Effects of Cooking on Anthocyanin Concentration and Bioactive Antioxidant Capacity in Glutinous and Non-Glutinous Purple Rice

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Abstract: Purple rice is a source of bioactive antioxidants for rice consumers. Loss of the major antioxidant compounds after a range of cooking processes was evaluated by measuring the change in anthocyanin concentration (ATC) and antioxidant capacity (DPPH activity) of four non-glutinous and four glutinous genotypes. However, soaking in water prior to cooking generally decreased ATC and DPPH activity more in non-glutinous than in glutinous genotypes. Wet cooking (WC) and soaking before wet cooking (S-WC) led to almost all the ATC and DPPH activity being lost with only slight variation between genotypes. In the glutinous genotype PES, which had the highest raw rice ATC, the highest ATC remained when cooked by the WC method. By contrast, almost no ATC remained after WC and S-WC in the low ATC genotypes such as KDK. Overall, the loss of ATC on cooking was greater in non-glutinous than glutinous genotypes for both WC and S-WC, but the reverse occurred for DPPH activity. Wet cooking using electric rice cooker retained higher ATC than the pressure cooking. Thus, for genotypes with high ATC and antioxidant capacity, the selection of cooking method is critical for retaining and stabilizing rice quality.

Key words: purple rice; rice cooking; anthocyanin; antioxidant capacity; wet cooking

Purple rice with pigmented grain has long been a unique and traditional food for desserts and for some medical purposes in many cultures (Rerkasem, 2015). Currently, its benefits have been widely acknowledged and pigmented rice is being used as commercial food products as well as in dietary supplements, cosmetics and pharmaceuticals, especially among Asian countries as well as in the USA and EU, leading to an increase in the demand for its value as a natural health food (Chaudhary, 2003; Appa Rao et al, 2006; Sukhonthara and Theerakulkait, 2009). Dietary antioxidants can protect against free radicals that may promote the aging process and disease progression (Sies, 1997). Furthermore, anthocyanins, polyphenols and other compounds in colored rice may have other beneficial effects for human (Chatthongpisut et al, 2015) and animal health (Xia et al, 2006; Suwannakul et al, 2015). Thus, purple rice has gained attention from food manufacturers in forms such as malt, flour, bread, ice cream and wine (Minh, 2014). However, the concentration of antioxidants and bioactivities varied with rice genotype, even though they had similar bran color (Surarit et al, 2015).

In general, rice is cooked before its consumption using methods such as rapid-boiling, pressure-cooking and steaming depending on the consumer’s preference and expectation of sensory quality (aroma, taste and
text). Each method can include pre-cooking processes such as rinsing to remove chemical residues and soaking to shorten the cooking time, but this differs between countries (Son et al, 2013). In Thailand, the cooking methods differ between non-glutinous and glutinous rice: wet cooking with an electric rice-cooker is commonly used for non-glutinous rice, while soaking before steaming is the traditional method for glutinous rice. However, both wet cooking and steaming methods can be applied both for non-glutinous and glutinous rice depending on consumer’s preference e.g., steaming for non-glutinous rice helps to remain firmness and chewiness texture, while wet cooking has less result. On the other hand, pressurized rice cookers are now widely used as an easy and rapid method. The cooking method may differentially affect the levels of antioxidants and other compounds in pigmented rice (Zaupa et al, 2016). For example, the decline response in anthocyanin content and antioxidant capacity of black rice differed between two home-cooked products (rice porridge and cooked rice) (Tang et al, 2016).

Compounds with antioxidant properties differ in their chemistry, with anthocyanins being water soluble and temperature labile, while phenolic acids occur in both soluble and insoluble forms with different properties from anthocyanins (Goufo and Trindade 2014). Notably, the anthocyanin in pigmented rice decreased by up to 80% after cooking with an electric rice cooker whereas phenolic compounds fell by 54% (Bhawamai et al, 2016). In comparison of the cooking methods, the risotto cooking retained more anthocyanin and other phenolic compounds than the boiling as most water is absorbed by the specific grain type with high porosity in the former method (Zaupa et al, 2015). So far, the effect of cooking process on the comparative antioxidant compounds in non-glutinous and glutinous rice genotypes has not been studied. Such knowledge would provide useful information for rice consumers in order to stabilize the antioxidant capacity during the cooking process. Therefore, this study was undertaken to evaluate the effect of cooking methods and soaking time on anthocyanin concentration and antioxidant capacity among non-glutinous and glutinous purple rice genotypes.

**MATERIAL AND METHODS**

**Rice samples**

Eight pigmented rice genotypes with purple pericarp color were used in this study: four were non-glutinous rice genotypes [Hom Nil (HN), Rice Berry (RBR), CMU168 and CMU107] and four were glutinous rice genotypes [Kum Doi Saket (KDK), CMU125, Kum Hom CMU (KHC MU), and Pieisu (PES)]. All genotypes were collected at maturity in the same growing condition at the research field of Agronomy Division, Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University in the wet season 2015. The paddy rice samples were sun dried to reach about 11%–12% moisture content before de-husked with a husker (Model P-1 from Ngek Seng Huat Co. Ltd., Thailand) to produce brown rice (the form of rice grain which palea and lemma are removed, but embryo and bran layers are still intact). Samples of the brown rice sample were analyzed for initial anthocyanin concentration and antioxidant capacity in the detail below. The amylose content was analyzed by iodine reaction (Juliano 1971) and it was in similar ranged from 14%-17% for non-glutinous genotype and from 2%-6% for glutinous rice, while total fat content in brown rice was also analyzed (Xu and Godber 1999) and it was in similar ranged at 1.5–2.0 mg/100 g among the eight genotypes used in this study. Grain was kept in zip-lock plastic bags and stored at -25 ºC before analysis.

**Experimental design**

The factorial randomized complete block design with two treatment factors was arranged in the experiment 1. The treatment factors were set as rice genotypes and the cooking method. The eight genotypes were included as in the above detail and the cooking method consisted of one process and two cooking methods, soaking (SR), wet cooking (WC) and combination of soaking and wet cooking (S-WC). Each treatment was carried out in triplicate. On the other hand, the completely randomized design was used in the experiment 2. The samples were cooked by using either an electric rice cooker (ERC) or a pressure rice cooker (PRC) in eight treatments with varying of soaking and cooking times. The treatment was also carried out in triplicate.

**Effects of cooking process on anthocyanin concentration and antioxidant capacity of non-glutinous and glutinous rice genotypes**
Samples (100 g) of brown rice of each genotype were soaked in water with the ratio 1:2 w/v at room temperature for 12 h based on the saturation point testing (Tang et al., 2016) which is also a traditional way to reduce cooking time and maintain the softness texture for sticky rice. The samples of soaked rice were collected to evaluate the effect of soaking (SR) before wet cooking. Then the samples were cooked by two methods (Table 1): (I) WC, grain was cooked in an electric rice cooker (Panasonic, SR-G101) at the ratio of grain to water of 1:4 w/v for non-glutinous rice and 1:5 w/v for glutinous rice for 25 min; and (II) S-WC, soaked rice was cooked by wet-cooking with 1:3 w/v in the above electric rice cooker for 20 min. The optimal ratio of grain to water was determined based on the amylase content of the grain (Yu et al., 2017), hence the ratios of grain to water for non-glutinous was lower than glutinous rice. The preliminary experiment was conducted to evaluate the ratio of grain weight to water volume as well as the cooking time. The initial volume of water was determined to confirm that the water was completely absorbed and rice grain was cooked as observed that the starch was completely disintegrated at the end of cooking. All samples of the cooked rice were dried at 75 °C for 48 h and then about 50 g samples of the brown rice were mechanically ground for 60 seconds in a hammer mill (Scientific Technical Supplies D-6072 Dreieich, West Germany). The flour was placed in zip-lock plastic bags stored at -25 °C until chemical analysis. In a preliminary experiment, there was no difference in the anthocyanin concentration and antioxidant capacity between freeze drying and chemical analysis. All cooked samples were dried at 75 °C for 48 h and then stored at -25 °C until analysis.

Effects of cooking process on anthocyanin concentration and antioxidant capacity of glutinous CMU125, a high anthocyanin concentration and yielding genotype

The preliminary experiment was conducted to determine steaming process by varying the soaking times before steaming process. The cooking time was recorded when the starch was broken down and the grains become soft and mushy consistently (Table 2). For the wet cooking (WC) method, brown rice (100 g) was cooked without soaking, with mass to water at the ratio of 1:3 w/v, in an electric rice cooker (Panasonic model SR-G101, Japan) (0-ERC) for 30 min or in a pressure rice cooker (SHARP model KS-G10A-W, Japan) (0-PRC) for 25 min. For the steaming cooking method, grain was soaked in water with ratio 1:2 w/v at room temperature for 4, 8 and 12 h and drained before being cooked in an electric rice cooker (Otto, CR-110T) (4-ERC, 8ERC and 12-ERC) for 60, 45 and 40 min, respectively or in a pressure rice cooker (SHARP, KS-GX10A-W) (4-PRC, 8-PRC and 12-PRC) for 35, 30 and 25 min, respectively. Each method was repeated three times and carried out in triplicate. All cooked samples were dried at 75 °C for 48 h and then stored at -25 °C until analysis.

Chemical analysis

Anthocyanin concentration

Anthocyanin concentration was determined using the modified pH-differential method of Abdel-Aal and Hucl (1999). About 2.5 g of brown rice was extracted with 24 mL acidified methanol (70% methanol and 30% 1.5 N HCl, v/v) on an orbital shaker (IKA KS 250 B) at room temperature for 1 h. After centrifugation, the liquid was filtered through Whatman No. 1 filter paper and the supernatant was collected and added to the two buffer solutions (0.025 mol/L potassium chloride buffer, pH 1.0 and 0.4 mol/L sodium acetate buffer, pH 4.5). The absorbance measured with a spectrophotometer (Biochrom Libra S22, England) at 520 and 700 nm, and results expressed as mg of cyanidin-3-glucoside, the main anthocyanin in purple rice, was calculated as follows:

**Table 1. Description of cooking method in Experiment 1.**

<table>
<thead>
<tr>
<th>Cooking method</th>
<th>Rice type</th>
<th>Soaking time (h)</th>
<th>Grain water ratio (w/v)</th>
<th>Cooking time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>NGR</td>
<td>0</td>
<td>1:4</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>0</td>
<td>1:5</td>
<td>25</td>
</tr>
<tr>
<td>S-WC</td>
<td>NGR</td>
<td>12</td>
<td>1:3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>12</td>
<td>1:3</td>
<td>20</td>
</tr>
</tbody>
</table>

WC, Wet cooking; S-WC, Soaking followed by wet cooking; NGR, Non-glutinous rice; GR, Glutinous rice.

**Table 2. Description of cooking method in Experiment 2.**

<table>
<thead>
<tr>
<th>Cooking method</th>
<th>Rice cooker</th>
<th>Soaking time (h)</th>
<th>Cooking time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>ERC</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>PRC</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>ST</td>
<td>ERC</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>25</td>
</tr>
</tbody>
</table>

WC, Wet cooking; ST, Steaming; ERC, Electric rice cooker; PRC, Pressure rice cooker.
Anthocyanin = (A × MW × DF × 1000)/ ε × L
where A, A520 nm – A520 nm; MW, 449.2 g/mol for molecular weight of cyanidin-3-glucoside; DF, the dilution factor; ε, 26 900 molar absorbance; and L, 1 cm for cell path length.

Antioxidant capacity
The DPPH radical scavenging activity (DPPH activity) was determined using the modified method of Amarowicz et al (2004). Rice flour (0.1 g) was extracted with 10 mL methanol solvent. The extract was shaken on an orbital shaker (IKA KS 250 B) for 30 min. The supernatant in each tube was collected by centrifugation at 4 500 rpm for 10 min, and filtered through a 0.22 µm Nylon syringe filter. Then 0.3 mL supernatant was reacted with 1.6 mL methanol and 0.5 mL 0.1 mmol/L DPPH solution, while blank tube were performed using 0.3 mL supernatant was mixed with 2.1 mL of methanol. The mixture was incubated in darkness at room temperature for 20 min and then measured using a spectrophotometer at 517 nm. The DPPH radical scavenging activity (%) of samples and standard (Trolox) were calculated as follows:

DPPH-scavenging activity (%) = [(AC– AS/AC) ×100]

where AC, absorbance of control; AS, the absorbance of sample. The DPPH radical scavenging activity was calculated using a calibration curve made using Trolox concentrations ranging from 10–62 µg/mL ($R^2 = 0.995$).

Statistical analysis
Statistix version 9.0 statistical software was used to calculate the means of all data and to perform analysis of variance (ANOVA) among raw and cooked samples. The LSD test at 95% confidence level ($P < 0.05$) was used to identify differences between each cooking method and genotype. All analyses were performed in triplicate.

RESULTS

Effects of cooking process on anthocyanin concentration and antioxidant capacity of non-glutinous and glutinous rice genotypes

Anthocyanin concentrations of raw rice ranged from 12 to 40 mg/100 g in non-glutinous and 42 to 271 mg/100 g in glutinous genotypes (Table 3). Soaking significantly decreased anthocyanin concentration in raw rice in all genotypes ($P < 0.01$) (Table 3), and the reduction was generally greater in non-glutinous (44–65%) than in glutinous genotypes (16–47%). The genotypes least affected by soaking were non-glutinous CMU107 and glutinous PES.

Both WC and S-WC cooking methods decreased ATC and there was an interaction between cooking method and genotype ($P < 0.01$) (Table 3). After cooking, the decrease in ATC was generally greater in non-glutinous (83–95%) than in glutinous genotypes (67–89%). Among the genotypes, only HN and PES cooked by the WC method retained significantly more anthocyanin than when processed by the S-WC method.

The activity of DPPH in raw rice ranged from 127 to 212 mg Trolox/100 g in non-glutinous and 221 to 616 mg Trolox/100 g in glutinous genotypes (Table 4). Soaking significantly decreased DPPH activity ($P < 0.01$) (Table 4) in raw rice. The loss after soaking was slightly higher in non-glutinous (13–30%) than in

Table 3. Anthocyanin concentration of purple rice after cooking with different methods among four non-glutinous and four glutinous genotypes.

<table>
<thead>
<tr>
<th>Rice type</th>
<th>Genotype</th>
<th>Raw rice</th>
<th>Cooking method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SR (mg/100 g)</td>
<td>%</td>
</tr>
<tr>
<td>NGR</td>
<td>HN</td>
<td>40 a</td>
<td>16 d</td>
</tr>
<tr>
<td></td>
<td>RBR</td>
<td>36 b</td>
<td>19 c</td>
</tr>
<tr>
<td></td>
<td>CMU168</td>
<td>16 d</td>
<td>6 fg</td>
</tr>
<tr>
<td></td>
<td>CMU107</td>
<td>12 c</td>
<td>7 f</td>
</tr>
<tr>
<td>GR</td>
<td>KDK</td>
<td>42 f</td>
<td>22 gh</td>
</tr>
<tr>
<td></td>
<td>CMU125</td>
<td>146 c</td>
<td>97 d</td>
</tr>
<tr>
<td></td>
<td>KHC MU</td>
<td>58 e</td>
<td>42 f</td>
</tr>
<tr>
<td></td>
<td>PES</td>
<td>271 a</td>
<td>227 b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype (G)</th>
<th>Cooking method (C)</th>
<th>G × C</th>
<th>5 % LSD (G × C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGR **</td>
<td>**</td>
<td>**</td>
<td>2</td>
</tr>
<tr>
<td>GR **</td>
<td>**</td>
<td>**</td>
<td>9</td>
</tr>
</tbody>
</table>

SR, Soaked rice; WC, Wet cooking; S-WC, Soaked wet cooking; %, Reduction percentage; NGR, Non-glutinous rice; GR, Glutinous rice.
Different letters in the each row (NGR and GR) indicate significant difference among cooking methods ($P < 0.05$).
* and ** indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively.
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Effects of Cooking on Anthocyanin and Antioxidant Capacity in Purple Rice

Purple rice (14%–22%). Concerning the non-glutinous and glutinous genotypes, the decrease in DPPH activity was least in CMU168 and PES, respectively.

As for ATC, there was also an interaction between cooking method and genotype (P < 0.01) on DPPH activity (Table 4). Compared to raw rice, the loss of DPPH activity in cooked rice was greater (P < 0.01) in glutinous (60%–73%) than in non-glutinous (23%–56%) genotypes (Table 4). However, the percent reduction in DPPH activity varied with genotype and cooking method. Among non-glutinous genotypes, CMU168 cooked by the WC method retained more DPPH activity than when cooked by the S-WC method, and this was similar to two glutinous genotypes, CMU125 and PES.

The relationship between anthocyanin and antioxidant capacity (DPPH activity) of non-glutinous and glutinous rice among different cooking methods was obtained in each genotype (Fig. 1). It was found that the anthocyanin of non-glutinous without CMU168 was highly correlated with the antioxidant capacity with a correlation coefficient of \( R^2 = 0.94, 0.89 \) and 0.94 at \( P < 0.05 \) in HN, RBR and CMU107, respectively. The similar strong correlation was also found in glutinous genotypes with a correlation coefficient of \( R^2 = 0.88, 0.95, 0.91 \) and 0.99 at \( P < 0.05 \) in KDK, CMU125, KHCMU and PES, respectively.

### Effects of cooking method on anthocyanin concentration and DPPH activity of glutinous CMU125

Most of the ATC (88%–95%) was lost during cooking, and this varied between cooking methods (P < 0.01) (Table 5). The loss of ATC was greater in rice cooked by PRC (93%–95%) than by ERC (88%–90%) at most soaking times used, excepted in 4-ERC and 4-PRC which were not different.

The DPPH also decreased (69%–81%) significantly after cooking, but the response differed from ATC (P < 0.01) (Table 5). Although the DPPH activity of cooked rice was similar for the ERC and PRC methods, using 0-ERC and 0-PRC retained more

### Table 4. DPPH radical scavenging activity (DPPH activity) of purple rice after cooking with different methods among four non-glutinous and four glutinous genotypes.

<table>
<thead>
<tr>
<th>Rice type</th>
<th>Genotype</th>
<th>Raw rice</th>
<th>Cooking method</th>
<th>SR (mg/100 g)</th>
<th>%</th>
<th>WC (mg/100 g)</th>
<th>%</th>
<th>S-WC (mg/100 g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGR</td>
<td>HN</td>
<td>205 a</td>
<td>SR</td>
<td>144 cd</td>
<td>30</td>
<td>104 fgh</td>
<td>49</td>
<td>91 hi</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>RBR</td>
<td>212 a</td>
<td>WC</td>
<td>156 be</td>
<td>27</td>
<td>116 efg</td>
<td>45</td>
<td>97 ghi</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>CMU168</td>
<td>127 de</td>
<td>S-WC</td>
<td>110 cefg</td>
<td>13</td>
<td>98 ghi</td>
<td>23</td>
<td>64 j</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>CMU107</td>
<td>173 b</td>
<td></td>
<td>125 e</td>
<td>28</td>
<td>100 fgh</td>
<td>42</td>
<td>82 i</td>
<td>53</td>
</tr>
<tr>
<td>GR</td>
<td>KDK</td>
<td>221 fg</td>
<td></td>
<td>128 hi</td>
<td>18</td>
<td>88 jk</td>
<td>60</td>
<td>80 k</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>CMU125</td>
<td>419 c</td>
<td></td>
<td>357 d</td>
<td>15</td>
<td>157 i</td>
<td>62</td>
<td>112 j</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>KHCMU</td>
<td>258 e</td>
<td></td>
<td>201 gh</td>
<td>22</td>
<td>100 jk</td>
<td>61</td>
<td>81 k</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>PES</td>
<td>616 a</td>
<td></td>
<td>529 b</td>
<td>14</td>
<td>232 ef</td>
<td>62</td>
<td>168 i</td>
<td>73</td>
</tr>
</tbody>
</table>

Different letters in the each row (NGR and GR) indicate significant difference among cooking methods (P < 0.05).

* and ** indicate significant difference at P < 0.05 and P < 0.01, respectively.

**SR**, Soaked rice; **WC**, Wet cooking; **S-WC**, Soaked wet cooking; %, Reduction percentage; **NGR**, Non-glutinous rice; **GR**, Glutinous rice.

Fig. 1. Relationship between anthocyanin concentration and the DPPH activity among non-sticky rice (A) and sticky rice (B) genotypes of purple rice genotypes (n = 12).
The response of purple rice to cooking differed between non-glutinous and glutinous rice genotypes. Even though, the difference of amylose content in non-glutinous and glutinous rice was found but the content of fat was not observed in this study. Therefore, the degradation of anthocyanin after cooking process is suggested to focus more in the content of amylose than fat. However, it would be interesting to increase number of rice genotypes from different original ecotype such as upland and wetland rice genotypes in the future study to confirm if the cooking process can be influence to the production of phospholipids modification and properties of the grain pericarp that can moderate water absorption and anthocyanin leaching.

In contrast to anthocyanin, the loss of antioxidant capacity in cooked non-glutinous rice was lower than in glutinous genotypes, suggesting that there could be higher concentration of the other antioxidants compounds than anthocyanin concentration in non-glutinous genotypes. There are different groups of bioactive compounds in purple rice. A number of studies have reported that phenolic acids and flavonoids showed higher antioxidant capacity (DPPH, ABTS and FIC assays) than anthocyanin in purple rice (Zhang et al, 2010; Surarit et al, 2015). It would be interesting to explore to what extent the non-glutinous genotypes used in our study contained insoluble forms linked to cell walls which are released on cooking as well as bound phenolic acid are distributed in the bran layer which are released on cooking and thus contribute to DPPH radical scavenging activity (Ryu and Koh, 2016).

Moreover, the cooking process can promote the degradation of anthocyanin after cooking process is suggested to focus more in the content of amylose than fat. Even though, the difference of amylose content in non-glutinous and glutinous rice was found but the content of fat was not observed in this study. Therefore, the degradation of anthocyanin after cooking process is suggested to focus more in the content of amylose than fat. However, it would be interesting to increase number of rice genotypes from different original ecotype such as upland and wetland rice genotypes in the future study to confirm if the cooking process can be influence to the production of phospholipids modification and properties of the grain pericarp that can moderate water absorption and anthocyanin leaching.

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Moreover, the cooking process can promote the

DPPH activity (31%) than the other methods (19%–25%).

### DISCUSSION

#### Effects of cooking process on anthocyanin concentration and antioxidant capacity of non-glutinous and glutinous rice genotypes

The cooking process depressed anthocyanin in purple rice thus reducing some of its antioxidant capacity. The response of purple rice to cooking differed between non-glutinous and glutinous genotypes. In general, a higher loss of anthocyanin was found in non-glutinous compared to glutinous genotypes. One factor that may influence the leaching of water-soluble compounds is their physical location in and properties of the grain pericarp and aleurone layer. Differences in grain chemistry, such as the lipid content, may also influence the degree to which antioxidant compounds may change during processing. For example, the cuticular layer can moderate water absorption and leaching in brown rice relative to peeled brown and white rice (Wu et al, 2016). Whether, glutinous rice has thicker pericarp and testa layers that are higher in lipid than non-glutinous rice remains to be determined. It is possible that the layer of bran thickness may be the critical factors affecting on the anthocyanin degradation as usually anthocyanin accumulate in the outer-most grain layer, while in the surface in some genotypes can also be occurred (Rerkasem et al, 2015). Notably, the fat content was double in glutinous rice with low in amylose compared to non-glutinous rice with high in amylose (Dutta and Mahanta, 2012). Even though, the difference of amylose content in non-glutinous and glutinous rice was found but the content of fat was not observed in this study. Therefore, the degradation of anthocyanin after cooking process is suggested to focus more in the content of amylose than fat. However, it would be interesting to increase number of rice genotypes from different original ecotype such as upland and wetland rice genotypes in the future study to confirm if the cooking process can be influence to the production of phospholipids modification and properties of the grain pericarp that can moderate water absorption and anthocyanin leaching.

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Moreover, the cooking process can promote the

#### Table 5. Anthocyanin concentration (ATC) and DPPH radical scavenging activity (DPPH activity) after cooking with different cooking methods of glutinous CMU125.

<table>
<thead>
<tr>
<th>Cooking method</th>
<th>Cooking type</th>
<th>ATC (mg/100 g)</th>
<th>%</th>
<th>DPPH activity (mg /100 g)</th>
<th>%</th>
</tr>
</thead>
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<tr>
<td>RR</td>
<td>WC</td>
<td>144 a</td>
<td>443 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-ERC</td>
<td>WC</td>
<td>14 bcd</td>
<td>90</td>
<td>136 b</td>
<td>69</td>
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<tr>
<td>0-PRC</td>
<td>WC</td>
<td>7 e</td>
<td>95</td>
<td>139 b</td>
<td>69</td>
</tr>
<tr>
<td>4-ERC</td>
<td>ST</td>
<td>14 bcd</td>
<td>90</td>
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<td>10 cde</td>
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<td>ST</td>
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<tr>
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<td>109 c</td>
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<tr>
<td>12-PRC</td>
<td>ST</td>
<td>7 e</td>
<td>95</td>
<td>96 c</td>
<td>78</td>
</tr>
</tbody>
</table>

Cooking method **
5% LSD 6 25

% Decreasing percentage; RR, Raw rice; ERC, Electric rice cooking; PRC, Pressure rice cooking; WC, Wet cooking; ST, Steaming.

0-ERC and 0-PRC represent wet cooking without previous soaking; 4-ERC and 4-PRC represent steaming with 4 h of previous soaking; 8-ERC and 8-PRC represent steaming with 8 h of previous soaking; 12-ERC and 12-PRC represent steaming with 12 h of previous soaking.

Different letters in the each row (NGR and GR) indicate significant difference among cooking methods (P < 0.05).

* and ** indicate significant difference at P < 0.05 and P <0.01, respectively.
partial migration of antioxidants from the outer bran fraction into the inner bran fraction of rice endosperm (Paiva et al., 2016). It is not known whether differences in the amylase content of non-glutinous and glutinous rice can affect the migration of phenolic acids and flavonoids. Nevertheless, the free phenolic acids increased in polished pigmented rice after parboiling (Paiva et al., 2016). Recently, it has been shown that antioxidant compounds, including phenolic acids and γ-oryzanol, were increased after steaming (Thammapat et al., 2016), however, no comparisons are available comparing the behavior of these compounds and bioactivities in non-glutinous and glutinous rice. Interestingly, higher phenolic acid levels have been measured in non-glutinous than in glutinous rice in some Indonesian and Korean pigmented rice (Surh and Koh 2014; Setyaningsih et al., 2015), suggesting non-glutinous rice may probably have greater increasing of phenolic acids during cooking. Further evidence is requiring in order determining the total phenols or flavonoids with a positive impact on antioxidant activity of pigmented grain.

Anthocyanins occur naturally as anthocyanidin aglycones and as anthocyanin glycosides, but can also be conjugated with other compounds such as aliphatic acid or phenolic acid. The presence of sugars and phenolic acids help to maintain anthocyanin stability (Andersen and Markham 2005). Hence, investigation of the anthocyanin chemistry within the Thai rice genotypes used in this study is worthwhile to pursue. The slightly greater stability of antioxidant capacity in cooked non-glutinous rice could also be a consequence of the hydrolysis of anthocyanin into phenolic acids (protocatechuic acid), one of the major anthocyanin degradation products as reported in cooked black rice (Bhawamai et al., 2016; Ryu and Koh, 2016). Therefore, it is possible that liberation of some phenolic acids from cell walls and anthocyanin uncoupling may compensate for some of the decrease in antioxidant capacity on cooking, contributing to observed differences between non-glutinous and glutinous rice genotype. The effect of cooking process on the degradation mechanism of anthocyanin and phenolic acid should be carefully identify and quantify in the further study.

**Effects of cooking method on anthocyanin concentration and DPPH activity of glutinous CMU125**

The decreasing was not observed proportionally between the anthocyanin and antioxidant capacity, which higher level of loss in the anthocyanin may due to the almost of anthocyanins are soluble form that located in the vacuole, especially higher in the inner bran fraction and not conjugate to cell walls, while the antioxidants such as phenolic acids and flavonoids are both soluble and insoluble forms (Goufo and Trindade, 2014), that lead to the almost anthocyanins are readily lost during cooking. Previous study reported on cherry that its anthocyanins are less stable compounds than phenolic acids when heating at 110 °C and variability in the degradation rate was observed among different compounds such as cyanidin-3-glucoside was the most unstable anthocyanin compared to cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside and the most stable phenolic acids were chlorogenic acid and ferulic acid (Zorić et al., 2014). Therefore, cooking temperature is very important to identify the suitable cooking method to maintain stable anthocyanin and phenolic acid in pigmented rice which is requiring further study.

The loss of anthocyanin and antioxidant capacity was reduced when the grain was cooked without presoaking. However, in Thailand soaking is a step in the production of parboiled rice that is widely consumed in some Middle East countries. Moreover, during the traditional process of parboiling, grain is subjected to soaking, steaming and drying resulting in a product that has advantage including higher milling yield, less stickiness and longer shelf-life. Nevertheless, cooking of parboiled grain lead to a greater loss of anthocyanin (88%–96%) compared to wet cooking, without preheating (60%–78%) (Min et al., 2014). Thus, it appears that all of the processes currently available for use before cooking significantly reduce the activity of antioxidants in the rice being consumed. However, the grain which make contact with the container margin when being processed may be exposed to higher heat loads than the grain further inside, hence it may be advantageous to use larger rice cookers to reduce the surface area of grain, especially in commercial situations. Further studies on rice cooker design and grain chemistry to enhance the retention of anthocyanin and other water soluble compounds in purple rice need to be conducted.

Use of a pressure rice cooker increased anthocyanin loss more than with an electric rice cooker, even though the former had a shorter cooking time.
Possibly, high pressure could destroy the bran structure and resulted in greater anthocyanin leaching, which was in accordance with some reports mentioned that high hydrostatic pressure (300 MPa) cause of changing in cell wall structure (Xia et al, 2017). In addition, a researcher showed that, anthocyanin in blueberry are highly sensitive to high temperature with pressure in comparison with individual high temperature (Buckow et al, 2010). However, the type of appliance did not affect DPPH activity. It seems that thermal condition consists of high pressure is the main factor that lead to greater anthocyanin degradation.

Wet cooking without presoaking lost less anthocyanin and antioxidant capacity than steaming, however, steaming glutinous grain improves the flavor and reduces the cooking time. In general, cooking attributes (e.g. optimum cooking time, water absorption, volume expansion ratio and total solids loss) contribute to rice texture (hardness and adhesiveness) and sensory appreciation of the consumer (Wu et al, 2016). Optimum conditions such as time, and amount of water and heat to use in the steamer are also important for the nutritional value of the rice and decisions need to be adjusted according to the rice genotype. There remains scope for defining cooking method with less thermal and pressure stress in order to retain higher anthocyanin and antioxidant capacity of purple rice to maximize its benefit to health conscious consumers.

CONCLUSIONS

Cooking methods significantly affected the anthocyanin and antioxidant capacity of non-glutinous and glutinous rice. The degree of anthocyanin and antioxidant capacity losses depended upon the cooking method used and the rice genotype. Cooking drastically decreased anthocyanin concentrations, especially in non-glutinous genotypes. In contrast, a higher loss of antioxidant capacity was found in glutinous genotypes. However, the present study concluded that consuming non-glutinous and glutinous purple genotypes by wet cooking method without presoaking could be the way to maximize dietary intake of antioxidants among rice consumers. Furthermore, wet cooking using an electric rice cooker without presoaking the grain lead to higher retention in both anthocyanin and antioxidant capacity than steaming the grain. Therefore, consideration should be given to the method of rice cooking particularly for purple genotypes with beneficial antioxidant compounds.

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REFERENCES


