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Morphological structure, starch fractions and starch digestibility of three pigmented rice cultivars cooked by microwave cooking

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ABSTRACT

Rice is the staple food that normally cooked before consumption. Rice cooking usually involving heating, influences on the microstructure and physicochemical property of grain. It would also affect the starch digestibility of cooked grain. This study aimed to investigate the effect of microwave cooking on the starch fractions, morphological damages and starch digestibility of cooked pigmented rice. Three cultivars of Thai pigmented rice, Hom Nin, Red Hommali and Kum Luempua, were used in this study. They were soaked in water at ratio 1:4.5 (rice:water, w/v) at 4°C for 19 hours. The soaked rice was cooked using microwave oven at 600W for 12 minutes, and then allowed to incubate at 30°C for 30 minutes to equilibrate moisture in the grain. The morphological structure of cooked grain was observed using fluorescent stereomicroscope. The moisture content, total starch content and resistant starch content were determined. The starch digestibility of cooked rice slurry during simulated *in vitro* digestion was also examined. The morphological structure of cooked grain revealed that cracks were found inside the grain during cooking and the bran layer of Hom Nin and Kum Luempua was more damaged than that of Red Hommali. Hom Nin showed significantly higher moisture content (%) (67.26±0.46) than Red Hommali (60.70±0.33) and Kum Luempua (60.54±1.19) ($P<0.05$). However, the total starch (%) and resistant starch content (%) found in Red Hommali was the highest (73.76±1.25; 0.18±0.03) followed by Hom Nin (71.44±0.75; 0.13±0.01) and Kum Luempua (71.23±2.09; 0.09±0.01), respectively. The starch hydrolysis (%) was increased during the simulated intestinal digestion process, in which Hom Nin obviously showed the highest value than the others. In conclusion, microwave cooking caused the morphological damages of grains with varying degree among cultivars which would affect to starch content.

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INTRODUCTION

Starch is known as a major carbohydrate in rice, accounts for approximately 90% (db.) (Arendt and Zannini, 2013). The starch digestion is a complex process that consists of different phase. This related to diffusion of pancreatic α-amylase enzyme into the starch substrate to hydrolyze starch into monosaccharide molecule (Lehmann and Robin, 2007). According to the rate and extent of starch digestion, they can be classified into three categories include rapidly digestible starch (RDS) defined as the amount of starch that is digested in the first 20 min, slowly digestible starch (SDS) defined as the amount of starch that is digested between 20 and 120 min, and resistant starch (RS) defined as the starch that escapes digestion in the small intestine (Dona et al., 2010). However, the rice starch has been reported that readily to be digested by enzyme after ingestion leading to high kinetic rate and glycemic index (GI) which relative increasing of blood glucose level and insulin secretion (Frei et al., 2003; Jung et al., 2009).

Numerous factors influence the starch digestibility including amylose/amylopectin content, processing/cooking method, degree of crystallinity, and type of starch component (Panlasigui et al., 1991). According to these factors, resistant starch has received much attention for influencing the starch digestibility due to its potential health benefits similar to soluble fiber. Resistant starch cannot be digested in upper gastrointestinal tract, but fermented by microflora in colon resulting in low starch hydrolysis rate and glycemic index which play an important role for anti-obesity and diabetic management (Mir et al., 2013). Resistant starch is generally classified into four types (RS1-RS4) depending on their physical and chemical characteristics (Mir et al., 2013). Among these types, the RS3 which defined as starch that has been processed and partially retrograded/re-crystalline structure, generally found in such kind of cooked starchy food like cooked rice, banana, and potatoes (Kim et al., 2006; Vatanasuchart et al., 2009). A study of Kim et al. (2006) also confirms that the occurring starch retrogradation of cooked rice during cooling could promote the formation of RS3. In addition, increasing of resistant starch has been found positively correlated with amylose content (Hu et al., 2004; Kim et al., 2006), because amylose molecules could easily aligning themselves during retrogradation (Sagum and Arcot, 2000). However, rice structure and their chemical properties changes during cooking/processing could affect the starch digestibility as well. For examples, the amylose-lipid complex formation during cooking (Parada and Santos, 2016) and the re-crystallinity of starch retrogradation (Frei et al., 2003), would increase resistant starch extent and reduce the starch digestibility.

The thermal cooking method also affects the change in morphological structure of rice kernel by developing internal crack of the grains during cooking leading to moisture absorption and destruction of cell wall structure (Tamura et al., 2014). Furthermore, our previous study (Thuengtung et al., 2018) also found that different of cooking condition and grain attribute would affect the starch hydrolysis and glycemic index of pigmented rice. Some previous study (Jaisut et al., 2008) demonstrated that thermal processing before cooking like drying treatment of paddy could also influence to decrease starch hydrolysis and glycemic index of cooked brown rice, by developing formation of the amylose-lipid complex during treatment. In addition to cooking method and processing, the different rice cultivars have been reported to significantly affect the change in cooked rice attributes, in particular, the morphological structure and physicochemical properties (Yadav et al., 2007). In the recent year, microwave cooking, one of thermal technique, has received interesting to apply in many food processing because less change in flavors and nutrition of foods, safe handling, easy operation, significant reduction in cooking time, and more uniform heating (Chandrasekaran et al., 2013; Vadivambal and Jayas, 2010). Zhong et al. (2013) who applied microwave treatment on brown and milled rice reveal that microwave treatment produced stress crack and some explosion in rice grain, notwithstanding, this treatment could preserve the composition in rice grain like free fatty acids during storage. Besides, the starch composition as well as other cooking qualities (i.e. color, flavor and water absorption capacity) of cooked rice by using microwave cooking has been reported comparable to conventional cooking (Khatoon and Prakash, 2007; Marsono and Topping, 1993).

In a world of rapidly changing food habits and stressful life styles, healthy digestive system is essential for overall quality of life. Recently, designing of healthy foods and cooking processes those contribute to a healthy digestive system are more developed. Among staple foods, pigmented rice that characterized as unpolished black, red, blue, or purple colored located in the pericarp and/or aleurone layers of rice (Kushwaha, 2016), received more interesting due to their health beneficial effects. Many previous studies (Abdel-Aal et al., 2006; Chatthongpisut et al., 2015; Yawadio et al., 2007) has been research on their phytochemicals and antioxidant activities, however, a few research on their starch properties and mechanism on starch hydrolysis during digestive system.

In this study, it was aimed to study the effect of microwave cooking method on the morphological structure, starch fractions, and starch digestibility of different cultivars of cooked pigmented rice.

MATERIALS AND METHODS

Chemicals and reagents

Pepsin (P7000, porcine gastric mucosal, ≥ 250 units/mg solid), pancreatin (hog pancreas, 4× USP), and invertase (Invertase, from baker's yeast, grade VII, ≥ 300/mg solid) were purchased from Sigma-Aldrich Ltd. (St. Louis, MO, USA). Amyloglucosidase (3260 U/ml) and pancreatic α-amylase (3000 ceralpha units/g) were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Formaldehyde solution and Lemosol reagent were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Immersion reagent (MP500) was purchased from Matsunami Glass Industries, Ltd. (Osaka, Japan).

Samples

Three cultivars of commercial Thai pigmented rice were used; cv. Hom Nin (black colored non-waxy rice) was purchased from Pensook Company, Bangkok, Thailand., cv. Red Hommali (red colored non-waxy rice), and cv. Kum Luempua (purple colored waxy rice) were purchased from Smile Rice Brand, Chaiyaphom, Thailand. All rice samples were stored at 4°C before analysis.

Sample preparation

Pigmented rice samples were soaked in water at a cooking ratio of 1:4.5 (rice:water, w/v) at 10°C for 19 h. All soaked rice samples were cooked using a microwave (Hitachi, MRO-DF6, Japan) at 600 W for 12 min. The cooked rice was compressed between two glass slides; no white core inside the grain was observed at cooked for 12 min, thus it was defined as completely cooked. They were then incubated at 30°C for 30 min to equilibrate the moisture in the grain. After incubation, cooked rice grain was divided into two portions. The first portion was determined moisture content, and prepared as slurry for *in vitro* digestion. The other was freeze dried by freeze dryer, ground, and sieved into the finest powder for total starch and resistant starch content analysis.

Observation of morphological structure of cooked rice grain

The observation of rice grain morphological structure was performed according to the previous study (Tamura and Ogawa, 2012). The cooked rice grain was immersed in 10% of formaldehyde for 10 min. The grain sample was then dehydrated by ethanol with varying concentration. The dehydrated grain was immersed in lemosol reagent for overnight before embedded into a paraffin block and sectioned by using a microtome (SM2000R; Leica, Wetzlar, Germany) at 20 µm of thickness. After sectioning, the remaining paraffin was removed by using lemosol reagent. The sample was placed on the glass slide and dropped by immersion reagent (MP500) before covered with a cover glass slide. Fluorescent stereomicroscope (MZ-FLIII; Leica, Wetzlar, Germany) was applied to observe the morphological structure of cooked rice grain, which were captured by digital camera (DS-5M; Nikon, Tokyo, Japan) attached on the microscope.

Determination of moisture content

Cooked rice, approx. 10 g, was placed in a moisture can and heated in an oven at 135±2˚C for 24 h. The moisture cans were allowed to cool in a desiccator and the moisture content in the sample was then calculated from the weight differences (AOAC, 1990). The measurement was performed in four replications.

Determination of total starch and resistant starch contents

The cooked rice samples were determined the total starch and resistant starch contents using the resistant starch assay kits (K-RSTAR 02/17, Megazyme International, Ireland). Cooked rice powder, 100±5 mg, was weighed directly into a screw cap tube. Samples were then incubated with 4 ml of pancreatic α-amylase (10 mg/ml) containing amyloglucosidase (3 U/ml) at 37°C in a shaking water bath. After 16 h of incubation, an equal volume of ethanol (99%, v/v) was added to terminate enzyme reaction and resistant starch was precipitated by centrifugation. The pellet was re-suspend twice in ethanol (50%, v/v) followed by centrifugation. The supernatant after each centrifugation step was carefully separated from the pellet. Resistant starch in the pellet was dissolved in 2M KOH with stirring in an ice-water bath and neutralized with 1.2M sodium acetate buffer. The starch solution was then hydrolyzed to glucose with amyloglucosidase and measure with glucose oxidase/peroxidase (GOPOD) reagent. The result was calculated and converted to resistant starch content. In addition, total starch content was calculated by sum of resistant starch and non-resistant starch content. Non-resistant starch was determined by pooling the supernatant after centrifugation, adjusting the volume to 100 ml and measuring glucose content with GOPOD reagent. The analysis was performed in five replications.

In vitro starch digestion

Two stages of *in vitro* digestion included simulated gastric digestion and simulated small intestinal digestion were performed according to the method of Dartois et al. (2010). Cooked rice slurry (contained 4% of starch concentration) was added into the reactor which the temperature was maintained at 37°C. The gastric fluid solution containing pepsin was added to start the simulated gastric digestion by maintaining the pH at 1.2. The supernatant was sampling at 0, 5, and 30 min of simulated gastric digestion in triplicate. After that, the digestive system was transformed to simulated small intestinal digestion by adjusting the pH to 6.0. The small intestinal fluid solution containing invertase, amyloglucosidase and pancreatin was then added into the reactor and adjusted the pH to 6.8. The supernatant was sampling at 5, 10, 15, 30, 60, 90, and 120 min during simulated small intestinal digestion in triplicate. The supernatants were mixed with 95% ethanol to terminate enzymatic reaction. The mixed solutions were centrifuged at 1800g for 10 min. The supernatant was collected and incubated with invertase/amyloglucosidase solution at 37°C for 10 min. The glucose content was then determined using the D-glucose assay kits (GOPOD Format K-GLUK 07/11, Megazyme International, Ireland). The result was calculated and expressed as starch hydrolysis (%) as below;

$$\%SH = Sh / Si$$

$$= 0.9 \times Gp / Si$$

where %SH is the percentage of starch hydrolysis, Sh is the amount of hydrolyzed starch, Si is the initial amount of starch, and Gp is the amount of produced glucose. A conversion factor of 0.9, which is generally calculated as the ratio of the molecular weight of the starch monomer to the molecular weight of glucose (162 / 180 = 0.9), was used (Goňi et al., 1997).

The kinetics of starch hydrolysis was also calculated according to first order equation model of Goni et al. (1997) as following;

$$C = C_{\infty}(1 - e^{-kt})$$

where k is the kinetic constant, t is time (min), C is corresponds to percentage of hydrolyzed starch at time t, C∞ is equilibrium concentration of starch in the simulated gastro-small intestinal digestion process.

Statistical analysis

Data were expressed as means ± standard deviation. The data were also subjected to analysis of variance (ANOVA) among mean by Duncan's multiple range tests using SPSS 20.0. The significance level of $P<0.05$ was considered significantly different. The kinetic constant and equilibrium of starch hydrolysis were tested by using Igor Pro 4.01 (Hulinks Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

Morphological structure of cooked pigmented rice

According to Figure 1, the grain damages were indicated by the visible cell wall disruption. The cracks of cooked grain were observed in all cultivars. This could be affected by the penetration of water from external surface into internal cell of cooked rice grain, and gelatinization facilitated by high heating temperature during cooking (Tamura et al., 2014). Furthermore, some cracks were produced along the edge of cooked rice pericarp, and there was explosion or fracture trough some part of the outer layer. In general, microwave heating occurred in food materials absorbs the microwave energy and convert to heat, resulting to moisture inside the grain moves out to the surface (Chandrasekaran et al., 2013). However, the pericarp layer would be a barrier for emanation of the moisture, caused the explosion and fracture in some outer layer of cooked rice (Zhong et al., 2013).

Comparison between rice cultivars found that the bran layer of cooked Red Hommali cultivar (Figure 1b) was less disrupted than cooked Hom Nin and Kum Luempua cultivars (Figure 1a, c) despite they were subjected to the same soaking and cooking condition. This may indicate that rice cultivar affect cooked rice characteristics namely thickness and/or robustness of rice bran layer.

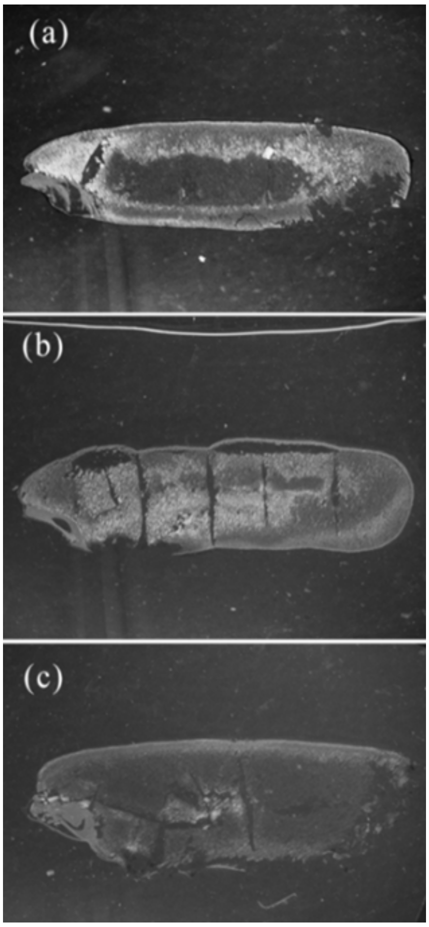

Figure 1. The morphological structure of cooked pigmented rice grain included Hom Nin cultivar (a), Red Hommali cultivar (b), and Kum Luempua cultivar (c), respectively.

Starch fractions of cooked pigmented rice

The moisture content (%), total starch content (%), and resistant starch content (%) of each pigmented rice after microwave cooking are shown in Table 1. Hom Nin cultivar showed significant highest in moisture content compared to the others ($P<0.05$). This would be related to the amount of water migration into the internal tissue of rice grain during soaking and cooking. As high temperature of cooking process, water could migrate rapidly from external toward the internal grain leading to produce the cracks, large void space, and increasing of moisture inside the grain (Horigane et al., 1999; Kasai et al., 2007). Hence, significantly higher moisture content (%) in Hom Nin cultivar could be affected by large amount of water migration to internal grain which observed as a larger void space produced in the central region of cooked grain.

Total starch content (%) and resistant starch content (%) were mostly found in Red Hommali cultivar, followed by Hom Nin and Kum Luempua, respectively ($P<0.05$), despite no significant difference ($P>0.05$) in total starch content (%) of Hom Nin and Kum Luempua cultivars (Table 1). A previous study (Yang et al., 2016) reported that the starch content would be easily leached out from the internal rice tissue during high temperature of cooking due to large fracture of cell wall structure at the exterior surface. This result indicated that leaching of starch material corresponded to cooked rice morphology. Therefore, less disruption of exterior layer of Red Hommali could more preserve the total starch content in cooked rice grain. In addition, Red Hommali and Hom Nin cultivars contained higher amylose content than Kum Luempua cultivar (Thuengtung et al., 2018) which might affect carbohydrate-protein interaction, starch retrogradation, and amylose-lipid complex formation, leading to an increase in the resistant starch content (Guha et al., 2011). After cooking and cooling of rice, the linear chains of amylose content encouraged the cross-linkage through the hydrogen bonds for re-crystallization process, resulting in the increment of resistant starch. Meanwhile, the branch chains of amylopectin would delay the re-crystallization (Singh et al., 2010).

Changes in starch hydrolysis of cooked pigmented rice during *in vitro* digestion

Figure 2 shows the changes in the starch hydrolysis (%) of cooked Hom Nin, Red Hommali, and Kum Luempua cultivars. The starch was not digested during 30 min of simulated gastric digestion because of the absence of pancreatic α-amylase enzyme. Meanwhile, the pancreatic α-amylase contained in the simulated small intestinal fluid solution can hydrolyze the starch into glucose and oligosaccharides (Dona et al., 2010), leading to outstandingly increase of starch hydrolysis during simulated small intestinal digestion.

The kinetics of starch hydrolysis could also be described by the kinetics constant and the completion of starch hydrolysis value. In this study, first order equation of exponential model described by Goni et al. (1997) was applied for calculation because this equation can describe closely the digestion of both cooked and raw grain starch (Dona et al., 2010). Table 2 shows that the kinetics constant (k) of all cooked rice cultivars during *in vitro* digestion were not significant different ($P>0.05$). The starch hydrolysis reached the equilibrium within 120 min of simulated small intestinal digestion and the equilibrium percentage of starch hydrolysis (C_{∞}) is shown in Table 2. Red Hommali cultivar showed the significantly lowest equilibrium percentage of starch hydrolysis (C_{∞}), followed by Kum Luempua and Hom Nin, respectively ($P<0.05$). Higher resistant starch content in Red Hommali cultivar might be one of several factors to encourage retarding of starch hydrolysis (Shi and Gao, 2011). However, a recent study (Shumoy and Raes, 2017) reported that the starch fraction by itself like resistant content or slow digestible starch could not always used to predict the starch hydrolysis, but the extent of starch hydrolysis also affected by type of crystallinity (A or B type), arrangement of crystalline and amorphous regions in starch granule, the structure of amylose and amylopectin, and size of blocklet that contains both crystalline and amorphous lamella. The smaller size of blocklet indicated high susceptibility of starch hydrolysis by related to the arrangement of crystalline and amorphous region (Baker et al., 2001; Tang et al., 2006), which A type crystal native starch has been reported more susceptible to enzymatic hydrolysis than B type crystal native starch (Shumoy and Raes, 2017). Moreover, a previous study (Kang et al., 2011) has been revealed that black pigmented rice varieties exhibited an A type crystalline structure. Consequently, this may influence why Hom Nin that belongs to a group of black pigmented rice had the highest equilibrium starch hydrolysis among the three cultivars.

Table 1. The moisture content (%), total starch content (%), and resistant starch content (%) of cooked three cultivars of pigmented rice.

Rice cultivar	Moisture content (%)	Total starch content (%)	Resistant starch content (%)
Hom Nin	67.26±0.46^{a}	71.44±0.75^{b}	0.13±0.01^{b}
Red Hommali	60.70±0.33^{b}	73.76±1.25^{a}	0.18±0.03^{a}
Kum Luempua	60.54±1.19^{b}	71.23±2.09^{b}	0.09±0.01^{c}

The results were expressed as mean±SD (n=4-5). The different letters in the same column indicate significant difference (P<0.05).

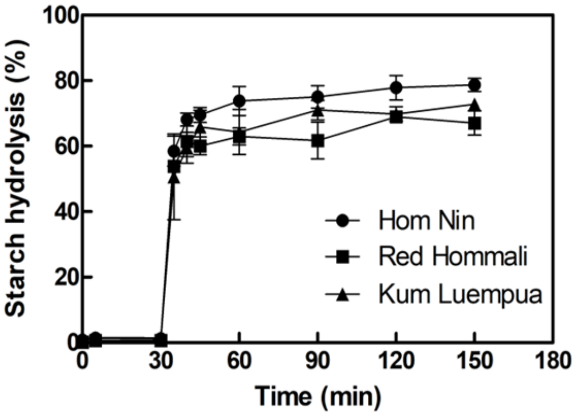

Figure 2. The starch hydrolysis (%) of cooked pigmented rice slurry during simulated *in vitro* digestion.

Table 2. The equilibrium percentage of starch hydrolysis (C_∞) and kinetics constant (k) of cooked pigmented rice slurry.

Rice cultivar	C_∞ (%)	k (min^{-1})
Hom Nin	75.08±2.38^{a}	0.28±0.01^{ns}
Red Hommali	64.28±1.78^{c}	0.35±0.07^{ns}
Kum Luempua	68.56±1.75^{b}	0.28±0.08^{ns}

The results were expressed as mean±SD (n=3). The different letters in the same column indicate significant difference (P<0.05). nssuperscript means no significant difference (P>0.05).

CONCLUSION

The results indicated that disruption of morphological structure of cooked rice caused by microwave cooking could relate to their moisture content with varying degree among cultivars. Less structural bran layer disruption of Red Hommali cultivar could more preserve their starchy component in cooked grain. Red Hommali cultivar showed the highest resistant starch content and lowest starch hydrolysis than the others, despite no significant difference in kinetic rate constant of digestion. This study could serve as baseline information for consumers to have such an alternative on selection of rice consumption. However, the estimated glycemic index of these three cultivars and the comparison of microwave cooking with conventional cooking would be conducted for further analysis.

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