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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.
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, 2018).

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 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 molecule 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, pentanol, 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.

2.1. 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 chemicals.
grade anhydrous glycerol (J.T. Baker, USA), reagent grade NaOH (Fischer Scientific, UK), HNO3 (63%, May and Baker, UK), H2SO4 (98%, Scharlau, Spain), crystalline phenol (99%, Waiko, Japan) and NaNO3 (99%, Acros Organics, USA). Liquid a-amilase from Bacillus licheniformis (500 U/mg protein, 10 mg protein/mL, Sigma Aldrich, USA) and amyloglucosidase from Aspergillus niger (P300 U/mL, Sigma Aldrich, USA) were diluted in acetate buffer (pH 5, 0.2 M) to obtain the stock solutions of 450 U/mL a-amilase and 5.25 U/mL amy-loglucosidase, respectively. Anhydrous NaCH3COO (P99%, Nacalai Tesque, Japan) and glacial acetic acid (P99%, Scharlau, Spain) were utilized for making the buffer. Standard for glucose analysis was anhydrous a-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 pullu-lan 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 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 ultrasonic 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 HNO3 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 g, 10 min) to remove some solid residues. Clear supernatant (20 mL) was taken and filtered to pass a nylon membrane (0.2 lm). 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 NaNO3 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 g, 20 min). The clear supernatant was decanted and collected for analysis of total sugar. The retained residue was hydro-lyzed using 4 mL of 450 U/mL a-amilase 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% H2SO4 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 proper-ties. 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 Diffraction-tometer.
(XRD) with the X-ray beam operating at 30 kV, 10 mA and ambient temperature. The X-ray generator was run at Cu Kα wave-length (k = 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. Area of crystalline peak Crystallinity ð%Þ 
¼ Total area under XRD curve 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 mL aluminum pan (TA Instruments, USA). Deionized water (9 mL) 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 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 (To), peak temperature (Tp), conclusion temperature (Tc) and enthalpy of gelatinization (DHgel). 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. Swelling power δw = w – 100

Weight of dried supernatant δp = weight of the dry flour δd = 100 Weight of dried supernatant and percent solubility were calculated using the following equation. Solubility δs 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 (tp), 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, and is closely related to the rheological prop- erties of flour (Yu, Ma, Menager, & Sun, 2012). Therefore, the objec- tive of this study was firstly to focus on reducing the amylose content of BRF to less than 2% and then examined the physico- chemical properties of the product. Each experiment on the treat- ment process was carried out in triplicate. The effects of glycerol concentration, NaOH, treatment time and ultrasonication on pro- duc-t 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) sub- stance for food and drugs application. The effect of glycerol con- centration 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 (1998), 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 dis- appearance 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 gly- cerol 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.% glyc- erol resulted in opposite trend due to increasing viscosity of the system, which may hinder ultrasonic mixing of rice flour with sol- vent. 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

<table>
<thead>
<tr>
<th>Glycerol concentration (%)</th>
<th>25</th>
<th>50</th>
<th>65</th>
<th>70</th>
<th>75</th>
<th>77</th>
<th>85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylose content (%)</td>
<td>20.46 ± 1.10</td>
<td>12.99 ± 0.67</td>
<td>7.84 ± 0.34</td>
<td>5.84 ± 0.20</td>
<td>4.09 ± 0.02</td>
<td>3.34 ± 0.04</td>
<td>3.34 ± 0.01</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>85.8 ± 0.3</td>
<td>84.8 ± 0.4</td>
<td>91.1 ± 0.4</td>
<td>90.4 ± 0.4</td>
<td>90.3 ± 0.3</td>
<td>90.6 ± 0.2</td>
<td>90.6 ± 0.1</td>
</tr>
<tr>
<td>Composition (%)</td>
<td>91.1 ± 0.4</td>
<td>91.2 ± 0.4</td>
<td>91.2 ± 0.4</td>
<td>91.3 ± 0.3</td>
<td>91.3 ± 0.2</td>
<td>91.6 ± 0.1</td>
<td>91.7 ± 0.01</td>
</tr>
</tbody>
</table>

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
Chinachoti, and Naivikul (2003) about the relationship between amylose content and gelatinization flours. This result agrees with the result of Varavinit, Shobsngob, Varanyanond, SYN-GRF COMM-GRF a.u. 5 10 15 20 25 30 35 40 2θ (degree) Fig. 2. X-ray diffraction pattern of rice properties of rice starch (Yu et al., 2013). Gelatinization temperature of COMM-GRF starch was lower BRF are insignificant due to low ultrasound power used in this study which had less effect on granule and range of the values reported in the study of amylose content. Gelatinization properties of BRF, COMM-GRF and SYN-GRF shown in Table 2 are in the percentage (Table 2), it can be concluded that the treatment increases crystallinity of BRF by reducing weak intensity peak at 2θ = 20° only for BRF (Zhu, Liu, Wilson, Gu, & Shi, 2011). Based on the crystallinity implying minimal effect of treatment on polymorphic crystalline structures. Amylose-lipid complex appears as 18°. Similar spec- tra of BRF and SYN-GRF indicate that the polymorphic form of starch remains the same, 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 amylo- plast. 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 16clump in the matrices of plant cell wall. 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 ty- pical peaks of A-type starch, which has strong diffraction peaks at 2θ = 15° and 23° and a doublet at 2θ = 17° and 18°. Similar spec- tra 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 2θ = 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 crys- tallinity 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 dif- ferences in 12To (onset temperature), Tp (peak temperature), Tc (con- clusion temperature) and DHgel (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 BRF SYN-GRF COMM-GRF a.u. 5 10 15 20 25 30 35 40 20 (degree) 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 gel-a-
broken rice flour also changed, towards those similar to the commercial glutinous rice flour. After the treatment, the pasting properties of the gelatinization temperature while decreased gelatinization enthalpy. The treated rice flour (SYN-GRF) has higher swelling power. The method used in this study increased relative crystallinity through amylose removal, but retained alkaline condition, and treatment time. SEM images revealed that the granule shape changed, with content in the treated flour. Low ultrasound intensity has less effect compared to glycerol concentration, protein, free sugar and lipid in rice flour were partly leached during treatment, which resulted in higher starch in water. While there was an increase in ash content by adsorption of Na+, some components, such as 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 DHgel 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 lowest 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-helical 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 tp in SYN-GRF compared to BRF showed acceleration due to lesser amylose content, which gave less resis-tance 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 dis-rupted than the non-waxy starch during heating. The lower set back and cold paste viscosity of SYN-GRF indicated weaker re-trogradation 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 sup-ported 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 can be reduced from 27.27% to 14.3% by treating the flour using 77 wt. % glycerol in 1 N NaOH under ultrasonication for 1 h. Glycerol-alkali 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 gran-ule surface due to application of alkaline condition and low sonica-tion power. The method used in this study increased relative crystallinity through amylose removal, but retained starch crys-talline 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.
References