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The utilization of ultrasound and chilling treatment to reduce GI in Thai glutinous rice (RD6)

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Abstract The source of carbohydrate in the North and North-Eastern areas in Thailand are predominantly based on glutinous rice which high glycemic index (GI75-92). Lower GI glutinous rice was interesting prospect to develop diabetics. There are modified methods to reduce GI by limit the accessibility of the digestive enzymes on starch molecule. The most common physical modification method used for reducing GI on starch are hydrothermal and gelatinization-retrogradation methods. Ultrasound is the sound wave at frequency exceeding audible threshold of the human hearing rang. It is studied on starch to change the molecular structure for improving some physicochemical properties. Therefore, this resecrch focused on the utilization of ultrasound and chilling treatment to reduce GI in Thai glutinousonrice (RD6). The glutinous rice was treated with ultrasound for 15 and 30 min and amplitude at 40, 70, and 100%. All of the ultrasound-treated rice was stored at 4 ^{o}Cfor 24 h. Then, thery were analyzed on the ratio crystalline to amorphous by FTIR, thermal properties by DSC, RVA pasting properties, and GI. With increasing time and amplitude of ultrasound, the ratio of crystalline to amorphous decreased from 0.779 to 0.662. The onset temperatureand enthalpy (ΔH) decreased from 62.38 to 58.10 ^{o}C, and 1.81 to 0.70 J/g, respectively. The peak viscosity, and final viscosity from RVA increased from 3079 to 3838.67 cP and 2407.33 to 2922 cP, respectively. When increasing time and amplitude of ultrasound, the hydrolysis index (HI) and eGI slightly increased with longer time and higher amplitude than the others. The chilled samplesafter ultrasound treatment showed that the ratio of crystalline to amorphous, and ΔH increased while HI and eGI decreased when compared to unchilled ultrasound treated rice.

Keywords: glutinous rice, ultrasound treatment, chilling, and glycemic index

Introduction

The glycemic index (GI) is a system that ranks food, particularly carbohydrate-based, on their actual postprandial blood glucose responsecompared with a reference, usually white bread or glucose. (Jenkins *et al.*, 1981; Miller *et al.*, 1992; Sugiyama *et al.*, 2003; Atkinson *et al.*, 2008). There are divided into three groups; low GI (≤55), medium GI (55-69), and

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high GI (≥70) (Foster-Powell *et al.*, 2002). GI is one important quality characteristics of rice. The low GI rice is recommended to improved blood glucose control by lowing the levels of blood glucose and the risk of type 2 diabetes (Shafaeizadeh *et al.*, 2018). Long-term intake of low GI foods was reported to associate with the reduced incidence and prevalence of heart disease, and diabetes (Brand-Miller, 2007; Jenkins, 2007; Roberts, 2000; Wolever&Mehling, 2002).

Rice (*Oryza sativa* L.) is one of the leading food crops of the world and is the staple food of more than half of the world's population. Thousands of rice varieties are available throughout the world. Glutinous rice (*Oryza sativa var. glutinosa*) is one of the most popular varieties. Normally known as sticky or waxy rice, its appearance are soft texture and sticky when cooked because contains low amount of amylose(<5% of amylose content) (Kadan, 1997; Guo *et al.*, 2015). Generally, glutinous rice used in fried cake, sliced cake, dessert, and they are also served as the staple food in Southeast Asia (Gao *et al.*, 2014). In North and North-Eastern area of Thailand, the glutinous rice is used for daily energy intake. Glutinous rice is presented a high glycemic index in range 75-92 (Frei *et al.*, 2003; Guo *et al.*, 2015), that depends on the ratio of amylose and amylopection, glutinous cultivars, growing zone (Wani *et al.*, 2012). The glutinous rice with low amylose content had high GI value as amylose content has a negative correlation with GI value (Denardin *et al.*, 2012). As a result, glutinous rice makes large contributions to glycemic index reflecting the blood glucose-raising potential from diet, which is presented in glycemic index (GI).

Generally, the methods have been used to reduce the glycemic index such as chemical modification (Zieba *et al.*, 2010), enzymatic modification (Berry, 1986; Guraya, James, & Champagne, 2001; Shin *et al.*, 2004; Shin, Kim, Ha, Lee, & Moon, 2005), physical modification (autoclaving) (Dundar & Gocmen, 2013), and hydrothermal treatment (Chung *et al.*, 2009).The suitable method used for modifying starch to reduce GI on food is the physical methods due to it is simple and health-safe (Zia-ud-Din *et al.*, 2017).The ultrasound is a physical method using mechanical waves with a frequency above the threshold of human hearing (>16 kHz.). Ultrasound is a highly effective means for the physical modification, which is a green technology. It shows beneficial effects in food process that includes shorter processing time and gaining higher product yields. Ultrasound wave could modify the composition, structure, properties and change morphologyof starch depending on frequency and intensiy of ultrasound (Flores-Silva *et al.*, 2017). It affected on the properties, and compositions of starch including solubility and swelling power, viscosity (Jambrak *et al.*, 2010; Sit *et al.*, 2014; Pinto *et al.*, 2015) and gelatinization temperature (Cui *et al.*, 2010; Pinto *et al.*, 2015; Zhu, 2015). It also increased the amount of linear chains by debranching amylopectin (Wang & Wang, 2004; Lu *et al.*, 2018).Therefore, those researches indicated that ultrasound treatment could induce important change on physicochemical properties of starch granule. Thus, it may be applied for obtaining lower GI. Furthermore, the storage rice with cool temperature might occur more ordered structure of the rice grains formed during temperature storage. It would be associated with the starch molecules to reassociate into compact structure (Wang *et al.*, 2015).

Therefore, the objective of this research was to study the effects of ultrasound treatment on some physicochemical properties (thermal properties, pasting properties) and glycemic index of Thai glutinous rice. The chilling treatment was also applied to rice after treating with ultrasound for enhancing lower GI.

Materials and methods

Grains were used as a cultivar RD6 which amylose content of 7.04% db. The glutinous rice was obtained from Ubonratchathani province. Pancreatic α-amylase (EC 3.2.1.1., 3000 U/g), amyloglucosidase (EC 3.2.1.3., 102 3300 U/mL) and glucose assay kit (GOPOD method) were purchased from Megazyme International Ireland Ltd.

The glutinous rice samples were treated with ultrasound using ultrasonic bath (WUC-D10H, Wisd, Daihan scientific, Korea) for 15 and 30 min with different amplitude levels at 40%, 70%, and 100% of ultrasonic power (400 W, 40 KHz). For ultrasonic treatment, 500 g of glutinous rice sample put in a wire basket was immersed in 3 L of water in the chamber at room temperature ($30 \pm 1^{o}C$). For chilling samples, after treating with ultrasound, rice was kept at $4^{o}C$ for 24 h and then, all of samples were dried at $40 \pm 1^{o}C$ for 8 h to reduce the moisture content $11 \pm 1\%$ wb. The dried rice samples were ground into flour with a pin mill (ZM-200, Retsch, America) fitted with a 0.25 mm sieve and screen by 160 µm sieve. The glutinous rice flour samples were used for analysis.

The treatments were divided into two groups. The first one was the glutinous rice treated with ultrasound. Another one was treated with ultrasound and chilling process. The code of the independent variables studied in this research are explained in Table 1.

Fourier transform Infrared (FTIR) spectroscopy: FTIR spectroscopy was performed on a FTIR spectrophotometer (NICOLET 6700, Thermo scientific, Germany). All glutinous rice flour samples were scanned in a range from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}, with 36 co-added scans per sample. The ratio of absorbance at 1045 to 1022 cm^{-1} was calucalated to represent the crystalline region and the ratio of absorbance at 1022 to 995 cm^{-1} represented amorphous region.

Table 1. The codes of independent variables used in the study

Treatments	Ultrasound conditions		Code
	Time (min)	Amplitude (%)	
Native			Native
Ultrasound treated (U)	30	40	U1
		70	U2
		100	U3
	15	40	U4
		70	U5
		100	U6
Ultrasound treated and chilled at 4°C for 24 h. (UCH)	30	40	U1CH
		70	U2CH
		100	U3CH
	15	40	U4CH
		70	U5CH
		100	U6CH

Thermal properties: the gelatinization parameters of glutinous rice flour were measured using a differential scanning calorimer (DSC 2 module, Mettler Toledo, Switzerland). Approximately 3 mg of rice flour samples and 9 µL of deioniaed water were added into DSC sample pans. The pans were sealed and equilibrated overnight at room temperature before heating in the DSC. For gelatinization, measurements were carried out at a heating rate of 5 °C/min from 20 to 120 °C. An empty pan was used as a reference.

The pasting properties of the glutinous rice flour samples were determined using a Rapid Visco Analyser (RVA) (RVA-4, Newport Scientific, Australia) according to Approved Method 61-02 (AACC, 2000). Rice flour slurry was prepared by mixing 3 g of rice flour and 25 mL of distilled water in aluminum canister. The slurry was heated from 50 to 95°C at a rate of 3 °C/min. The initial speed was 960 rpm for first 10 sec to thoroughly mix the slurry and the test speed was 160 rpm. Parameters of pasting properties including pasting temperature (°C), peak viscosity (cP), breakdown (cP), final viscosity (cP), and setback (cP) were determined.

The hydrolysis index (HI) and expected glycemic index (eGI) were analyzed according to Megazyme Resistant Starch Assay Kit (AOAC, 2000). The grounde samples 100±5 mg was added with 4.0 mL of pancreatic α-amylase into screw cap tupe and then incubated at 37°C in shaking water bath for 30, 60, 90, 120, 150, and 180 min. The samples were removed from water bath, washing twice with ethanol (50%) and the supernatants were combined, and their glucose content was measured using glucose oxidase-peroxidase kit. Each treatment was analyzed in duplicate.

A non-linear model established by Goñi *et al.* (1997) was applied to describe the kinetics of starch hydrolysis. The first order equation was$C = C_\infty (1 – e^{-kt})$, where C, C_∞, and k were the percentage of starch hydrolyzed at time t (min), the equilibrium percentage of starch hydrolyzed after 180 min, and the kinetic constant, respectively. The hydrolysis index (HI) was calculated by dividing the area under the hydrolysis curve (0-180 min) of each sample by the corresponding area of a reference sample (white bread). eGI was calculated using the equation: eGI = 39.71+.549HI (Goñi *et al.*, 1997).

The experimental data were analyzed using SPSS for window (Statistical Package for the Social Sciences). The means were compared using Duncan's multiple comparison with a confidence level of 95% to perform the analysis of variance (ANOVA).

Results

The FTIR spectra of the native and ultrasound treated glutinous rice samples are shown in Fig 1. The obvious IR patterns of the native and ultrasound treated glutinous rice were presented the characteristic absorption patterns within a frequency band at 400-4000 cm^{-1}. The IR patterns of ultrasound treated glutinous rice samples were not changed as compared with the native. The ratios of absorbance at 1047/1022 cm^{-1} and 1022/995 cm^{-1} from FTIR spectra as shown in Table 2 associated with ordered (crystalline) and amorphous region, respectively. All the glutinous rice samples did not show significant differences in the ratio of absorbance at 1047/1022 cm^{-1} and 1022/995 cm^{-1}. However, both ratios of ultrasound treated samples tended to decrease as compared with the native. Moreover, the increasing amplitude levels of ultrasound treatment showed that the values of the ratio of absorbance 1045/1022 cm^{-1} slightly decreased for both ultrasound time, while, the ratio of absorbance 1022/995 cm^{-1}slighltly increased to the range 0.857 to 0.887 for 15 min of ultrasound time. For ultrasound-and-chilled rice samples, the ratio of absorbance 1022/995 cm^{-1} decreased as compared with ultrasound treated rice samples.

The thermal properties of the native, ultrasound treated, and ultrasound-and-chilled treated glutinous rice samples are shown in Table 3. The To, Tp, Tc, and ΔH of the native was 62.38 ^{o}C, 69.03 ^{o}C, 75.53 ^{o}C and 1.81 J/g, respectively. The ultrasound treated samples showed To (58.10-60.42^{o}C), Tp (68.06-68.73^{o}C), Tc (74.26-74.67^{o}C), and ΔH (0.7-1.43 J/g) which were lower than those of the native. The To and ΔH of ultrasound treated samples were higher in the rice samples prepared for 30 min of ultrasound treatment as compared with the rice samples prepared for 15 min of ultrasound treatments. For ultrasound-and-chilled rice samples, it had ΔH higher than that of ultrasound treated samples.

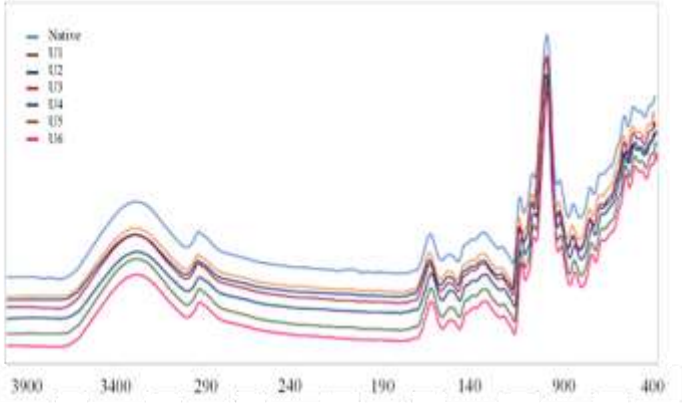

Figure 1. FTIR spectra of the native and ultrasound treated glutinous rice samples

Table 2. The ratio of absorbance at 1045/122 cm^{-1} and 1022/995 cm^{-1}of the native, ultrasound treated, and ultrasound-and-chilled treated glutinous rice

Samples	1045/1022^{ns} (cm^{-1})	1022/995^{ns} (cm^{-1})
Native	0.779±0.001	0.908 ±0.128
U1	0.671±0.002	0.857±0.063
U2	0.662±0.001	0.880±0.073
U3	0.664±0.001	0.887±0.073
U4	0.680±0.001	0.873±0.054
U5	0.665±0.001	0.851±0.069
U6	0.665±0.001	0.851±0.069
U1CH	0.672±0.001	0.837±0.014
U2CH	0.662±0.192	0.834±0.002
U3CH	0.667±0.005	0.847±0.001
U4CH	0.682±0.011	0.834±0.002
U5CH	0.666±0.001	0.839±0.002
U6CH	0.661±0.001	0.841±0.003

Values are means of triplicate measurement ± standard deviation.

^{ns} Non-significantly different at 95% confidence level in the same column.

The pasting properties of the native, ultrasound treated, and ultrasound ultrasound-and-chilled treated glutinous rice samples are shown in Table 4. The ultrasound treated rice presented higher pasting temperature, peak viscosity, breakdown, final viscosity than the native, while its setback decreased. The peak viscosity tended to increase when the amplitude levels at 40 to 70% for 30 min of ultrasound. The pasting temperature, peak viscosity, breakdown, final viscosity and setback were in the ranges of 67.28-69.35 ^{o}C, 2922.67-3244.00 cP, 917.00-1188.33, 2481.00-2612.33 cP and 465.00-497.33 cP, respectively. The pasting temperature increased while peak viscosity, breakdown, final viscosity, and setback decreased when the the rice was chilled after ultrasound treated.

Table 3. Thermal properties and crystallinity of the native, ultrasound treated, and ultrasound-and-chilled treated glutinous rice

Samples	**Gelatinization temperature**			**ΔH**
	T_o (^{o}C)	**T_p (^{o}C)**	**T_c (^{o}C)**	**(J/g)**
Native	62.38^{a} ±1.54	69.03^{a} ±0.19	75.53^{a} ±0.32	1.81^{a} ±0.19
U1	60.42^{b} ±0.40	68.06^{b} ±0.25	74.59^{b} ±0.25	1.43^{b} ±0.08
U2	60.18^{b} ±0.04	68.73^{ab} ±0.48	74.67^{b} ±0.44	1.04^{cd} ±0.04
U3	60.42^{b} ±0.40	68.21^{ab} ±0.00	74.57^{b} ±0.02	0.96^{c-f} ±0.01
U4	58.10^{b} ±0.27	68.06^{b} ±0.25	74.59^{b} ±0.25	0.86^{d-f} ±0.04
U5	58.76^{c} ±0.37	68.73^{ab} ±0.48	74.67^{b} ±0.49	0.74^{ef} ±0.01
U6	58.10^{c} ±0.27	68.56^{ab} ±0.12	74.26^{b} ±0.95	0.70^{f} ±0.30
U1CH	60.10^{b} ±0.47	68.88^{c} ±0.01	74.85^{c} ±0.32	1.16^{c} ±0.47
U2CH	62.03^{a} ±0.98	68.82^{c} ±0.01	75.42^{c} ±0.45	1.84^{a} ±0.02
U3CH	62.02^{a} ±0.18	68.60^{c} ±0.18	75.34^{c} ±0.03	1.99^{a} ±0.01
U4CH	60.37^{b} ±0.11	68.51^{d} ±0.08	75.35^{c} ±0.00	1.93^{a} ±0.01
U5CH	58.71^{c} ±0.33	68.55^{d} ±0.00	75.00^{c} ±0.05	1.80^{a} ±0.12
U6CH	58.54^{c} ±0.49	68.55^{c} ±0.00	74.12^{c} ±0.11	1.00^{c-e} ±0.01

Values are mean of triplicate measurements ± standard deviation; Mean values in the same column with different letters are significantly different (p<0.05).

The hydrolysis index (HI) and expected glycemic index (eGI) of the native, ultrasound treated rice, and ultrasound-and-chilled treated glutinous rice samples are shown in Table 5. The HI and eGI of the native were 75.58 and 81.08, respectively. The HI and eGI of ultrasound treated rice increased as compared with the native, especially the rice samples prepared for 30 min of ultrasound treatment. The HI and eGI values of ultrasound treated rice were in the ranges of 71.26-77.38 and 79.84-83.64, respectively. Moreover, ultrasound time treatments showed eGI increased by increasing time of ultrasound. The highest eGI value (83.64) was found in ultrasound treated rice for 30 min with 100% amplitude of ultrasound. Also, at all ultrasound times the eGI was higher in the ultrasound treated rice at amplitude level 100% of ultrasound power as compared with the amplitude levels at 40% and 70% of ultrasound power. Furthermore, ultrasound and chill-treated glutinous rice had lower HI (64.89-70.76) and eGI (78.56-75.34) which was significantly different (p<0.05), as compared with the ultrasound treated rice.

Table 4. The pasting properties of the native, ultrasound-treated, and ultrasound-and-chilled treated glutinous rice

Samples	Pasting temp (^{o}C)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
Native	65.95^{c-f} ±0.91	3079.00^{f} ±87.469	1186.33^{de} ±42.193	2407.33^{g} ±57.29	514.66^{ab} ±10.96
U1	66.48^{b-e} ±0.10	3493.00^{b} ±59.56	1240.67^{cd} ±21.57	2745.00^{bc} ±23.30	492.67^{c} ±57.18
U2	66.52^{b-e} ±0.78	3894.33^{a} ±9.27	1419.67^{a} ±35.81	2891.67^{a} ±3.79	417.00^{e} ±25.24
U3	66.22^{c-f} ±0.93	3532.33^{b} ±107.97	1307.67^{bc} ±56.50	2787.67^{b} ±60.54	563.00^{ab} ±12.77
U4	65.92^{d-f} ±0.83	3419.00^{bc} ±20.95	1226.00^{cd} ±50.47	2638.00^{cd} ±19.70	445.00^{cd} ±42.29
U5	64.65^{f} ±1.21	3346.67^{cd} ±41.88	1253.33^{cd} ±60.62	2562.00^{c-e} ±44.19	472.67^{cd} ±68.13
U6	65.40^{ef} ±0.43	3838.67^{a} ±47.65	1387.00^{ab} ±26.00	2922.67^{a} ±30.53	471.00^{cd} ±35.04
U1CH	67.77^{b-e} ±1.16	3177.67^{ef} ±132.27	1069.67^{f} ±88.75	2559.67^{c-e} ±87.18	497.00^{bc} ±55.32
U2CH	69.35^{a} ±0.43	3244.00^{de} ±44.58	1117.67^{ef} ±41.88	2612.33^{cd} ±7.51	486.00^{cd} ±9.85
U3CH	67.60^{bc} ±0.87	3061.67^{f} ±79.03	1188.33^{de} ±65.77	2489.00^{e} ±38.97	570.33^{a} ±21.55
U4CH	67.87^{ab} ±0.49	3156.00^{ef} ±50.69	1077.00^{f} ±38.97	2544.00^{de} ±63.59	465.00^{cd} ±26.15
U5CH	67.28^{b-d} ±0.80	3133.67^{ef} ±14.47	917.00^{g} ±72.55	2583.00^{ef} ±13.45	475.33^{cd} ±17.62
U6CH	68.03^{ab} ±1.33	2922.67^{g} ±59.81	1048.00^{f} ±39.28	2481.00^{cd} ±37.99	497.33^{bc} ±37.69

Values are mean of triplicate measurements ± standard deviation; Mean values in the same column with different letters are significantly different (p<0.05).

Table 5. The hydrolysis index (HI) and expected glycemic index (eGI) of the native, ultrasound-treated, and ultrasound and chill-treated glutinous rice

Samples	**HI**	**eGI**
Native	75.58^{ab} ±0.38	81.08^{bc} ±0.14
U1	73.09^{bc} ±1.33	80.64^{bc} ±0.76
U2	73.56^{bc} ±1.72	79.84^{cd} ±0.73
U3	77.38^{a} ±1.46	82.19^{ab} ±0.80
U4	71.26^{cd} ±0.58	82.20^{ab} ±0.80
U5	75.58^{ab} ±0.38	81.92^{ab} ±0.80
U6	76.10^{ab} ±0.15	83.64^{a} ±1.80
U1CH	69.33^{de} ±0.24	77.773^{e} ±0.1
U2CH	70.76^{cd} ±0.56	78.56^{de} ±0.31
U3CH	67.83^{ef} ±0.75	76.94^{df} ±0.41
U4CH	64.89^{f} ±0.81	75.34^{f} ±0.44
U5CH	65.42^{f} ±1.92	75.62^{f} ±1.05
U6CH	65.27^{f} ±0.41	75.54^{f} ±0.23

Values are mean of duplicate measurements ± standard deviation; Mean values in the same column with different letters are significantly different ($p<0.05$).

Discussion

The FTIR spectra presented bands with associated to stretch, flexion, and deformation corresponding to the main functional groups characteristic of the polymer of rice flour (Monroy *et al.*, 2018). For the native and ultrasound treated rice, the IR spectrum was presented in region 400-4000 cm^{-1}.The characteristic absorption peak of ultrasound treated rice were not changed as compared with that of the native.The similar results were reported by Bai *et al.* (2017). The wide band presented at 3300-3400 cm^{-1} that corresponded to vibration of OH group (Flores-Silva *et al.*, 2017). The intensity band at 2923 cm^{-1} which can be attributed to C-H vibration, absorption band of fat and the band at ~1532 cm^{-1} (amide II). The band at 1643 cm^{-1}, 1344 cm^{-1}, and 997 cm^{-1} were corresponded to the stretching vibration of C-O bond, C-O-H and C-O-C group, respectively. The band at 900-1300 cm^{-1} corresponding mainly to C-O and C-C stretching vibration (Warren *et al.*, 2016; Monroy *et al.*, 2018). Furthermore, the ratio at 1045/1022 cm^{-1} associated with crystalline, while the ratio at 1022/995 cm^{-1} associated with amorphous region (Wang *et al.*, 2015). The ratio of absorbance 1045/1022 cm^{-1} and 1022/995 cm^{-1} of ultrasound treated rice showed that they were not significantly different ($p>0.05$) as compared with the native. However, the ratio at 1045/1022 cm^{-1} and 1022/995 cm^{-1} of the ultrasound treated rice samples slightly tended to decrease as compared with the native. This results indicated that the crystalline region disrupted and it promoted proportion of amorphous to crystallinity region by action of ultrasound (Flores-Silva *et al.*, 2017; Monroy *et al.*, 2018). Similar result was reported by Monroy *et al.* (2018). However, the factors of ultrasound time and amplitude did not apparently affect the ratio at 1045/1022 cm^{-1} and 1022/995 cm^{-} of ultrasound treated rice which implied to the crystalline and amorphous region of rice flour molecule treated by ultrasound. The ultrasound following chilled rice showed that the ratio of absorbance 1045/1022 cm^{-1} and 1022/995 cm^{-1} not significantly different ($p \geq 0.05$). However, ratio at 1022/995 cm^{-1} of ultrasound-and-chilled rice decreased indicating that a lower proportion of amorphous to crystalline structure. Furthermore, the ratio 11045/1022 cm^{-1} of ultrasound-and-chilled rice tended to slightly increase. This the ratio represented a proportion of crystalline to amorphous (Wang *et al.*, 2015; Warren *et al.*, 2016). Therefore, chilling proesses after utilization treatment could attributed to crystalline structure.

The thermal properties of the native, ultrasound treated glutinous rice, and ultrasound-and-chilled treated glutinous rice samples were measured by DSC. The onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and enthalpy of gelatization (ΔH) were obsereved. The To, Tp, Tc, and ΔH of ultrasound treated rice was lower than those of the native due to the internal crystalline and amorphous region of starch were destroyed, and some of the internal double-helical structure disappeared by ultrasound (Hu *et al.*, 2014). Moreover, the longer time (30 min) of ultrasound treated rice resulted in lower ΔH than that of the shorter time (15 min) of ultrasound treated rice. Qiang *et al.* (2007) also reported that increasing time of ultrasound resulted in the crystalline region of starch glanule disrupted. For the amplitude effect, the ΔH of ultrasound treated rice slightly decreased as compared with ultrasound treated rice with higher amplitude level. Thus,, the amplitude level at 100% of ultrasound treated rice showed the lowest ΔH values for each ultrasound time. Manchun *et al.* (2012) found that increase of the amplitude level (50 to 100%) of ultrasound power resulted in the ΔH decreased in tapioca starch. ultrasound-and-chilled treated glutinous rice showed significantly increase of ΔH as compared with that of the ultrasound treated rice. That could be explained that the mechanical rearrangement of molecular was packed to double helixes within the granule microstructure when the ultrasound treated rice was chilled (Flores-Silva *et al.*, 2017).

The pasting properties of the native, ultrasound-treated, and ultrasound-and-chilled treated glutinous rice samples shows in Table 4. During heating and mixing of starch in RVA, starch gelatinization results in the water uptake and swelling of the granules and consequently reaching to peak viscosity. At this stage, continuous mixing of starch pastes results in the rupture of the granules and rappid decrease in the viscosity. At the cooling stage, assosiation between starch chains result in the gel formation and the viscosity increases rapidly to reach final viscosity. The increase of the peak viscosity, breakdown, and final viscosity while the decrease of setback was found in the ultrasound treated glutinous rice samples as compared with those of the native due to the ultrasound treatment caused the disruption of starch granules by cavitation forces which made the starch granule more permeable to water and swelling during the heating step. This caused to increase in peak viscosity (Sit *et al.*, 2014). Pinto *et al.* (2015) reported that an increase in peak viscosity that attributed to a possible loosening of interaction between amylose and amylopectin chains causing higher breakdown value. Furthermore, the peak viscosity, breakdown, and final viscosity tended to increase when the amplitude levels of ultrasound treatment increased from 40 to 70% for 30 min of ultrasound treatment. Bernardo *et al.* (2018) reported that higher amplitude of ultrasound processing resulted in starch granule disrupting interaction between chain hence compromising granule integrity and reducing swelling leading to higher peak viscosity. The peak viscosity, breakdown, and final viscosity of ultrasound-and-chilled treated glutinous rice were lower than those of the ultrasound treated rice samples. These results could be due to the reassosiation of starch molecules during storage at chill temperature which led to strong interactions between the starch chain within the granules (Klein *et al.*, 2013; Pinto *et al.*, 2015). This might be attributed to greater disruption of the granule structure for more sonication time, which allowed more water to be absorbed and thereby increasing the PV.

The ultrasound treated rice showed higher HI and eGI than those of the native. That could be the mechanical power of ultrasound affected on the crystalline region to be weaker resulting in the susceptibility of α-amylase and amyloglucosidase starch hydrolysis (Shumoy and Raes, 2017; Lu *et al.*, 2018). Thus,the ultrasound treated rice had higher eGI than that of the native. In addition, HI and eGI increased with increasing time and amplitude of ultrasound treatment. These results were due to more power of ultrasound provideed crystalline region easily destroyed (Czechowska-Biskup *et al.*, 2005). which promoted more accessibility of enzyme to hydrolyze starch molecules (Cui *et al.*, 2010; Trinh *et al.*, 2013). For ultrasound-and-chilled treated glutinous rice presented lower HI and eGI as compared with ultrasound treated samples. These results was also observed in enthalpy (ΔH) (Table 3). Flores-Silva *et al.* (2017) reported that higher enthalpy value indicated that the greater number of double helices led to more compact rearrangement of the double-helice structure resulting in less enzymatic susceptible attack on starch granule. Therefore ultrasound treatment destroyed crystalline region, its molecule rearranged to compact structure after being treated by chilling which led to the stronger starch molecule structure and resulted in lower GI than that of the native rice.

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