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Production of glutinous rice flour from broken rice via ultrasonic assisted extraction of amylose

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ABSTRACT

In this study, a modified aqueous leaching method by complex formation of amylose with glycerol was employed for reducing the amylose content of starch in broken white rice to less than 2%, so that the resulting starch can be classified to that of glutinous rice flour. By employing ultrasonication in alkaline condition, extraction of amylose could be performed by washing at lower temperature in shorter time compared to the existing aqueous leaching method. The effects of glycerol concentration, alkali concentration, ultrasonication and treatment time on the amylose content of the treated starch were systematically investigated. Under optimum condition, amylose content of broken white rice starch can be reduced from 27.27% to 1.43% with a yield of 80.42%. The changes in the physicochemical properties of the rice flour before and after treatment were studied.

1. Introduction

Glutinous rice or sticky rice is a type of rice which has sticky texture after being cooked. This rice type contains a very small content of amylose (1.0–2.3%) in its starch (Wani et al., 2012). Glutinous rice flour is excellent for many applications in food products such as thickening agent for white sauces, gravies, puddings and waxy rice dumpling (Ding, Wang, Zhang, Shi, & Wang, 2015; Prasad, Anil, Singh, & Sinha, 2013). The wide application of this flour has raised the demand of glutinous rice flour in the market. Despite its high value in food processing industry, the productivity of this flour seems to be low and in some countries, such as Thailand, glutinous rice price was significantly high in the past years (Titapiwatanakun, 2012; Zhu et al., 2000).

Non-waxy rice or white rice is more popularly known as one of the staple foods in the world and is predicted to increase 40% in demand by 2030 (Khush, 2005). This type of rice commonly contains 12.2% to 28.6% amylose in its starch (Wani et al., 2012). During rice milling, some rice grains break and become the major byproduct known as broken rice. This byproduct is often mixed with bran and ground rice husk to become cattle feed (Shih,

Champagne, Daigle, & Zarins, 1999). As the rice production and demand for polished white rice increase, the amount of broken rice produced worldwide also increases.

Due to higher price of glutinous rice flour in the market, there is an incentive to develop a method to obtain rice flour with similar characteristic to glutinous-rice flour from the under-utilized broken rice. Since an amylose content less than 2% is the only criterion for a rice to be classified as sticky rice, extracting of amylose from the starch of white rice is one way to turn white rice flour into glutinous rice flour. The existing techniques to selectively extract amylose from starch, especially for structural analysis purpose, include aqueous dispersion followed by selective precipitation and aqueous leaching (Doblado-Maldonado, Gomand, Goderis, & Delcour, 2015; Pigman & Wolfrom, 1945). There are several disadvantages in the first method such as long gelatinization time, requirement of an elaborate procedure of starch dispersion under controlled pH in an autoclave and the necessity to use a large amount of water. While aqueous leaching method offers easier and non-destructive approach, slow extraction may occur as the process is based on diffusional mass transfer (Doblado-Maldonado et al., 2015).

During aqueous leaching, the diffusion rate of amylose is affected by external factor (operating condition) and internal factor (structure of solid matrix). By manipulating operating condition and disruption of solid matrix, diffusion resistance of amylose

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can be reduced. In this method, in addition to low starch/water concentration, high temperature and long processing time were required to obtain high amylose removal and yield. Better molecular mobility by introducing organic molecules as plasticizer without affecting starch crystalline structure has been successfully performed by heating starch in 85 wt.% aqueous solution of glycerol, n-butanol, pentasol, cellosolve or dioxane prior to the extraction step (Montgomery & Senti, 1958). However, two to three subsequent extraction steps with very low starch/water concentration (2 wt.%) in nearly boiling water were required to completely extract amylose.

Ultrasonication has been acknowledged as a diffusion-enhancer unit process in food processing (Knorr, Zenker, Heinz, & Lee, 2004). In this study, the mechanical force of ultrasonic wave was harnessed to erode the diffusion barrier such as protein on the surface of starch granule, create a cavity in the dense solid matrix and induce chain scission on polysaccharide matrices so that amylose could diffuse out more easily under lower processing temperature. The effect of glycerol concentration was investigated with the aim to produce rice flour with similar amylose content to that of glutinous rice flour. Alkalinity, ultrasonication and processing time were found to affect amylose removal. Physicochemical properties of the treated rice flour starch, including proximate composition, pasting properties, morphology, crystallinity structure, swelling power and solubility, thermal decomposition and gelatinization properties were further analyzed.

2. Materials and methods

2.1. Materials

Broken white rice was obtained from a local mill in Taiwan. The broken rice was ground first by a home blender. Particles passed through a 25-mesh stainless steel screen (particle size < 0.71 mm) was then dried in a freeze dryer ($-40\,°C$, 0.05 mbar) for 2 days and stored in an air-tight container at room temperature for later use. The moisture content of broken rice flour was analyzed according to the AOAC 925.09 procedure and was found to be 11.87%. All chemicals were used as received, including ACS grade anhydrous glycerol (J.T. Baker, USA), reagent grade NaOH (Fischer Scientific, UK), HNO_3 (63%, May and Baker, UK), H_2SO_4 (98%, Scharlau, Spain), crystalline phenol (99%, Wako, Japan) and $NaNO_3$ (99%, Acros Organics, USA). Liquid α-amylase from *Bacillus licheniformis* (500 U/mg protein, 10 mg protein/mL, Sigma Aldrich, USA) and amyloglucosidase from *Aspergillus niger* ($\geqslant 300$ U/mL, Sigma Aldrich, USA) were diluted in acetate buffer (pH 5, 0.2 M) to obtain the stock solutions of 450 U/mL α-amylase and 5.25 U/mL amyloglucosidase, respectively. Anhydrous $NaCH_3COO$ ($\geqslant 99\%$, Nacalai Tesque, Japan) and glacial acetic acid ($\geqslant 99\%$, Scharlau, Spain) were utilized for making the buffer. Standard for glucose analysis was anhydrous α-D(+)-glucose (99 + %, Acros Organics, USA). Ethanol (95%, Echo, Taiwan) and n-hexane (95%, Tedia, USA) were used as the solvents to extract sugar and lipid from rice flour, respectively. Commercialized glutinous rice flour was purchased from a local supermarket and used without any pretreatment. Standard pullulan set with known molecular weights (708,000, 107,000, 21,100 and 6100) was purchased from Showa Denko, Japan.

2.2. Effect of glycerol concentration on amylose leaching

Rice flour sample (0.6 g, dry basis) was placed in a reaction tube and mixed with 2.4 g of various concentrations (25, 50, 70, 75, 77 and 85 wt.%) of glycerol in 1 N NaOH. Solvent and rice flour were mixed using a vortex mixer and then placed in an ultrasonic bath (Lissome LS-300H, 40 kHz, 300 W) at 70 °C for 1 h. The mixture

was subsequently mixed every 15 min using a vortex mixer. After 1 h, the sample was centrifuged ($2000 \times g$, 10 min). The supernatant was removed and the solid residue was washed 3 times each using 15 mL of deionized water to remove glycerol and NaOH. The washed residue was freeze dried for 2 days before analysis.

2.3. Effect of NaOH on amylose leaching

The procedure is exactly the same as in Section 2.2 except 85 wt.% glycerol in 1 N NaOH or in water was used.

2.4. Effect of treatment time on amylose leaching

The procedure is exactly the same as in Section 2.2 except 85 wt.% glycerol in 1 N NaOH was used and the mixture of solvent and rice flour was ultrasonicated for various times (30, 45, 60, 75 and 90 min).

2.5. Effect of ultrasonication on amylose leaching

The procedure is exactly the same as in Section 2.2 except 85 wt.% glycerol in 1 N NaOH was used and either the mixture was put in a ultrasound bath or in a water bath.

2.6. Amylose content quantification by gel permeation chromatography (GPC)

About 100 mg of flour sample was dispersed in 5 mL of 1 N NaOH solution and stirred at 380 rpm overnight. Several drops of 0.5 M HNO_3 were added to neutralize the mixture. The mixture was then transferred into a 50 mL volumetric flask and diluted. The aliquot was first centrifuged ($2000 \times g$, 10 min) to remove some solid residues. Clear supernatant (20 mL) was taken and filtered to pass a nylon membrane (0.2 μm). The filtered aliquot containing starch was injected into a GPC system, which consists of a series of standard pullulan-calibrated GPC column (Waters Corp., 7.8×300 mm, WAT011520, WAT011530, WAT011540 and WAT011545) at 40 °C, an RI detector (Waters 2414) and a binary HPLC pump (Waters 1525). The mobile phase was 0.1 N $NaNO_3$ with a flow rate of 0.8 mL/min. Acquisition time was programmed to 45 min. Amylopectin and amylose were identified based on molecular weight. The amylose content of starch was determined as the ratio of the peak area correlated to amylose to the total peak area.

2.7. Proximate analysis

Total free sugar and starch were determined using the method of Castillo et al. (2000) with a slight modification. Dried ground sample (100 mg) was extracted twice each with 30 mL of 80% v/v aqueous ethanol at 80 °C with constant stirring at 275 rpm for 30 min. After cooled, the solid was separated by centrifugation ($1800 \times g$, 20 min). The clear supernatant was decanted and collected for analysis of total sugar. The retained residue was hydrolyzed using 4 mL of 450 U/mL α-amylase in a boiling water bath for 30 min. After that, hydrolysis was continued using 4 mL of 5.25 U/mL amyloglucosidase at 55 °C for 16 h. Total free sugar and starch as glucose were analyzed using phenol sulfuric acid method by adding 1 mL of 5% phenol followed by 5 mL of 96% H_2SO_4 into 1 mL of sample solution. UV–vis spectrophotometer measurement of the supernatant was conducted at 490 nm. Blank solution for total free sugar and starch analysis was made by replacing the sample with 1 mL of deionized water. In addition to total free sugar and starch content analysis, the contents of crude fiber, protein, ash, lipid were also analyzed following the AOAC Official method 962.09, AOAC Official method 955.04, AOAC

Official method 923.03 and AOAC Official method 920.39, respectively.

2.8. Physical properties analysis

Physical properties of the rice flours investigated in this study include microscopic appearance, starch crystallinity, gelatinization properties, solubility in water, swelling power, and pasting properties. In taking electron microscopy image of rice flour sample, about 2 mg of the sample was applied onto a double-sided tape attached to a specimen stub. The starch granules were then evenly distributed on the surface of the tape and coated with gold. SEM micrographs were obtained with a JEOL SEM (JSM-6390LV) at an accelerating voltage of 20 kV. The crystalline structure of rice flour samples were analyzed by using a Bruker D2 Phaser X-ray Diffractometer (XRD) with the X-ray beam operating at 30 kV, 10 mA and ambient temperature. The X-ray generator was run at Cu K$_\alpha$ wavelength (λ = 1.5406 Å) with scanning region of diffraction angle (2θ) from 5° to 35°. The specific step size and counting time were set at 0.1° and 2 s, respectively. The relative crystallinity of starch in rice flour was calculated from the following equation (Nara and Komiya (1983) using Origin 9.1 (Microbial, Northampton, MA, USA) for peak smoothing and peak integration.

$$\text{Crystallinity } (\%) = \frac{\text{Area of crystalline peak}}{\text{Total area under XRD curve}} \times 100\%$$

Gelatinization properties of rice flour were analyzed using a Jade Perkin Elmer Differential Scanning Calorimetry (DSC) according to the method described by Yuliana, Huynh, Ho, Truong, and Ju (2012) with slight modification. About 3.5 mg of dry sample was loaded into a 40 μL aluminum pan (TA Instruments, USA). Deionized water (9 μL) was added into the pan and the pan was covered. The sample was then kept for 1 h at room temperature before analysis. Prior to this measurement, the DSC instrument was calibrated by using indium and an empty aluminum pan was prepared as the reference. During this analysis, the sample-containing pan and reference pan were heated from 30 °C to 100 °C at a rate of 10 °C/min. Nitrogen at 50 mL/min was passed through the system. The results obtained by this analysis comprised onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}).

The method of Nadiha, Fazilah, Bhat, and Karim (2010) was employed to determine the swelling power and solubility. About 0.4 g of pre-dried rice flour was weighed and 40 mL of deionized water was added. This mixture was heated at 90 °C for 30 min in a water bath. The sample was cooled to room temperature and centrifuged (2000×g, 15 min). The wet residue was collected and weighed to calculate the swelling power by the following equation.

$$\text{Swelling power } (\text{g/g}) = \frac{\text{weight of the wet residue (g)}}{\text{weight of the dry sediment (g)}}$$

In order to determine the solubility of starch, supernatant obtained from the centrifugation was dried at 110 °C. The dried residue was weighed and percent solubility was calculated using the following equation.

$$\text{Solubility } (\%) = \frac{\text{weight of dried supernatant (g)}}{\text{weight of the dry flour (g)}} \times 100$$

The pasting properties of representative rice flours were measured in triplicate by using a Rapid Visco Analyzer (RVA Model 4D, Newport Scientific, Australia). Rice flour sample (3 g) was mixed with 25 g of deionized water in an Al canister. The suspension was stirred using constant paddle speed of 160 rpm with heating and cooling program according to AACC Approved Method 61-02. The measured viscosities during the program were expressed in rapid viscometer unit (RVU) and translated into pasting properties, which comprised pasting temperature (PT), peak time (t_p), peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), breakdown viscosity (BDV = PV–HPV), and set back (SB = CPV–HPV).

3. Results and discussion

Table 1 shows the proximate analysis results (based on dry flour weight) of the original broken rice flour (BRF) and glutinous rice flour (COMM-GRF) used in this study. It is apparent that the most distinct characteristic between BRF and COMM-GRF is the amylose content. The amylose content in the starch of broken rice flour is 27.27%, which can be categorized as high amylose rice starch (Wani et al., 2012). Although there are variances in the contents of other components, only amylose content of starch may affect the properties of rice flour since starch constitutes about 90% of both rice flours. In addition, only amylose can form complex with lipid and protein, which is closely related to the rheological properties of flour (Yu, Ma, Menager, & Sun, 2012). Therefore, the objective of this study was firstly to focus on reducing the amylose content of BRF to less than 2% and then examined the physicochemical properties of the product. Each experiment on the treatment process was carried out in triplicate. The effects of glycerol concentration, NaOH, treatment time and ultrasonication on product composition are listed in lower part of Table 1.

3.1. Effect of glycerol concentration on amylose removal

Glycerol is regarded as GRAS (Generally Regarded as Safe) substance for food and drugs application. The effect of glycerol concentration used in treatment on amylose content of starch is presented in Table 1. Amylose content of rice flour starch decreased after treatment. By employing 77% alkaline glycerol, amylose content of BRF can be reduced to 1.43% with a yield of 80.42%, which indicates that glutinous rice flour (coded as SYN-GRF) was successfully obtained. The result of this study conforms with the result of Montgomery and Senti (1958), which reported that solution containing 70–85 wt.% organic solvent can effectively free amylose from amylose–amylopectin strong bond, although some amylopectin can be leached out. This non-selectivity also can be seen from the GPC curve of SYN-GRF (Fig. S3) with the disappearance of low molecular weight part of amylopectin peak. No protein peak was seen in the GPC curve as observed also in the result of previous study that used non-purified rice flour directly for GPC analysis (Lu, Chen, & Lii, 1996). The absence of protein interference may be due to the precipitation of protein during the neutralization of flour dispersion (Ju, Hettiarachchy, & Rath, 2001; Shyur, Zia, & Chen, 1988). Precipitated protein can be removed easily by membrane filtration prior to injection to the GPC apparatus.

Treatment using low glycerol concentration failed to considerably reduce amylose content. This may be because at lower glycerol concentration, fewer plasticizer (glycerol) can diffuse into the starch through flour matrix. Although more plasticizer is expected to ease amylose leaching, treatment using 85 wt.% glycerol resulted in opposite trend due to increasing viscosity of the system, which may hinder ultrasonic mixing of rice flour with solvent. In addition to the physical property of the mixture, decreased removal of surface protein and lipid under less alkaline condition may also hinder diffusion of amylose out of starch.

The glycerol concentration affects not only to the extent of alkalinity, but also to the water content in the system. Sufficient water in the alkaline system can promote gelatinization of starch components, solubilization of protein, and reaction with

Table 1
Proximate composition of rice flour samples.

Parameters	Amylose content (%)	Yield (%)	Composition (%)					
			Starch	Free sugar	Fiber	Protein	Lipid	Ash
Characterization								
BRF	27.27 ± 1.36	–	88.8 ± 0.4	0.84 ± 0.01	2.40 ± 0.01	0.70 ± 0.02	6.67 ± 0.03	0.60 ± 0.01
COMM-GRF	1.89 ± 0.29	–	91.2 ± 0.3	0.31 ± 0.00	2.01 ± 0.02	0.50 ± 0.01	5.20 ± 0.02	0.74 ± 0.01
Glycerol concentration (%)								
25	20.20 ± 0.42	37.1 ± 0.2	66.4 ± 0.3	0.20 ± 0.01	5.93 ± 0.02	7.32 ± 0.02	0.56 ± 0.01	19.59 ± 0.03
50	12.01 ± 0.35	68.5 ± 0.3	82.1 ± 0.4	0.08 ± 0.00	3.30 ± 0.01	3.97 ± 0.02	0.25 ± 0.01	10.28 ± 0.04
65	7.38 ± 0.41	72.2 ± 0.2	84.5 ± 0.4	0.09 ± 0.00	3.14 ± 0.01	3.94 ± 0.02	0.18 ± 0.00	8.11 ± 0.05
70	5.84 ± 0.18	76.5 ± 0.3	90.9 ± 0.3	0.03 ± 0.00	2.98 ± 0.01	3.85 ± 0.01	0.15 ± 0.00	2.07 ± 0.01
75	3.84 ± 0.20	82.6 ± 0.3	91.5 ± 0.3	0.04 ± 0.00	2.77 ± 0.01	3.65 ± 0.01	0.13 ± 0.01	1.91 ± 0.01
77 (SYN-GRF)	1.43 ± 0.30	80.4 ± 0.4	91.0 ± 0.3	0.07 ± 0.00	2.85 ± 0.02	3.99 ± 0.01	0.11 ± 0.01	1.95 ± 0.02
85	7.84 ± 0.34	80.7 ± 0.4	90.6 ± 0.4	0.30 ± 0.00	2.92 ± 0.01	4.09 ± 0.02	0.18 ± 0.01	1.96 ± 0.01
Alkalinity								
Without NaOH[a]	16.92 ± 0.31	84.8 ± 0.4	91.1 ± 0.4	0.13 ± 0.01	2.73 ± 0.01	5.06 ± 0.01	0.31 ± 0.00	0.70 ± 0.00
Time (min)[b]								
30	20.46 ± 1.10	82.5 ± 0.3	89.6 ± 0.2	0.05 ± 0.00	2.89 ± 0.01	6.09 ± 0.03	0.47 ± 0.01	0.89 ± 0.01
45	12.99 ± 0.67	81.2 ± 0.4	90.3 ± 0.3	0.09 ± 0.00	2.91 ± 0.02	5.21 ± 0.03	0.27 ± 0.02	1.23 ± 0.02
60	7.84 ± 0.98	80.7 ± 0.4	90.6 ± 0.4	0.30 ± 0.00	2.92 ± 0.01	4.09 ± 0.02	0.18 ± 0.01	1.96 ± 0.01
75	6.24 ± 0.90	79.9 ± 0.2	91.5 ± 0.8	0.19 ± 0.01	2.95 ± 0.00	3.34 ± 0.04	0.09 ± 0.00	1.97 ± 0.00
90	6.17 ± 0.70	79.8 ± 0.4	91.6 ± 0.4	0.00 ± 0.00	2.95 ± 0.03	3.34 ± 0.01	0.09 ± 0.00	2.04 ± 0.01
Mixing mode[b]								
Ultrasonication	7.84 ± 0.98	80.7 ± 0.4	90.6 ± 0.4	0.30 ± 0.00	2.92 ± 0.01	4.09 ± 0.02	0.18 ± 0.01	1.96 ± 0.01
Without ultrasonication	10.22 ± 0.46	84.4 ± 0.4	91.3 ± 0.4	0.07 ± 0.00	2.78 ± 0.01	3.92 ± 0.01	0.21 ± 0.01	1.76 ± 0.01

[a] Glycerol concentration 85%.
[b] Glycerol concentration 85%, 1 N NaOH.

alkaline-vulnerable moieties (lipid saponification and lignin hydrolysis). In the treatment process of starchy feedstock, starch gelatinization can irreversibly dissolve starch granule in water, thus less or no starch remains in the solid residue. In Table 1, treatment using 25 wt.% glycerol resulted in the lowest yield and the highest ash content. This was attributed to the substantial loss of starch by starch gelatinization and the inclusion of more Na$^+$ into residual flour solid from NaOH introduced to the system. Severe loss of starch granules in the 25 wt.% glycerol treatment is portrayed microscopically in Fig. S1. By decreasing the water content (increasing glycerol concentration to 50 wt.% and so on), starch gelatinization could be minimized so that only partial gelatinization occurred, which preserved the yield of residual solid. Investigation on the mass balance indicated that fiber was mostly retained during the treatment, while protein and lipid were remarkably reduced due to alkaline extraction mechanism.

3.2. Effect of NaOH on amylose removal

The absence of NaOH in glycerol solution resulted in more than two times final amylose content (16.92%) in starch than that of treated in the presence of NaOH (7.84%). Alkaline condition also had significant impact on the solubilization of lipid and protein (Table 1). Since NaOH can dissolve both amylose and amylopectin, significant drop of amylose content may only be attributed to the increase of solubility of amylose in water, which can occur due to random substitution of hydroxyl group of amylose. Increasing solubility of amylose in water can be explained by the increase of its hydrophilicity and stability in water (Wulff & Kubik, 1992). Addition of two hydroxyl groups from deprotonated glycerol for each substituted hydroxyl group in native amylose may enhance the hydrophilicity of amylose in water, while random position of substituent prevents amylose molecule from forming helical coil with non-polar outer side, which can precipitate out of water easily. Therefore, removal of amylose from starch granules using this approach can be achieved just by simple washing. The proposed reaction mechanism between deprotonated glycerol and the hydroxyl group of amylose is illustrated in Fig. S2. The differ-

ence in nature between linear and branched structure of amylose and amylopectin allows amylose to react with other organic molecules, thus amylose is more easily extracted from starch (Reid, Tatsuta, & Thiem, 2008). After standing at 4 °C for 7 days, the retrograded amylose in the supernatant can be recovered for other applications. Qualitative iodine test on the pooled supernatant and retrograded amylose showed the existence of leached amylose as shown in Fig. S4.

3.3. Effect of treatment time on amylose removal

During the treatment, it is obvious that amylose content decreased with increasing treatment time (Table 1). At the same time, protein and lipid that hinder amylose extraction decrease concurrently with the diffusion of amylose. Ash in the treated flour also increases due to the adsorption of Na$^+$, while fiber content only increases slightly, which may be due to reduction of other components. Amylose content changed insignificantly after 60 min due to saturation, which is also reflected in the relatively unchanging proximate compositions (Table 1). Thus, in this study 60 min was chosen as the effective treatment time.

3.4. Effect of ultrasonication on amylose removal

When white rice flour was treated with 85 wt.% glycerol in 1 N NaOH for 60 min, the amylose content of the resulted starch was 7.84% if ultrasonication was used while 10.22% was obtained if ultrasonication was not used. The result indicates that ultrasonication has effect on decreasing amylose content due to the well-known effect of formation, growth, and implosive collapse of bubbles in ultrasonic mixing. However, the result in Table 1 showed that ultrasonication has less effect on amylose reduction than other factors studied. This may be due to the indirect sonication provided by a classic ultrasonic bath used in this study which has low intensity. There are insignificant differences in the proximate compositions between flours treated by sonication and without sonication (Table 1).

3.5. Physical properties of treated flour

SEM images of the original white rice flour (BRF) and those of the treated rice flour (SYN-GRF) are shown in Fig. 1(a, c) and (b, d), respectively. It can be seen that before the treatment, starch granules appear as clump in the matrices of plant cell wall. Sonication treatment releases starch granules by breaking plant cell wall matrices (Fig. 1b). Starch granule shape changed from nearly spherical before treatment (Fig. 1c) to polygonal (Fig. 1d). This polygonal shape can be reasoned as the artifact of NaOH abrasion of starch granule surface, which is commonly identified as amyloplast. Since amyloplast is a non-pigmented plant organelle that is strongly associated with protein bodies (i.e., enzyme) for starch synthesis and stored lipid, removal of amyloplast also contributes to the reduction of protein and lipid content in rice flour after treatment. Since low power ultrasound was utilized in this study, the surface of starch did not develop any remarkable pore or pitch (Jambrak et al., 2010).

XRD peaks of BRF, COMM-GRF and SYN-GRF (Fig. 2) reflect typical peaks of A-type starch, which has strong diffraction peaks at $2\Theta = 15°$ and $23°$ and a doublet at $2\Theta = 17°$ and $18°$. Similar spectra of BRF and SYN-GRF indicate that the polymorphic form of starch remains the same, implying minimal effect of treatment on polymorphic crystalline structures. Amylose-lipid complex appears as weak intensity peak at $2\Theta = 20°$ only for BRF (Zhu, Liu, Wilson, Gu, & Shi, 2011). Based on the crystallinity percentage (Table 2), it can be concluded that the treatment increases crystallinity of BRF by reducing amylose content.

Gelatinization properties of BRF, COMM-GRF and SYN-GRF shown in Table 2 are in the range of the values reported in the study of Singh, Singh, Kaur, Sodhi, and Gill (2003). However the differences in T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature) and ΔH_{gel} (gelatinization enthalpy) are insignificant due to low ultrasound power used in this study which had less effect on granule and properties of rice starch (Yu et al., 2013). Gelatinization temperature of COMM-GRF starch was lower
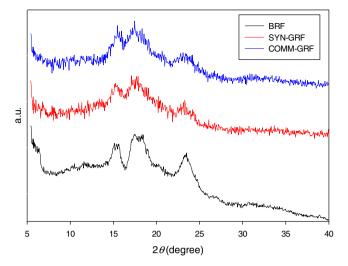

Fig. 2. X-ray diffraction pattern of rice flours.

than that of BRF starch. This result agrees with the result of Varavinit, Shobsngob, Varanyanond, Chinachoti, and Naivikul (2003) about the relationship between amylose content and gelatinization temperature of starch. Theoretically, gelatinization is initiated by granule swelling due to hydration and swelling of the amorphous region (Wani et al., 2012). During gelatinization, hydrogen bonds between amylose and amylopectin in semi-crystalline region in starch need to be disrupted first for water to be inserted, which inflicts higher gelatinization temperature in normal rice. Glutinous rice contains very small amylose content and since its crystalline region consists of short chain of glucose, thus it needs lower temperature to melt or dissociate. From Table 2, it can be seen that SYN-GRF has a slightly higher gelatinization temperature than that of the BRF. Sodium ions remained in starch, which cannot be removed completely by washing, enhanced the
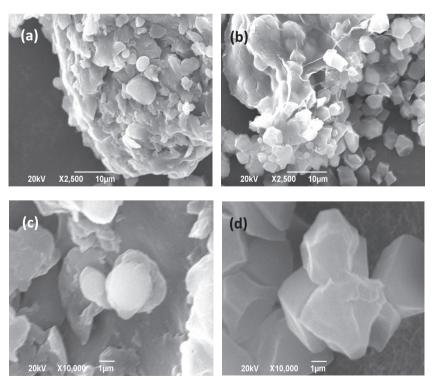

Fig. 1. SEM images of BRF (a and c) and SYN-GRF (b and d).

Table 2
Physical properties of BRF, SYN-GRF and COMM-GRF.

Flour samples	Gelatinization (°C)					Swelling power (g/g)	Solubility (%)	Crystallinity (%)
	T_o	T_p	T_c	T_c-T_o	ΔH_{gel} (J/g)			
BRF	61.43	70.17	75.74	14.31	8.327	10.79	5.17	18.41
SYN-GRF	62.04	70.53	77.27	15.23	8.014	14.06	21.54	21.30
COMM-GRF	61.10	69.04	75.33	14.23	8.673	12.10	15.22	20.83

Table 3
Pasting properties of BRF, SYN-GRF and COMM-GRF.

Flour samples	PT (°C)	t_p (min)	PV (RVU)	HPV (RVU)	BD (RVU)	CPV (RVU)	SB (RVU)
BRF	62.9	6.4	104.8 ± 0.8	92.7 ± 0.5	12.1 ± 0.3	133.2 ± 2.4	40.5 ± 1.9
SYN-GRF	62.6	4.8	198.9 ± 2.4	93.4 ± 1.1	105.5 ± 1.3	106.1 ± 0.3	15.7 ± 0.8
COMM-GRF	62.2	2.9	195.6 ± 1.4	98.1 ± 1.2	97.5 ± 0.2	123.1 ± 3.3	25.0 ± 2.1

stability of starch granules through electrostatic interaction between these ions and hydroxyl groups of starch (Ahmad & Williams, 1999; Karim et al., 2008) that resulted in the slight increase of onset and peak temperatures. Broader temperature range between onset and conclusion temperature of SYN-GRF is in accordance with its increase in relative crystallinity (Zobel, 1992).

ΔH_{gel} represents the phase transition of granules from an ordered state to a disordered one during heating in excess water. It involves melting of both crystalline and double-helical regions (Jambrak et al., 2010). The endothermic enthalpy of COMM-GRF starch is higher than that of the BRF starch because COMM-GRF contains higher portion of amylopectin, which forms crystalline region that needs more energy to be disordered than that of BRF. It is interesting to observe that ΔH_{gel} of the treated sample is lower than that of original sample, although it possesses higher crystallinity. This can be interpreted as the effect of diffused ions in the starch granule that shaped molecular gap allowing water to readily diffuse through the compact structure of crystalline zone (Ahmad & Williams, 1999).

The data obtained in this study show that SYN-GRF has the largest swelling power, followed by COMM-GRF and BRF. The lower swelling power and solubility of BRF than other types of rice flour is due to its high amylose content, which tends to inhibit water mobility by the formation of less-opened double-helix structure (Sodhi & Singh, 2003). For the treated rice flour, swelling power increased significantly due to the removal of amylose, which permits starch granules to absorb more water and swell to a greater extent. The same trend in solubility and swelling power suggests that the increase in starch solubility is due to the effect of granule swelling, allowing the exudation of amylose into water (Techawipharat, Suphantharika, & BeMiller, 2008).

The pasting properties reflect the behavior of starch granule during cooking or processing. The viscosity of starch dispersion may change during water penetration, swelling of granule, breaking down of starch granular structure (gelatinization), and retrogradation of starch components. The pasting properties of BRF, SYN-GRF, and COMM-GRF measured by RVA are listed in Table 3. Reduced PT and t_p in SYN-GRF compared to BRF showed accelerated swelling due to lesser amylose content, which gave less resistance towards water penetration (Park, Ibanez, Zhong, & Shoemaker, 2007). SYN-GRF showed relatively similar PV as COMM-GRF, indicating similar swelling behavior of the amylopectin-rich starch. Higher HPV and remarkable BD of SYN-GRF signified a waxy starch identity, which is easier to be disrupted than the non-waxy starch during heating. The lower set back and cold paste viscosity of SYN-GRF indicated weaker retrogradation potential, which correlated to the lower amylose content.

It is also worth noting that no decrease in the HPV value of SYN-GRF proves that the integrity of starch granule was not disrupted by depolymerization during ultrasound-alkaline glycerol treatment (Lai, Karim, Norziah, & Seow, 2006). This statement is supported also by the result of GPC analysis that showed insignificant shifting of amylopectin curves and their peaks towards lower molecular weight direction (Fig. S3). Thorough observation on the chromatogram showed that only the amylose peak experienced considerable depolymerization by alkaline glycerol that may cause overestimation in the result of iodine colorimetric technique.

4. Conclusion

The amylose content of starch in broken white rice flour could be reduced from 27.27% to 1.43% by treating the flour using 77 wt.% glycerol in 1 N NaOH under ultrasonication for 1 h. Glycerol and alkali are responsible for amylose to have more solubility and stability in water. While there was an increase in ash content by adsorption of Na⁺, some components, such as protein, free sugar and lipid in rice flour were partly leached during treatment, which resulted in higher starch content in the treated flour. Low ultrasound intensity has less effect compared to glycerol concentration, alkaline condition, and treatment time. SEM images revealed that the granule shape changed, with insignificant destruction on granule surface due to application of alkaline condition and low sonication power. The method used in this study increased relative crystallinity through amylose removal, but retained starch crystalline structure as A-type starch. Sodium ions stabilize starch structure which increased gelatinization temperature while decreased gelatinization enthalpy. The treated rice flour (SYN-GRF) has higher swelling power and solubility than other rice flours. After the treatment, the pasting properties of the broken rice flour also changed, towards those similar to the commercial glutinous rice flour.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.02.068.

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