EFFECT OF PROCESSING PROCEDURES ON IN VITRO DIGESTIBILITY AND COLONIC FERMENTATION OF RICEBERRY RICE

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ABSTRACT
Riceberry is a new rice variety which has recently become popular in Thailand and Asia. Cooking cooled rice influences its digestion; however colonic fermentation studies comparing freshly cooked rice (FCR), refrigerated rice (RR) and frozen rice (FR) hydrolysates are limited. Here, in vitro digestion rate and colonic fermentation of freshly cooked Thai riceberry rice prepared by conventional rice cooker (RCM) and boiling method (BM), and reheated after 3 days storage (4 °C; RR and -20 °C; FR) were investigated. Starch fractions (% wet basis) differed between cooking methods due to varied moisture contents. After storage, resistant starch (RS) contents in RR and FR were not significantly different compared to FCR; however, increase in slowly digestible starch (SDS) was accompanied by reduction in rapidly digestible starch (RDS) in riceberry rice cooked by BM. SDS increased from 7.56% to 16.00% in refrigerated rice (RR-BM) and by 15.81% in frozen rice (FR-BM). Riceberry rice hydrolysates after simulated human upper gut hydrolysis, were not significantly different among treatments and 49.90% escaped hydrolysis. During in vitro colonic fermentation, riceberry rice hydrolysates significantly enhanced probiotic strains; B. animalis TISTR 2194, B. bifidum TISTR 2129 and L. reuteri KUB AC-5 than pathogens; E. coli E101 and S. serovar Enteritidis S003. Colonic fermentation was similar among treatments. Results indicated that cooking riceberry rice by BM and storage reduced starch digestion but colonic fermentation was not dependent on cooking and storage conditions.

Keywords: riceberry rice, cooking and storage, digestibility, colonic fermentation

INTRODUCTION
Composition of gut microbiota impacts on host health through the supply of nutrients which alter metabolism and interact with host cells (Flint, Duncan, Scott and Louis, 2007). Imbalanced human microbiota is associated with inflammatory bowel disease, gastroenteritis, and colon cancer (Venter, 2007). A proper balance of the microbiota is important and this can be achieved by dietary carbohydrates that escape digestion in the small intestine and enter the colon where they are selectively used by probiotics (Cervantes-Pahm, Liu and Stein, 2014; Marotti et al., 2012). Probiotics are found in the gut microbiota as mostly lactobacillus acid bacteria consisting of multiple strains of the genera Lactobacillus and Bifidobacterium which confer health benefits through their activities. Conversely, pathogenic strains from the genera Escherichia coli and Salmonella are associated with several human diseases. Colonic fermentation of indigestible host diet fraction by probiotic bacteria limits pathogen growth, thereby reducing the risk of colonic cancer and regulating the immune system. Hence, the impact of diet fraction in manipulating gut microflora for host well-being has attracted multiple research interest (Gibson et al., 2004).

Fermentable carbohydrates that escape digestion in the upper gut and selectively stimulate growth and activities of probiotic bacteria in the colon, resulting in host health benefits, are known as prebiotics (Gibson et al., 2017; Gibson and Roberfroid, 1995). Prebiotics serve as food for probiotic bacteria in the colon. Prebiotics are considered important compared to other dietary fibers due to their unique property to be selectively utilized by bifidobacteria or lactobacilli in the colon (Tuohy, Rouaud, Bruck and Gibson, 2005). Probiotics are indigestible carbohydrates including resistant starch (RS), which enhances host health through modulation of probiotic bacteria (Y. K. Lee and Salmi, 2009).

Resistant starch (RS) is defined as total amount of starch and starch degradation products not absorbed in the upper gastrointestinal (GI) tract of healthy humans (Englyst, Kingman and Cummings, 1992). RS is presented to colonic microbiota as a fermentable carbohydrate (Fuentes-Zaragoza et al., 2011). As a result of the relationship between gut microbiota and the host, RS may have the ability to reduce several human diseases (DuPont and DuPont, 2011). RS occurs in rice but the amount depends on processing conditions, especially cooking and low-temperature storage.

Riceberry rice is a purple-pigmented variety cross-bred from Thai Hom Mali rice, Hom Hin rice and Khaos Dawk Mali 105 by the Rice Research Center, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. This variety is popular in Thailand and its environs due to its high nutritive value. Riceberry rice is eaten freshly cooked or reheated after storage. Cooking methods (Rashmi and Urooj, 2003; Reed, Ai, Leutcher and Jane, 2013) and storage temperatures (Frei, Siddhuraju and Becker, 2003; Sonia, Witjaksono and Ridwan, 2015) both affect rice digestion. Cooking renders the starch rapidly digestible by digestive enzymes due to the gelatinization process and storage gradually converts RDS to SDS and RS through the retrogradation mechanism (Frei et al., 2003; Sonia et al., 2015). Thai jasmine rice; Hom Mali rice digestion was affected by cooking and storage conditions; SDS increased significantly but RS did not increase after storage (Ayimbila and Keawsompong, 2018).

Digestibility of rice varies with cooking and storage conditions; however, information regarding in vitro digestion and fermentation of riceberry rice is limited regarding the combined effects of cooking methods and storage temperatures. Here, digestion rates of freshly cooked and reheated stored riceberry rice were compared and the impacts of their hydrolysates on colonic bacteria fermentation were assessed.
MATERIALS AND METHODS

Materials

From a local shop in Bangkok, Thai ricerry rice was purchased, sealed in polyethylene bags and stored at 4 °C prior to analysis. All chemicals sourced from Sigma-Aldrich (USA) were of analytical grade.

Sample preparation

Freshly cooked rice FCR( as control was obtained using the boiling method BM/Pilaf or oriental method on a gas cooker with water to rice ratio 1:2, and rice cooker method RCM( by electric rice cooker Otto, Kingglass Co., Ltd., Thailand) based on rice to water ratio 1:1.5. Refrigerated rice JR( and frozen rice JRF( samples were obtained by storing 100 g of control sample at 4 °C and 20 °C for 3 days, respectively. Prior to analysis, stored rice was reheated for 30 s at 100% power using a microwave JLG Electronics Co., Ltd., Thailand). Six treatments were prepared in triplicates from a bag of rice. Apparent amylose content in the raw rice was analyzed by the iodine colorimetric method [Juliano et al., 1981] before cooking.

Quantification of starch fractions

Approximately 0.5 g of minced rice was analyzed [Englyst et al., 1992] with slight modifications. The sample was combined with sodium acetate buffer 0.1 M, pH 5.2, containing an enzyme mixture of 10 mg pancreatic α-amylase, 37–7545; Sigma-Al which of starch remaining undigested after 120 min of incubation was obtained as the starch content.

Rice hydrolysis in simulated gastrointestinal GI conditions

In the buccal cavity or mouth condition, approximately 20 g minced rice was combined with 160 mL of artificial saliva JMGDF, Megazym per mL with glass beads and incubated horizontally in a shaking water bath for 2 h at 37 °C. Aliquots of 250 µL were taken at 20 and 120 min and each was placed in a 4 mL vial of 95% ethanol. Glucose content was measured using the glucose oxidase-peroxidase method JK-GLUC, Megazymel. Resistant starch was obtained as the starch remaining undigested after 120 min of incubation. Starch was classified as rapidly digestible starch JRDS, slowly digestible starch JSDS, and resistant starch JRS per 100 g on wet basis.

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Table 1 Bacterial strains tested and their cultivated conditions

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Medium</th>
<th>Growth condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus reuteri KUB-AC5</td>
<td>MRS (Difco, USA)</td>
<td>Incubated; 37 °C, 16 h</td>
</tr>
<tr>
<td>Bifidobacterium bifidum TISTR 2129</td>
<td>MRS+005 L-cysteine (Difco, USA)</td>
<td>Incubated; 37 °C, 24 h, anaerobically</td>
</tr>
<tr>
<td>Bifidobacterium animalis TISTR 2194</td>
<td>MRS+005 L-cysteine (Difco, USA)</td>
<td>Incubated; 37 °C, 24 h, anaerobically</td>
</tr>
<tr>
<td>Escherichia coli E010</td>
<td>Nutrient broth (Merck, Germany)</td>
<td>Incubated; 37 °C, 16 h, shaking</td>
</tr>
<tr>
<td>Salmonella serovar Enteritidis S003</td>
<td>Nutrient broth (Merck, Germany)</td>
<td>Incubated; 37 °C, 16 h, shaking</td>
</tr>
</tbody>
</table>

KUB: culture collection at Department of Biotechnology, Kasetsart University, Thailand. TISTR: Thailand Institute of Scientific and Technological Research. De Man, Rogosa and Sharpe JMR.

Data analysis

Replicate data were analyzed by one-way analysis of variance (ANOVA) with cooking methods and storage temperatures as factors using statistical analysis of SPSS version 19. Significant differences in rates of digestion between means of treatments were determined. Degrees of significances were set at p = 0.05 for all experiments.

Six riceberry rice hydrolysates and a basal medium without carbon source as control were evaluated. The criterion for data analysis was defined as follows; if specific growth rate of the bacterial strain with hydrolysate was statistically lower or equal to growth of the control, then the hydrolysates did not enhance growth of the strain; however, if specific growth rate was statistically higher than the control, then the hydrolysates enhanced bacterial growth.

RESULTS AND DISCUSSION

Effect of cooking and storage conditions on starch fractions

Values are mean ± standard deviation of rapidly digestible starch JRS, slowly digestible starch JSDS and resistant starch JRS of three replicates. Error bars show standard deviation, p<0.05.
Starch fractions of freshly cooked rice (FCR), reheated refrigerated rice (RR) and frozen rice (FR) of riceberry rice prepared by boiling method (BM) and rice cooker method (RCM) are displayed in Figure 1. Mean percentage of amylase before cooking was 13.68±0.58. Mean percentage moisture content of riceberry rice cooked by boiling method was 55.33±0.88 and reheated rice cooked by rice cooker method was 44.83±0.78. For the boiling method, FCR-BM recorded 20.32% rapidly digestible starch (RDS), 7.56% slowly digestible starch (SDS) and 6.78% resistant starch (RS). While FCR-RCM produced 28.67% RDS, 9.0% SDS and 8.11% RS. Between cooking methods, total starch (TS) content varied significantly, hence starch fractions were significantly different. This was attributed to significant variation of moisture content (p<0.05). Cooking caused starch gelatinization of riceberry rice as water intake replaced hydrogen bonds between the starch molecules. Increased amounts of water used in BM resulted in well-cooked rice, and high temperature caused greater intake of water which reduced TS content.

Refrigeration and freezing decreased RDS content and significantly increased SDS content in both cooking methods compared to FCR but RS (p<0.05) was not significantly different (Figure 1). Compared to FCR-BM, RR-BM gave a lower RDS content of 12.89%, higher SDS of 16.0% and RS of 7.99%. Likewise, FR-BM gave lower RDS of 12.02%, higher SDS of 15.81% and RS of 5.89%. Compared to FCR-RCM, RR-RCM gave a lower RDS content of 26.59%, higher SDS of 11.50% and similar RS of 8.54%. FR-RCM recorded similar RDS of 28.59%, SDS of 10.34% and RS of 8.33%. Storage affected riceberry rice digestion as the retrogradation mechanism caused reorganization of gelatinized starch molecules. Starch retrogradation caused an imperfect crystalline structure through interaction between long-chain and highly-branched amylopectin (Park, Baik and Lim, 2009; Zhang, Hu, Xu, Jin and Tian, 2011). This increased SDS in riceberry rice depends on cooking methods but with no significant difference between storage temperatures. Differences were attributed to varied amounts of water which play a crucial role in starch gelatinization and retrogradation mechanisms during cooking and storage of starchy foods. The rate of starch retrogradation was highly influenced by the amount of water absorbed (Wang and Copeland, 2013).

Figure 2: Scanning electron micrographs of cross-sectional and longitudinal surfaces of cooked riceberry rice grains by boiling method, FCR-BM, RR-BM and FR-BM and rice cooker method, FCR-RCM, RR-RCM and FR-RCM. Cross sections (boiling method; A-C, rice cooker method; M-O) and longitudinal surfaces (boiling method; D-F, rice cooker method; P-R). Lowercase letters show higher magnification of samples labeled with uppercase.
Impact of cooking methods and storage on starch fractions as evidenced by SEM micromorphology

From Figure 2, FCR-BM rice images and DI showed more and larger cracks on the cross-sectional surface; image A and longitudinal surface; image D than FCR-RCM images and P rice cross-sectional surface; image M and longitudinal surface; image S indicating the effect of water quantity used during cooking. Cracks in grains provided a medium for water penetration into the grains during cooking. Using lower amounts of water resulted in dense regions with less starch gelatinization in FCR-RCM riceberry rice, while higher amounts of water in BM caused dense voids in FCR-BM. This confirmed the differences in starch fractions between cooking methods.

RR-BM riceberry rice; images B and E, FR-BM riceberry rice; images C and F, RR-RCM riceberry rice; images N and Q and FR-RCM riceberry rice; images O and R gave more and larger voids than their respective FCR grains. During storage, water relocation inside the gelatinized rice starch occurs as hydrogen bonds between water and starch molecules dissociate through a process of retrogradation. The disruption of hydrogen bonds between water and starch is the first step for retrogradation (H. Lee, Lee and Kim, 2017; Ogawa, Glenn, Orts, and Wood, 2003). The re-association of the starch molecules after 3 days storage increased cracks in riceberry rice grains, which influenced by cooking conditions.

In vitro rate of hydrolysis in simulated gastrointestinal (GI) conditions

![Figure 3 Rate of hydrolysis (% of riceberry rice prepared by boiling method (BM) in simulated gastrointestinal (GI) conditions.](image)

Freshly cooked rice; FCR-BM, refrigerated rice; RR-BM, frozen rice; FR-BM. Human salivary α-amylase in the mouth condition (M1: 1 min, M2: 2 min, M5; 5 min, M20; 20 min, M30; 30 min; pH 6.8, pepsin in human gastric juice (G30); 30 min, G120;120 min, G240; 240 min; pH 2.0 and porcine pancreatic α-amylase in the intestinal condition (I1; 1 h, I2; 2 h, I6; 6 h; pH 6.9. Error bars show standard deviation, p >0.05.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>FCR-BM</th>
<th>RR-BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>49.79±0.72</td>
<td>51.40±0.72</td>
</tr>
<tr>
<td>M2</td>
<td>51.40±0.72</td>
<td>50.62±0.57</td>
</tr>
<tr>
<td>M5</td>
<td>52.41±1.16</td>
<td>51.97±0.64</td>
</tr>
<tr>
<td>M30</td>
<td>50.56±1.18</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Percentage resistance to hydrolysis after simulated upper gut digestion

Data are mean from three replicates ± standard deviations, p >0.05.

Hydrolysis rate of riceberry rice in artificial human gastric juice

Riceberry rice hydrolysis in gastric juice containing pepsin was studied. Hydrolysis rates of freshly cooked riceberry rice (FCR-BM) at 30, 120 and 240 min were 11.51, 11.63 and 11.99, respectively, while those of refrigerated riceberry rice (FR-BM) were 9.49, 9.82 and 9.98, respectively. Similarly, hydrolysis rates of frozen riceberry rice (FR-BM) were 10.57, 10.74 and 11.11, respectively. Riceberry rice cooked by BM gave 89.00% resistance in gastric juice. Likewise, hydrolysis rates of freshly cooked riceberry rice (FCR-BM) were 9.72, 10.08 and 10.26, respectively, while those of refrigerated riceberry rice (FR-BM) were 10.91, 11.17 and 11.52, respectively. Similarly, frozen riceberry rice (FR-BM) recorded 9.70, 10.32 and 10.87, respectively. A total of 89.11% of BM rice was resistant. Hydrolysis rates of FCR, RR and FR for both cooking methods were also similar and reached a maximum of 9.98-11.99 at 240 minutes. Riceberry rice was resistant to hydrolysis in the gastric condition because starch is the main component; however, some hydrolysis occurred due to the acid effect (Singh, Kaur and Singh, 2013).

Hydrolysis rate of Riceberry rice in intestinal condition

Riceberry rice was rapidly hydrolyzed under simulated small intestine condition (I) after 1 h of incubation as shown in Figure 3. Hydrolysis rates of freshly cooked riceberry rice (FCR-BM) at 1, 2, 5, 20 and 30 min were 0.72, 1.35, 2.47, 8.71 and 10.98, respectively, while refrigerated riceberry rice (RR-BM) gave 0.82, 1.45, 2.58, 6.88 and 9.45, respectively. In the same trend, frozen riceberry rice (FR-BM) gave 0.95, 1.35, 3.06, 9.48 and 11.99, respectively. Also, hydrolysis rates (Figure 4) of freshly cooked riceberry rice (FCR-BM) was 0.66, 1.20, 3.85, 7.61 and 9.70, respectively. Likewise, percentage hydrolysis of refrigerated riceberry rice (RR-BM) were 0.53, 1.09, 2.83, 6.55 and 9.14, respectively, while that of frozen riceberry rice (FR-BM) were 0.77, 1.19, 2.25, 6.71 and 9.68, respectively. These results suggested that hydrolysis rates of freshly cooked, reheated, refrigerated and frozen riceberry rice in the baccal cavity were similar and reached a maximum of 9.14-10.98%. Hydrolysis was not significantly influenced by cooking methods and storage. Riceberry rice prepared by BM and RCM showed 89.19% and 90.49% resistance in simulated human baccal cavity conditions. Salivary α-amylase acted partially on riceberry rice. A similar effect on Thai jasmine rice was reported by Ayimbila and Keawsompong (2018).

![Figure 4 Rate of hydrolysis (%) of riceberry rice prepared by rice cooker method (RCM) in simulated gastrointestinal (GI) condition. Freshly cooked rice; FCR-RBM, refrigerated rice; RR-RCM, frozen rice; FR-RCM. Human salivary α-amylase in the mouth condition (M1; 1 min, M2; 2 min, M5; 5 min, M20; 20 min, M30; 30 min; pH 6.8, pepsin in human gastric juice (G30); 30 min, G120;120 min, G240; 240 min; pH 2.0 and porcine pancreatic α-amylase in the intestinal condition (I1; 1 h, I2; 2 h, I6; 6 h; pH 6.9. Error bars show standard deviation, p >0.05.](image)
escaped hydrolysis in simulated gastrointestinal tract (Ayimbila and Keawsompong, 2018). In this study, mean percentage amylose content of riceberry rice was 13.68±0.58. Ayimbila and Keawsompong (2018) reported the mean percentage amylose of jasmine rice (Thai Hom Mali rice) to be 15.54±1.02. Both rice varieties are comparable in terms of amylose and are also genetically related; riceberry rice was cross-bred from three rice varieties including Thai Hom Mali rice, thus, this has resulted in similar percentage resistant to hydrolysis in the simulated GI tract.

SEM micromorphology of riceberry rice hydrolysates showing hydrolysis in the human simulated condition

Figure 5 Scanning electron microscope images of different magnifications of minced cooked riceberry rice before (A) and after hydrolysis during simulation of human buccal cavity or mouth (M), gastric (G) and small intestine (I) conditions.

Figure 5 shows that the structure of minced cooked riceberry rice (FCR) before hydrolysis (B) had large connective structures. After digestion by artificial human saliva (M), these structures disconnected into smaller units as a result of salivary amylase activities. Subsequently, further breakdown of structures was observed with voids on the surface of starch compounds after gastric condition (G), possibly due to the acid effect on the bran layer. Finally, much smaller units were observed in the small intestine condition, indicating maximum hydrolysis effect of pancreatic α-amylase on riceberry rice. Finally, most compound granules retained their shapes and structures and were resistant to hydrolysis in the simulated human upper gut. These results confirmed that salivary amylase, gastric condition and pancreatic α-amylase manage to digest riceberry rice although most hydrolysis was carried out by pancreatic α-amylase.

Bacteria fermentation

Changes in bacteria population

![Bacteria fermentation chart](image)
tic bacteria, while

\[ AC5 \]

'B. 2129' as

\[ f \]

stobacilli rather than pathogens indicated the

\[ pH \]

acids such as SCFA

\[ L \]

Acid bacteria releases organic acids such as SCFA

\[ B. \]

animalis

\[ S. \]

Enteritidis S003 were

\[ p<0.05 \]

Bifidobacterium bifidum TISTR 2129 (X), Escherichia coli E010 (Y) and Salmonella serovar Enteritidis S003 (Z).

**Figure 6** Mean population (log CFU/mL) of bacteria in medium with or without (control) riceberry rice hydrolysates over time (h) of fermentation. Rice cooker method (RCM), FCR-RCM (a), RR-RCM (b), FRR-RCM (c), and boiling method (BM), FCR-BM (d), RR-BM (e), FR-BM (f). Values are mean ± standard deviation. Error bars show standard deviations. *P<0.05. Lactobacillus reuteri KUB-AC-5 (V), Bifidobacterium animalis TISTR 2194 (W), Bifidobacterium bifidum TISTR 2129 (X), Escherichia coli E010 (Y) and Salmonella serovar Enteritidis S003 (Z).

**Figure 7** pH reduction in medium with or without (control) riceberry rice hydrolysate over time of fermentation by Lactobacillus reuteri AC5 (AC5); 4h, 8h and 16h, Bifidobacterium animalis TISTR 2194 (B. 2129) and Bifidobacterium bifidum TISTR 2129 (B. 2129); 6h, 12h and 24h. Initial pH was 6.8±0.1.

The population log CFU/mL of probiotic and pathogenic bacterial strains during fermentation of riceberry rice hydrolysates over time is shown in Figure 6 above. All strains exhibited similar changes in population among treatments, which were strain-dependent. Based on the respective control of probiotics, range of growth changes log CFU/mL among treatments after 4 h, 8 h and 16 h for *L. reuteri* KUB-AC5 were 0.76-0.79, 1.02-1.06 and 1.01-1.05, respectively. Moreover, growth changes log CFU/mL after 6 h, 12 h and 24 h for *B. animalis* TISTR 2194 were 0.32-0.35, 0.86-0.89 and 0.64-0.69, while those of *B. bifidum* TISTR 2129 were 0.62-0.67, 0.92-0.94 and 1.32, respectively. On the other hand, growth changes log CFU/mL of pathogenic strains among treatments after 4 h, 8 h and 16 h for *E. coli* E010 were -0.01-0.02, 0.17-0.2 and 0.01-0.02, while those of *S. serovar Enteritidis* S003 were -0.05-0.02, 0 and 0.00-0.04, respectively. Significantly *p<0.05* changes of *Bifidobacterium animalis* TISTR 2194, Lactobacillus reuteri KUB-AC-5 and *Bifidobacterium bifidum* TISTR 2129 were observed over time compared to control of basal medium. Likewise, populations of *Escherichia coli* E010 and *Salmonella serovar Enteritidis* S003 changed over time but were not significantly *p<0.05* different from control. Thus, riceberry rice hydrolysates enhanced the growth of probiotic bacteria, while pathogens were neither enhanced nor inhibited by riceberry hydrolysates. Also, hydrolysates FCR, RR and FR of riceberry rice gave similar fermentation for each strain.

Growth of probiotic strains decreased pH value over time as shown in Figure 7 above, but significant differences *p<0.05* were not observed among FCR, RR and FR hydrolysates of riceberry rice. *L. reuteri* KUB-AC5 fermentation of riceberry rice hydrolysates decreased pH ranging from 0.2 to 0.5 at 4 h, 2.0 to 2.3 at 8 h and 2.2 to 2.4 at 16 h of incubation, while *B. animalis* TISTR 2194 decreased pH ranging from 0.2 to 0.3 at 6 h, 1.6 to 1.8 at 12 h and 2.2 to 2.5 at 24 h of incubation. Also, *B. bifidum* TISTR 2129 caused pH reduction of 0.2 to 0.3 at 6 h, 1.7 to 1.8 at 12 h and 2.1 to 2.3 at 24 h of incubation. Reductions in pH were attributed to liberation of organic acids, reflecting production of short chain fatty acids (SCFA) by the fermentation of riceberry rice hydrolysates. The effect on growth of bifidobacteria and lactobacilli rather than pathogens indicated the fermentable ability of riceberry rice hydrolysates by probiotics. Studies conducted on humans fed diets rich in resistant starches revealed changes of major groups of bacteria including bifidobacteria and lactobacilli (Bertocci, 2004; Phillips et al., 1995; Tomlin and Read, 1990). Also, hydrolysates of Thai jasmine rice selectively enhanced probiotic bacterial growth in tandem with pH reduction (Asymbila and Keawsompong, 2018). Fermentation of starch by lactic acid bacteria releases organic acids such as SCFA’s that reduce pH (Sušković, Kos, Goreta and Matošić, 2001).

### Table 3 Specific growth rate (μ) of test strains of probiotics and pathogens grown in medium with or without riceberry rice hydrolysates.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Basal medium</th>
<th>FCR-RCM</th>
<th>RR-RCM</th>
<th>FCR-BM</th>
<th>RR-BM</th>
<th>FR-BM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. reuteri</em> AC5</td>
<td>0.91±0.00a</td>
<td>0.97±0.01a</td>
<td>0.96±0.001a</td>
<td>0.95±0.001a</td>
<td>0.95±0.02a</td>
<td>0.96±0.01a</td>
</tr>
<tr>
<td><em>B. bifidum</em> TISTR 2129</td>
<td>0.94±0.02b</td>
<td>1.04±0.02</td>
<td>1.03±0.01</td>
<td>1.02±0.02</td>
<td>1.03±0.01</td>
<td>1.01±0.004</td>
</tr>
<tr>
<td><em>B. animalis</em> 2194</td>
<td>0.86±0.03c</td>
<td>0.95±0.01c</td>
<td>0.94±0.02c</td>
<td>0.93±0.02c</td>
<td>0.94±0.01c</td>
<td>0.94±0.03c</td>
</tr>
<tr>
<td><em>E. coli</em> E010</td>
<td>0.92±0.01a</td>
<td>0.96±0.01a</td>
<td>0.95±0.02a</td>
<td>0.96±0.01a</td>
<td>0.96±0.02a</td>
<td>0.97±0.01a</td>
</tr>
<tr>
<td><em>S. Enteritidis</em> S003</td>
<td>0.93±0.003b</td>
<td>0.94±0.01c</td>
<td>0.95±0.02c</td>
<td>0.95±0.02c</td>
<td>0.93±0.03</td>
<td>0.94±0.02</td>
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</table>

Freeze-dried hydrolysates of freshly cooked riceberry *FCR-RCM, refrigerated riceberry* *JFR-RCM*, frozen riceberry *FR-RCM* by rice cooker method, and freshly cooked riceberry *FCR-BM, refrigerated riceberry* *JFR-BM*, and frozen riceberry *FR-BM* by boiling method. Different letters as superscripts a, b, c and d show significant differences.

Table 3 shows specific growth rates (μ) h⁻¹ of strains tested on riceberry rice hydrolysates compared to the control without hydrolysate. Differences in specific growth rates compared to the control among treatments for *L. reuteri* KUB-AC5 ranged from 0.03 to 0.05, while *B. animalis* TISTR 2194 ranged from 0.05 to 0.07. Likewise, specific growth rates of *B. bifidum* TISTR 2129 ranged from 0.08 to 0.10. Also *E. coli* E010 gave specific growth rates ranging from 0.01 to 0.03, whereas those of *S. serovar Enteritidis* S003 ranged from 0.01 to 0.02. Results indicated that probiotic strains gave higher specific growth rates than pathogens. Among the probiotics, *B. animalis* subsp. *B. animalis* TISTR 2194 produced the highest specific growth rates, followed by *L. reuteri* AC-5 and lastly, *B. bifidum* TISTR 2129. Riceberry rice hydrolysates enhanced rapid multiplication of probiotics and provided growth advantage for probiotic strains. Changes in bacteria growth in the microbiota occur rapidly after dietary changes.
Bacteria that can ferment resistant starch generate energy which provides them with growth advantages in the gut microbiota. \textit{Walker et al., 2011}. Thai jasmine rice hydrolysate consistently promoted probiotics growth in \textit{in vitro} fermentation. \textit{Aymibila and Keawsompong, 2018}.  

**CONCLUSIONS**  
Riceberry rice RS content was not significantly different between cooking methods and storage temperatures. However, SDS increased in riceberry rice cooked by boiling method due to a decrease in RDS after storage. SDS increased from 7.56% to 16.00% in RR-BM and by 15.81% in FR-BM. Also, riceberry rice hydrolysate after simulated hydrolysis in the human upper gut was not significantly different and 49.90% escaped hydrolysis. During \textit{in vitro} fermentation, riceberry rice hydrolysate significantly enhanced growth of probiotic bacteria and \text{p}\text{} differences in growth changes over time were not observed among treatments. Probiotic strains gave specific growth rates ranging from 0.02-0.08, higher than pathogens, and coupled with \textit{pH} reduction. This demonstrated that starch fractions of riceberry rice were affected by processing conditions but no colonic fermentation of the hydrolysate. A deeper understanding of the prebiotic properties of riceberry rice hydrolysate is urgently required.  

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