrolyzed to glucose by pancreatic α-amylase and amyloglucosidase for 16 h at 37°C. The RS was a subtracted fraction from the non-RS part as described elsewhere (McCleary et al., 2002).

Total phenolic determination

The concentration of total phenolics was determined by the method adapted from Chidambara Murthy and co-workers (Chidambara Murthy et al., 2002). 1.5 ml 10% diluted Folin-Ciocalteu reagent and 3 ml of 7.5% sodium carbonate solution were added to the extract samples and adjusted total volume to 10 ml. Then the mixture was allowed to stand for 30 min (until it turned from yellow into blue). The absorbance was measured at 765 nm using a spectrophotometer. Estimation of the phenolic compounds was performed in duplicate and averaged.

Antioxidant activity measurements

The antioxidant activities were measured by 2 methods – 2,2-Diphenyil-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. For the DPPH method, 2 ml of sample was added to 3 ml of 0.025 mg/ml methanol DPPH solution. The mixture was then kept in the dark for 30 min. The absorbance was determined at 517 nm. 0.312-2.5 mg/ml tocopherol was used as

standard solution. Antioxidant activity was expressed as % inhibition and calculated from the following equation.

$$\% \text{ Inhibition} = (A_{control} - A_{sample})/A_{control} \times 100 \quad (1)$$

For the FRAP method, 150 µl of sample was added to 3 ml of FRAP solution. The mixture was then kept in the dark for 30 min. The absorbance was determined at 593 nm. 20-100 mg/ml tocopherol was used as standard solution. Antioxidant activity was expressed mg tocopherol equivalent/g extract.

Estimation of glycemic index with starch hydrolysis procedure

Estimation of glycemic index with starch hydrolysis procedure was adapted from the method of Goñi and colleagues (Goñi et al., 1997). Briefly, 50 mg of each sample was added with 0.2 ml of pepsin (≥2500 units/mg) and 10 ml HCl–KCl buffer (pH 1.5) solution. Then the mixture was incubated in a shaking water bath at 40 °C for 60 min. Then 5 ml of α -amylase (≥10 units/mg solid) and 15 ml of Tris–Maleate buffer (pH 6.9) were added into the mixture and incubated in a shaking water bath at 37 °C for 120 min. A 60 µl of amyloglucosidase was used to hydrolyze the digested starch. A standard curve of glucose solution was prepared. The absorbance of the sample was read against the reagent blank at 514 nm using a UV–visible spectrophotometer. D-Glucose was calculated from the following equation.

$$\text{D-Glucose (µg/100 mL)} = (Abs_{Sample}/ Abs_{Glucose\ standard\ (100\ µg)}) \text{ x } 100 \quad (2)$$

Then, amount of starch was calculated from the equation.

$$\text{Starch} = \text{D-Glucose} \times 0.9 \quad (3)$$

Starch hydrolysis rate was expressed as the percentage of total starch hydrolyzed at different times (30, 60, 90, and 120). Starch hydrolysis rate was calculated from the equation.

$$\text{Starch hydrolysis rate (\%)} = (\text{Starch}/ \text{Total starch}) \times 100 \quad (4)$$

The hydrolysis index (HI) and estimated glycemic index (pGI) were calculated as the relation between the area under curve (AUC) from the following equations.

$$HI = (AUC_{sample}/AUC_{reference}) \times 100 \quad (5)$$

$$pGI = 39.71 + (0.549HI) \quad (6)$$

Statistical analysis

Multiple group analysis was performed using One-way ANOVA. Duncan's new multiple range post-hoc test was used to measure specific differences between pairs of means on IBM® SPSS Statistics version 22.

Results

Autoclave and annealing method had highest yield of resistant starch

Percentage of RS yielded from 3 methods is shown in Table 1. Results showed that the autoclave and annealing processing method gave 27.33±8.56%, followed by freeze-thawing, 15-day acid methanol, and 30-min acid methanol methods.

Table 1
Percentage of resistant starch yields from 3 methods of production processes

Method	Resistant starch (g/100 g sample)	
	Before process	After process
Autoclave and annealing	1.67±0.54	27.33 ± 8.56 [a]
Freeze-thawing	1.67±0.54	23.70 ± 3.32 [a]
30-min acid methanol	1.67±0.54	10.10 ± 0.56 [c]
15-day acid methanol	1.67±0.54	20.75 ± 1.97 [a, b]

Data are expressed percentage ± SD. Different alphabets indicate significant differences between the groups in the same column ($p<0.05$).

Resistant starch from the autoclave and annealing method had highest total phenolic content and antioxidant activity

Total phenolic contents in resistant starch (RS) from autoclave and annealing, freeze-thawing, 30-min acid methanol, and 15-day acid methanol methods are listed in Table 2. Tocopherol was used as standard control. Results showed that the RS from autoclave and annealing method had highest total phenolic content 61.55±1.19 mg gallic acid/g sample, followed by freeze-thawing and 30-min acid methanol methods. Antioxidant activity was also highest in the RS from autoclave and annealing method when measured by DPPH (IC_{50} of 7.40±0.24 mg/ml) and FREP (75.47±0.51 mg tocopherol equivalent/g extract) assays.

Table 2
Total phenolic contents and antioxidant activity tests with DPPH assay and FRAP assay

Sample	Total phenolic (mg gallic acid/g sample)	IC_{50} (mg/ml)	FREP value (mg tocopherol equivalent/g extract)
Tocopherol	-	0.01 ± 0.03	182.98 ± 42.19
Autoclave and annealing	61.55±1.19[a]	7.40 ± 0.24[b]	75.47 ± 0.51 [a]
Freeze-thawing	57.93 ±0.93[a, b]	11.06 ± 1.95[b]	42.08 ± 1.12 [b]
30-min acid methanol	57.53±1.90[b]	25.27 ±7.37[a]	40.83 ± 7.37 [b, c]
15-day acid methanol	52.82±1.20[c]	29.47 ± 1.44[a]	38.59 ± 0.08 [c]

Different alphabets indicate significant differences between the groups in the same column ($p<0.05$).

Resistant starch from the autoclave and annealing method had lowest glycemic index

Percentage of hydrolysis in 120 min was illustrated in Fig. 1. The HI and pGI were calculated from the area under curve (equations 5 and 6). Results showed that the autoclave and annealing processing method had lowest HI of 32.07 ± 1.36, followed by freeze-thawing (51.69±0.06), 15-day acid methanol (53.80±0.05), and 30-min acid methanol (56.91± 1.20) methods. Similarly, pGI of the autoclave and annealing method was 57.32±0.74, followed by freeze-thawing (68.09±0.03), 15-day acid methanol (69.25±0.03), and 30-min acid methanol (70.95±0.66) methods (Fig. 2).

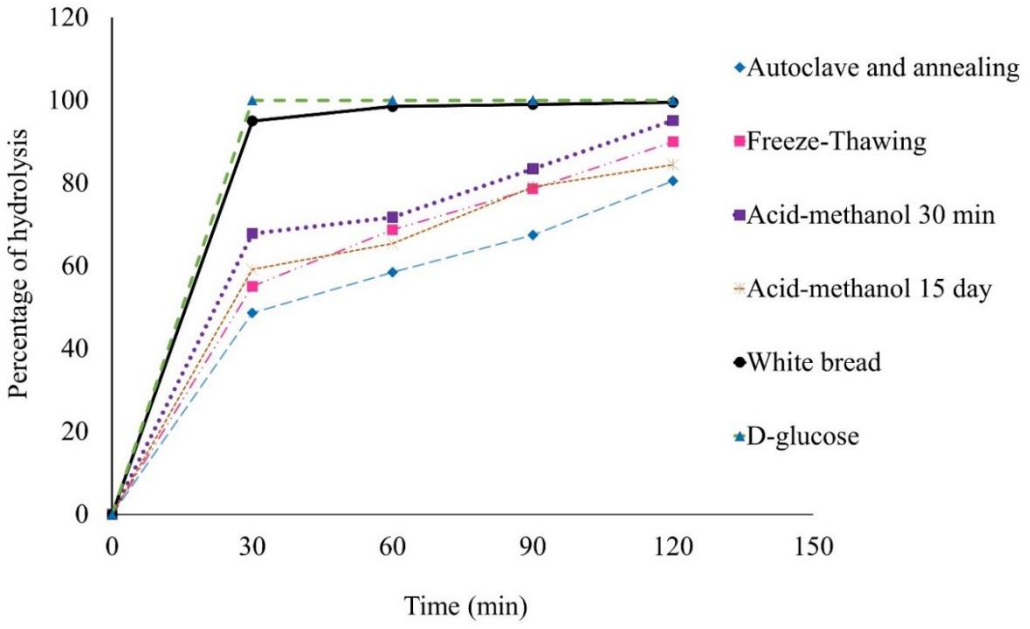

Figure 1 Percentage of hydrolysis in 120 min of resistance starch from autoclave and annealing, freeze-thawing, acid methanol 0-day, acid methanol 15-day, white bread, and D-glucose

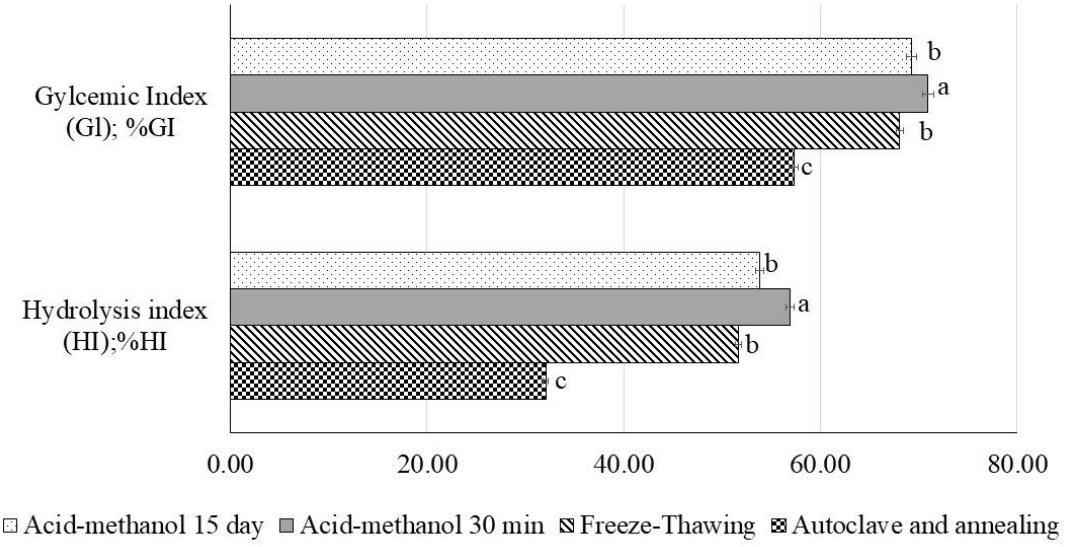

Figure 2 Hydrolysis index (HI) and estimated glycemic index (pGI) of resistance starch from autoclave and annealing, freeze-thawing, acid methanol 0-day, and acid methanol 15-day

Discussion

Our present study illustrates that the autoclave and annealing process is the best method to produce RS. This conclusion is based on the following findings – the autoclave and annealing process had 1) highest percentage of RS yield, 2) highest total phenolic content, 3) highest antioxidant activity, and 4) lowest GI over the freeze-thawing and acid methanol methods.

Rice is the main staple food for more than half of the world's population, especially for people in Asia (Osman et al., 2017). However, rice has a low RS content and a high GI value; thus is not an ideal staple food for T2DM (Kumar et al., 2018; Meera et al., 2019). Our baseline RS content in black glutinous rice in the present study (1.67%) was in line with the previous report by Kumar and co-workers (between 0.35% and 2.57%) (Kumar et al., 2018). Nowadays, the RS3 production methods consist of the autoclave method, enzymatic method, extrusion method, microwave conversion method; and heating and cooling method (Zheng et al., 2020). Among other methods, the autoclave method has the highest potential for commercialization; thus the food industry focuses on how to increase the content of RS by optimizing the autoclave processing technology (Zhao et al., 2013). In the present study, autoclaving black glutinous rice at 121 °C for 1 h, followed by annealing process of incubation and frozen storage was effective to yield of 27% comparable to corn RS produced by an autoclave-enzymatic hydrolysis method with a yield of 26% (Hu & Zhang, 2010).

Pigmented rice contains a high amount of phenolic compounds such as cyanidin, malvidin, and peonidin (Bae et al., 2017; Pedro et al., 2016). Total phenolic content in black glutinous rice varies with pre-harvest factors (planting location,

irrigation, soil type, fertilizer, and pesticides), post-harvest factors (storage condition and temperature), starch modification processes, and analytical methods (Goufo & Trindade, 2014). Here we showed that the autoclave and annealing process is the best method to preserve total phenolic content and hence the antioxidant activity. It was also reported that GI of rice was between 60.07 and 70.36 (Kumar et al., 2018). This present study showed that the autoclave and annealing was the only method able to reduce the GI to approximately 57. Interestingly, Similä and colleagues reported that replacement of moderate GI starch for high GI starch was inversely associated with risk for T2DM. Interestingly, the low GI replacement for medium or high GI starch was not associated with the risk. This finding emphasizes a pivotal role of the moderate GI starch in glycemic control.

In conclusion, the autoclave and annealing process had yielded highest percentage of RS with high total phenolic content and antioxidant activity, and most importantly with the lowest GI over the freeze-thawing and acid methanol methods. This information on RS production should be disseminated to people prone to hyperglycemia to enhance their practice in preventing and alleviating T2DM.

Conflict of Interest

The authors have no conflicts of interest to declare.

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