International Journal of Agricultural Technology 2019 Vol. 15(6):933-946
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The effects of dual modification with ultrasound and annealing treatments on the properties and glycemic index of the Thai glutinous rice cultivar ‘RD6’.

Kunyanee, K. and Luangsakul, N.[*]

Faculty of Agro-Industry, King Mongkut’s Institute of Technology Ladkrabang, Bangkok, 10520, Thailand.

Kunyanee, K. and Luangsakul, N. (2019). The effects of dual modification with ultrasound and annealing treatments on the properties and glycemic index of the Thai glutinous rice cultivar ‘RD6’. International Journal of Agricultural Technology 15(6):933-946.

Abstract Glutinous rice is a popular food and an important source of carbohydrate, especially in the North and North-Eastern areas of Thailand. However, glutinous rice can be considered unhealthy because it has a high glycemic index (GI=75-92), which makes it unsuitable for people suffering with type II diabetes. Therefore, a method of processing glutinous rice that reduces its GI could make it a more acceptable food and more suitable for diabetics. Ultrasound, which applies mechanical waves with a frequency above 16 kHz, and annealing (ANN), which is a hydrothermal treatment, have both been successfully used to modify starch. Ultrasound impacts starch granules and can change molecular structure and properties. ANN can reduce starch hydrolysis and improve its relative crystallinity, which affected its thermal properties. Therefore, the combination of ultrasound and ANN treatments were investigated to determine their effects on the glycemic index of the Thai glutinous rice cultivar ‘RD6’. Samples of ‘RD6’ were treated with ultrasound, at 100% amplitude, for 15 min followed by storage at 4^{o}C for 24 h and then exposed to ANN at 45, 50 or 55^{o}C. The samples were then evaluated for their pasting properties (pasting temperature, pasting viscosity, breakdown, final viscosity and setback), gelatinization properties, starch hydrolysis and glycemic index. All the pasting properties of the ‘RD6’ samples that treated with ultrasound and ultrasound plus ANN had significantly (p<0.05) increased levels of all compared to the untreated samples (control). These treatments also significantly (p<0.05) increased onset temperature and gelatinization enthalpy, particularly the ANN at the higher temperatures (50 and 55^{o}C). In addition, ultrasound plus ANN significantly (p<0.05) decreased starch hydrolysis and promoted a decrease in their glycemic index from (76.78-77.62) as compared to the control (84.14) and ultrasound alone (80.71), but none of these treatments reduced glycemic index to below 70, which is ranked as high.

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

Introduction

Glutinous rice (*Oryza sativa L.*), also called waxy rice or sticky rice, is composed mainly of starch whose dry weight is 98-100% amylopectin

[*] **Corresponding Author:** Luangsakul, N.; **Email:** naphatrapi.lu@kmitl.ac.th

(Widiastuti Setyaningsih *et al.*, 2015), which gives it a sticky texture after cooking (Guo *et al.*, 2015). Glutinous rice is mainly grown in South East and Eastern Asia, the eastern parts of South Asia and the North and North Eastern parts of Thailand. Glutinous rice is consumed as part of the daily diet for breakfast, lunch and dinner (Lefferts, 2005). However, this high consumption of glutinous rice may lead to an increased risk of type II diabetes and insulin resistance (Panlasigui *et al.*, 1991) since glutinous rice has a high glycemic index (GI) in a range of 75–92 (Frei *et al.*, 2003; Guo *et al.*, 2015).

GI is a relative ranking of carbohydrate in foods and is an established indicator of the effects on blood glucose level of foods. The GI is ranked as high (≥70), medium (55-96) and low (≤55), based on their glucose raising potential as compared to a reference (glucose or white bread) (Jenkins *et al.*, 1981). Glutinous rice is therefore ranked as high GI and Boers *et al.* (2015) reported that higher levels of postprandial glycemic exposure have been associated with the development of type II diabetes mellitus as well as cardiovascular disease and Chen *et al.* (2010) and Kaur *et al.* (2016a and 2016b) reported that there was good evidence suggesting that the consumption of low-GI rice can decrease the incidence of diabetes. Many methods have been used to reduce the GI in foods including chemical modification (Zieba *et al.*, 2010), enzymatic modification (Berry, 1986; Guraya *et al.*, 2001; Shin *et al.*, 2005), physical modification (autoclaving) (Dundar and Gocmen, 2013) and hydrothermal treatment (Chung *et al.*, 2009). Also exposing rice at cool temperatures might affect the ordered structure of starch granule so that the starch molecules re-associate, resulting in a more compact structure of starch granules (Wang *et al.*, 2015). Also, Zia-ud-Din *et al.* (2017) reported that the most suitable methods that can be used for modifying starch to reduce GI of food are physical methods since they are simple and safe.

Ultrasound is sound waves at a frequency above the threshold of human hearing (>16 kHz) that can be used as a physical method for starch modification. It impacts on starch granules and starch properties have shown that it can change their molecular structure and properties (Wang and Wang, 2004; Zuo *et al.*, 2009; Polesi and Sarmento, 2011). Jambrak *et al.* (2010) reported that ultrasound treatment affected on the properties of starch including solubility and swelling power, and viscosity and Huang *et al.* (2007) reported ultrasound treatment increased gelatinization temperatures of maize starch and increased gelatinization enthalpy. Annealing (ANN) is hydrothermal treatment, in the presence of higher water (>60%) or medium water content (40-55%), for an extended period of time that is performed at a temperature above the glass transition but below the gelatinization temperature of starch (Gomes *et al.*, 2005; Liu *et al.*, 2009; Dias *et al.*, 2010). This treatment changes the physicochemical properties of starch without destroying starch structure (Dias *et al.*, 2010). Moreover, ANN affects changes in the properties of starch including gelatinization temperatures (especially onset temperature), changes in gelatinization enthalpy, pasting properties and *in vitro* enzyme hydrolysis (Gomes *et al.*, 2004; Waduge, R. N. *et al.*, 2006; Wang *et al.*, 2017). The effects of ANN treatment on starches were described by Dias *et al.* (2010) as follow: (1) a reorganization of the granule structure; (2) an increase in the stability of the granules; (3) an increase in crystallinity; (4) an increase in the interaction between the starch chains in amorphous and crystalline regions of the granule; (5) the formation of double helices; (6) an elevation of the starch gelatinization temperature and a sharpening of the gelatinization range; (7) a reduction in the swelling of the granules and the potential and extent of amylose leaching; (8) a reduction in peak viscosity and setback; (9) the formation of resistant starch. For these reasons, the annealing treatment is interesting to apply for reducing the glycemic index of rice.

Previously, the combination of ultrasound and ANN treatments on physicochemical properties of starch have shown increases in the relative crystallinity and gelatinization temperatures of Pinhão seeds (*Araucaria angustifolia*) (Pinto *et al.,* 2015). Babu *et al.* (2019) reported the combination of ultrasound-chilled foxtail millet (*Panicum italicum*) starch with ANN treatment showed higher the relative crystallinity than its control and increased the onset temperature (To), gelatinization enthalpy (ΔH) and pasting temperature (PT) as compared to the control. However, showed positive effects of the combination of ultrasound and ANN on crystallinity, thermal proprieties and gelatinization temperatures, but their effects on GI were limited. Consequently, the primary objective of this study was to test the effects of the combination of ultrasound and ANN on the GI of the Thai glutinous rice cultivar ‘RD6’ and the pasting properties, thermal properties, and expected glycemic index (eGI) using rapid visco analysis, differential scanning calorimetry and *in vitro* analysis, respectively.

Materials and Methods

Materials

Glutinous rice grains of cultivar ‘RD6’ that had an amylose content of 7.04 %wb were obtained from Ubonratchathani province, Thailand. They were vacuum sealed in polyethylene film bags and stored at 4^oC until used. Pancreatic α-amylase (EC 3.2.1.1., 3000 U/g), amyloglucosidase (EC 3.2.1.3., 102 3300 U/mL) and a glucose assay kit (GOPOD method) were purchased from Megazyme International, Ireland Ltd. Other chemicals used in this study were analytical grade.

Ultrasound treatment (U)

500 g of the 'RD6' rice grains were placed in a wire basket (19 x 25 x 9 cm^3) and immersed in 6L of water in the chamber of ultrasonicate bath (WUC-D10H, Wisd, Daihan Scientific, Korea) at room temperature (30±1°C) for 15 min with amplitude at 100% of ultrasound power (665 W, 60 KHz.) After treatment, the rice grains were removed and drained for 1 min then packed in polyethylene film bags and stored at 4°C for 24 h and subsequently dried at 40±5°C in a tray dryer (progress co., Ltd, Thailand) to reduce the moisture content to 11±1%wb. The dried rice was ground in a pin mill (ZM-200, Retsch, America) fitted with a 0.25 mm sieve and screened using a 160 µm sieve for analysis of pasting properties and thermal properties.

Ultrasound and Annealing treatment (U-ANN)

Samples from the ultrasound and chilling treatments, above, were exposed to the annealing treatment (ANN) by placing them in an aluminum trays (21 cm long x 30 cm wide), covering with aluminum foil and placing them in an incubator (MIR-23, Sunyo, Japan) at 45, 50 or 55°C (U-ANN45, U-ANN50 or U-ANN55, respectively) for 16 h. At the end of incubation period, all the samples were dried at 40±5 °C to moisture content 11±1% wb in a tray dryer (progress co., Ltd, Thailand). The samples were then ground with a pin mill (ZM-200, Retsch, America) fitted with a 0.25 mm sieve and screened by 160 µm sieve for analysis of pasting properties and thermal properties.

Pasting properties

The pasting properties of U sample and U-ANN samples were determined using a Rapid Visco Analyser (RVA) (model 4, Newport Scientific, Australia), according to the Approved Method 61-02 (AACC, 2000). The ground rice (3.0 g) was weighed directly into the RVA canister, and 25 mL of distilled water was added. The samples were heated to 50°C and stirred at 160 rpm for 10 s for thorough dispersion. The slurry was held at 50°C for 1 min and then the temperature was linearly increased up to 95°C and held at 95°C for 7.5 min, and then cooled to 50°C and then held at 50°C for 4 min. The pasting properties parameters, including pasting temperature (°C), peak viscosity (cP), breakdown (cP), final viscosity (cP) and setback (cP), were determined. Setback is simply the gelling of the cooked starch mass that occurs as straight chain amylose starch molecules begin to realign and form a stable gel structure.

Thermal properties

The gelatinization parameters of U and U-ANN rice samples were measured using a differential scanning calorimeter (DSC 2 module, Mettler Toledo, Switzerland). Approximately 3 mg of ground rice and 9 µL of deionised water were placed into the DSC sample pan. The pan was sealed and equilibrated overnight at room temperature before heating in the DSC. Gelatinization measurements were carried out at a heating rate of 5^{o}C/min from 20 to 120^{o}C. An empty pan was used as a reference.

The percent of Starch hydrolysis and the expected glycemic index (eGI)

Analysis for expected glycemic index (eGI) was by the AACC method 32-40.01 (AACC, 2000) with slight modifications. The dried treated U and U-ANN samples were ground with a grinder (SG-10HK, Cuisinart, USA) then sieved through 1.0 mm screen. Each ground sample (100±5 mg) was placed with 4.0 mL of pancreatic α-amylase into screw cap tube and incubated at 37^{o}C in a shaking water bath for 30, 60, 90, 120, 150 or 180 min after which 8.0 mL of ethanol (99% v/v) was added and centrifuged at 1500 xg for 10 min. The supernatant was decanted into a volumetric flask and each pellet was washed with 8 mL of ethanol (50% v/v). The supernatant was combined with the initial supernatant and the volume adjusted with sodium acetate buffer (pH 4.5) to 100 mL. Then, 0.1 ml of this solution was put into a test tube with 10 µL of dilute amyloglucosidase solution (300 U/mL) in 100 mM sodium maleate buffer (pH 6.0) and incubated for 20 min at 50^{o}C. The glucose content was measured using a glucose oxidase-peroxidase kit (GOPOD-kit).

The percentage of starch hydrolysis was calculated using the following equation (AACC, 2000):

$$\text{Starch hydrolysis (\%)} = \Delta E \times (F/W) \times 90 \quad (1)$$

Where: ΔE = absorbance (reaction) read against the reagent blank.
F = conversion from absorbance to micrograms.
W = dry weight of sample analysed

The absorbance obtained for 100 µg of D-glucose in the GOPOD reaction was determined and F = 100 (µg of D-glucose) divided by the GOPOD absorbance for 100 µg of D-glucose.

The kinetics of starch hydrolysis of the rice samples were calculated using the model established by Goñi *et al.* (1997):

$$C = C_{\infty} (1 - e^{-kt}) \quad (2)$$

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 curve area (AUC) was calculated using the following equation:

$$AUC = C_{\infty} (t_f - t_0) - (C_{\infty} / k)(1 - \exp^{(-k(tf - t0))}) \quad (3)$$

Where t_f and t_0 were the final time (180 min) and the initial time (0 min), respectively. The hydrolysis index (HI) was calculated by dividing the area under the hydrolysis curve of each sample by the area of a reference sample (white bread).

The eGI was calculated using the equation by Goñi *et al.* (1997):

$$eGI = 39.71+(0.549HI) \quad (4)$$

Statistical analysis

The experimental data were analyzed using SPSS for window (Statistical Package for the Social Sciences). The means were compared using the Duncan's Multiple Comparison with a confidence level of 95% ($p \geq 0.05$) when there were significant differences in the analysis of variance (ANOVA).

Results

Pasting properties

Pasting properties of the control, U and U-ANN treatments of the rice samples are shown in Table 1. The U and U-ANN treatments showed significantly higher ($p<0.05$) levels in all the pasting properties tested, pasting temperature (PT), pasting viscosity (PV), breakdown (BD), final viscosity (FV), and setback (SB) than the control. The U-ANN treated samples had increased levels of PT, except for U-ANN45, whereas U-ANN treated samples had decreased levels of PV, BD, and FV. Also, the higher temperatures of the ANN treatments resulted in increases in PT, PV, BD, FV and SB as compared to their U-ANN equivalents with the highest PT in U-ANN at 55^{o}C.

Table 1. The pasting properties of glutinous rice treated with ultrasound treatment (U) and combination of ultrasound with annealing treatment (U-ANN)

Samples	PT[1] (°C)	PV[1] (cP)	BD[1] (cP)	FV[1] (cP)	SB[1] (cP)
Native (control)	65.63±0.78^{d}	3152.33±39.55^{e}	1277.33±37.02^{e}	2459.33±34.67^{e}	523.00±10.58^{c}
U	72.07±0.03^{c}	5120.67±25.42^{a}	2092.00±59.03^{a}	3625.33±9.29^{a}	596.67±49.72^{ab}
U-ANN45	72.83±0.06^{c}	4506.67±17.50^{d}	1809.33±36.91^{d}	3249.33±11.93^{d}	552.00±29.46^{bc}
U-ANN50	74.15±0.48^{b}	4780.00±37.03^{c}	1932.33±3.78^{c}	3497.33±27.83^{c}	649.67±31.56^{a}
U-ANN55	75.72±0.41^{a}	4958.33±16.74^{b}	2011.33±14.47^{b}	3552.67±21.83^{b}	605.67±15.63^{ab}

Data are mean ± standard deviation (n=3). Values within the same column with different letter were significantly different at p<0.05.

[1] PT: pasting temperature, PV: peak viscosity, BD: breakdown, FV: final viscosity, and SB: setback.

Thermal properties

Thermal properties (pasting temperature, peak temperature, conclusion temperature and enthalpy gelatinization) of the control, U and U-ANN treatments are shown in Table 2. The U treated rice samples had higher onset temperature (T_o) than that the control but showed no significant differences (p≥0.05) in peak temperature (T_p), conclusion temperature (T_c) and ΔH. The U-ANN treated rice samples had increased levels of T_o, T_p, and ΔH compared to the U treatment. However, for T_o, T_p, and ΔH the U-ANN45 treated samples were not significant (p≥0.05) different. In addition, the increase in temperatures of U-ANN treatment contributed to increases in T_o, T_p, and ΔH. The highest T_o and ΔH were found in the samples treated with U-ANN with ANN temperatures of either 50 or 55°C (U-ANN50 and U-ANN55, respectively).

Table 2. The gelatinization properties of glutinous rice treated with ultrasound treatment (U) and combination of ultrasound with annealing treatment (U-ANN)

Samples	Gelatinization temperatures			ΔH[1] (J/g)
	T_o[1] (°C)	T_p[1] (°C)	T_c[1] (°C)	
Native (control)	58.76±0.37^{c}	69.03±0.19^{d}	74.32±0.13^{c}	1.87±0.06^{a}
U	62.21±1.30^{b}	68.13±0.51^{cd}	74.48±0.29^{c}	1.92±0.06^{a}
U-ANN45	62.79±0.12^{b}	68.74±0.18^{c}	75.53±0.32^{b}	1.82±0.06^{a}
U-ANN50	63.03±0.40^{ab}	70.00±0.16^{b}	75.36±0.12^{b}	2.35±0.07^{b}
U-ANN55	64.14±0.63^{a}	71.65±0.54^{a}	76.46±0.04^{a}	2.30±0.05^{b}

data are mean ± standard deviation (n=3). Values within the same column with different letter are significantly different at p<0.05.

[1] T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature and ΔH.

Starch hydrolysis and expected glycemic index (eGI)

The starch hydrolysis of the U and U-ANN treated samples are shown in Figure 1. The curves of starch hydrolysis showed that there were different kinetics of starch hydrolysis in different treatments. The starch hydrolysis curves of the control and U treatment were slightly different to the kinetics of starch hydrolysis. Samples with the U treatment had lower starch hydrolysis after 90-180 min compared to the control. The combination of ANN followed by the U treatment (U-ANN) had lower percentage starch hydrolysis after 30 min compared to the control and U treatment. Rice samples treated at the higher temperature of U-ANN treatment promoted a decrease starch hydrolysis rate, particularly at the higher temperature of the U-ANN treatments (U-ANN50 and UANN55).

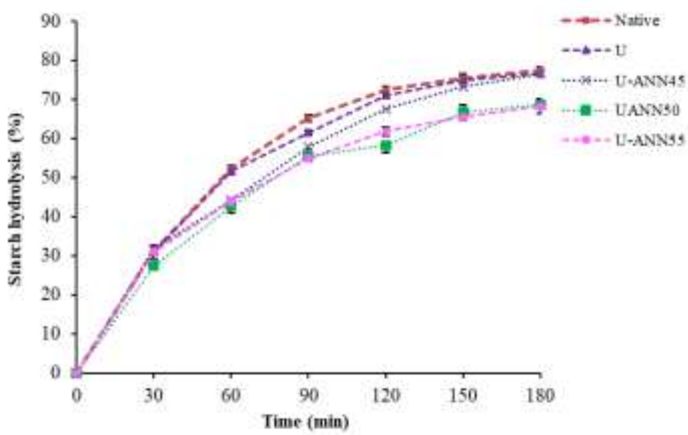

Figure. 1 Starch hydrolysis curve of glutinous rice treated with ultrasound treatment (U) and a combination of ultrasound with annealing treatment (U-ANN)

The eGI of the control, U treatment and U-ANN treatments of the rice samples (Table 3) show that there were significant differences (p<0.05) with the highest eGI for the control, which decreased with glutinous rice treated U treatment and U-ANN treatments. The eGI value decrease with the combination of ANN and ultrasound treated rice (U-ANN) from 80.71-76.78. However, the increase in ANN temperatures of U-ANN treatment were not significantly different (p≥0.05).

Table 3. The expected glycemic index of glutinous rice treated with ultrasound treatment (U) and combination of ultrasound with annealing treatment (U-ANN)

Samples	**eGI[1]**
Native (control)	84.14 ± 0.80[a]
U	80.71 ± 0.77[b]
U-ANN45	76.78 ± 1.63[c]
U-ANN50	77.15 ± 1.39[c]
U-ANN55	77.62 ± 1.02[c]

data are mean ± standard deviation (n=2). Values within the same column with different letter are significantly different at $p<0.05$.
[1] eGI: expected glycemic index.

Discussion

Pasting properties

The fact that all the treatments tested increased pasting properties could be a temperature effect since it has previously been shown that exposure of starch granules to low temperature can result in reassociation of starch molecules that could lead to a strong interaction between starch chains within starch granules (Klein *et al*., 2013; Pinto *et al*., 2015). Also, the effects of ultrasound have been shown to cause surface cracking of rice grains and/or fragmentation of starch granules (Park and Han, 2016) leading to the starch granules absorbing more water resulting in increased pasting properties. However, the added effect of some of the treatments to the temperature effects could be explained by the positive effects of the annealing treatment promoting the bond strengthening resulting in higher pasting temperatures (Dias *et al*., 2010). Pinto *et al*. (2015) reported that annealing treatment increased the granular structure stability of starch granules, resulting in a decrease in peak viscosity, breakdown and final viscosity. There were differences in pasting due to temperature in the range tested (45-55^{o}C) supported the results found by Dias *et al*. (2010) who previously found that the pasting temperature of annealed rice starch increased with increasing temperature for annealing treatment. The reduction of pasting viscosity, breakdown and final viscosity with increasing the annealing temperature indicated that organization of the starch molecules had increased stability resulting in them having reduced swelling during heating and mechanical stirring as previously reported by Gomes *et al*. (2005) and Hormdok and Noomhorm (2007).

Thermal properties

The results of the analyses of the thermal properties indicated that during storage at 4^{o}C the way the starch molecules were packed was rearranged in the order of the double helices within the granules, as previously described by Flores-Silva *et al*. (2017), resulting in higher peak temperature and enthalpy of gelatinization. This is also supported by the increasing annealing temperatures (45, 50 and 55^{o}C) resulting in significantly increases in onset temperature, peak temperature and conclusion temperature. Liu *et al*. (2009) also reported that the onset temperature and gelatinization enthalpy of potato starch increased after annealing treatment at higher temperature. Effects of annealing temperature can also be attributed to changes to the perfection of the crystalline structure and lead to formation of new double helices by amylose-amylose, and/or amylose-amylopectin interaction within starch granules as described by Waduge *et al*. (2006) and Zavareze and Dias (2011). Gomes *et al*. (2004) also reported that the strengthening of the bonds created by the annealing treatment meant a higher temperature required to gelatinize starches.

The percent of starch hydrolysis and expected glycemic index (eGI)

Storing the rice samples at 4^{o}C for 24 h resulted in a reduction in the percentage of starch hydrolysis. This effect could be explained by the rearrangement of the starch to form a stronger structure, which in turn could result in decreased enzyme attack on starch granules (Flores-Silva *et al*., 2017). Also, the combined reduced temperature/annealing treatments resulted in lower starch hydrolysis rate compared to the control and reduced temperature treatments alone and indicated that the annealing treatment promoted changes in the starch granules pack into perfect crystalline structures as explained by Ji *et al*. (2019). Chung *et al*. (2009) also found that starch hydrolysis decreased after starch grains were exposed to annealing treatment. It can be interpreted that these effects might be due to the higher temperature of annealing, over the range tested, contributed to the increase in the formation of double helices to form perfect crystalline structures, which was evidenced by the increase in pasting temperature, gelatinization temperatures, and gelatinization enthalpy that could lead to decreased starch hydrolysis. Overall these results imply that the treatments limited enzyme accessible to starch granules compared to the control.

The glycemic index was highest in the control, which indicates that all the treatments resulted in the starch chains being rearranged to the perfect crystalline regions, causing lower starch hydrolysis. According to Gomes *et al*.

(2004) the amorphous and crystalline region of the starch granules become more ordered after the annealing treatment causing lower starch hydrolysis and consequently decreased glycemic index. The ultrasound treatment reduced the GI and the combined ultrasound and annealing treatments reduced it even further (with no difference between the three temperatures) but none of the treatments were found to reduce GI to or below 70, which is the standard that is required to improve glutinous rice's acceptability for diabetics.

Acknowledgement

The authors gratefully thank Professor Keith Thompson for his help and language corrections in the preparation of this paper.

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(Received: 2 July 2019, accepted: 30 October 2019)