Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară 2014, **15** (2), pp. 135 – 148

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

PREPARATION AND PHYSICOCHEMICAL CHARACTERIZATION OF MODIFIED (ACETYLATED) GADUNG (*DIOSCOREA HISPIDA DENNST*) FLOURS

Andri C. Kumoro*, Rizka Amalia, Diah S. Retnowati, Catarina S. Budiyati, Ratnawati Ratnawati

Diponegoro University, Faculty of Engineering, Department of Chemical Engineering, Prof. H. Soedarto, SH Road-Tembalang, Semarang, 50275, Indonesia

*Corresponding author: andrewkomoro@undip.ac.id

Received: June, 04, 2014
Accepted: July, 18, 2014

Abstract: Acetylation is one of methods to alter the physicochemical properties of starch. This work aimed to investigate the effect of reaction time, glacial acetic acid/gadung flour (GAA/GF) mass ratio and *p*H on gadung (*Dioscorea hispida dennst*) flour acetylation at ambient temperature. The acetylation was carried out by reacting gadung flour slurry with GAA under alkaline condition. The results show that degree of substitution and swelling power of the acetylated flours increased with reaction time, while the solubility was not affected by reaction time after 10 minutes acetylation. The GAA/GF mass ratio inversely affected the solubility of acetylated flour, but did not affect the swelling power and degree of substitution. Acetylation changed the structure, morphology and crystallinity of gadung flour starch granules. The swelling power and solubility of all acetylated flours obtained in this work were higher than the native one.

Keywords: *acetylation, morphology, solubility, starch, swelling power*

INTRODUCTION

Gadung (*Dioscorea hispida Dennst.)* remains as one of the underutilized tubers, that is easily found in most Asia and Pacific regions during the dry season. Thanks to its carbohydrate content, this tuber has been used as a staple food during World War II, especially by people in the tropical and sub-tropical regions [1]. The resistant starch contained in this food source promotes slow digestion in the lower parts of the human gastrointestinal tract, resulting in slow liberation and absorption of glucose. Aprianita *et al*. [2] suggested that this digestive property has shown the potentials of gadung tuber in reducing the risks of being obesity, diabetes and other related diseases. In addition, gadung tuber also does not contain gluten, which makes it becomes an important substance in the reduction in the incidence of celiac disease (CD) or other allergic reactions [3].

Apart to its superiorities, gadung tuber also possesses drawbacks, specifically related to its antinutritional substances contents, such as dioscorine and cyanides. To prevent serious dietary cyanides exposure, the glucosides and their derivative substances, popularly known as cyanogens, must be removed from the food source before being consumed. Effective processing modes may reduce all cyanogens in cassava and gadung tubers to below the safe level of 10 mg HCN equivalent per kg body weight as set by WHO 1988 [4, 5].

Tuber flours, which contain native starches, have been used since ancient times as raw material to prepare a variety of products. Starch is utilized for its various functionalities in thickening, stabilizing, texturizing, gelling, film forming, encapsulation, moisture retention and shelf life extension [6]. They are also used to help retain moisture in baked goods, as anticaking agents in baking powder and as a moulding medium in confections [7]. However, they have limitations i.e. low shear stress resistance, thermal decomposition, high retrogradation, and syneresis that reduce their use in industrial applications. The hydrophilic nature of starch is a major constraint that seriously limits the development of starch based products; so starch modification is a way to solve the problem and produce water-resistant materials [8].

Starches can be modified by chemical, physical, and enzymatic methods to improve desirable functional properties. The modified starches generally show better paste clarity, stability, increased resistance to retrogradation, and increased freeze-thaw stability [9]. Acetylated starch is a granular starch ester obtained by esterification of native starch with acetic anhydride at low temperature [6, 10]. Rutenberg and Solarek reported that the introduction of acetyl groups upon acetylation reduces the bond strength between starch molecules and thereby increases the swelling power and solubility of the starch granule, decreases the coagulation of the starch, and provides improved freeze-thaw stability [11]. Phillips *et al*. found that the extent of physicochemical property changes in the acetylated starch compared to the native starch is proportional to the degree of acetylation or degree of C=O substitution incorporated into the starch molecules [12]. Acetylated starches are widely used in a large variety of foods including baked goods, canned pie fillings, sauces, retorted soups, frozen foods, baby foods, salad dressings, and snack foods [13]. As in all chemical reactions, acetylation depends upon factors such as reactant concentration, reaction time, *p*H, and the presence of catalyst, which finally determine the number of acetyl groups incorporated into the molecule [14]. The objective of this study was to evaluate the effect of reaction time, glacial acetic acid: gadung flour mass ratio and *p*H on the swelling power, solubility and morphology of glacial acetic acid modified gadung tuber flour.

MATERIALS AND METHODS

Materials

Native gadung flour (GF) used in this work was obtained from milling and sieving of treated gadung tuber chips using a method suggested by Kumoro *et al*. [5, 15]. Glacial acetic acid (GAA) and other chemicals used in this work were of analytical grade (≥99.90 % w/w), purchased from Sigma-Aldrich Pte. Ltd. (Singapore) and directly used without further purification.

Methods

The acetylation was carried out by dispersing 20 grams of gadung flour in 100 mL distilled water in a 500 mL beaker glass to obtain gadung flour slurry with 20% consistency. To facilitate good flour dispersion, a magnetic stirrer was employed for agitation. The *p*H of the dispersed system was then adjusted to a certain value (8, 9 or 10) by addition of 1 M NaOH. Then, a predetermined mass of glacial acetic acid was added to the reaction mixture which caused a drastic reduction of *p*H. The *p*H was brought back to the initial condition (8, 9 or 10) by addition of 1 M NaOH. The acetylation reaction was let to occur for 10 to 60 min before being terminated by bringing the *p*H to 5.5 through addition of 1 M HCl. The acetylated flours were obtained by filtration of the reaction mixture and followed by threefold washing of the flours using distilled water. The acetylated flours were then dried in an electric oven at 50 ^{o}C till dryness before subjected to degree of substitution, swelling power, solubility, SEM and XRD analysis.

The degree of substitution (*DS*) was determined titrimetrically, following the method of Sodhi and Singh [16]. One gram of acetylated starch was placed in a 250 mL flask and 50 mL of 75 % (v/v) ethanol was added. The loosely stopper flask was agitated, warmed to 50 ^{o}C for 30 min, cooled and 40 mL of 0.5 M KOH were added. The excess alkali was back-titrated with 0.5 M HCl using phenolphthalein as an indicator. A blank, using the original unmodified starch, was also used. Degree of substitution is defined as the average number of sites per glucose unit that possess a substituent group.

$$Acetyl\ \ (\%) = \frac{[(blank\,(mL) - sample\,(mL)) \times Molarity\ of\ \ HCl \times 0.043 \times 100]}{Sample\ weight\,(g)} \qquad (1)$$

$$DS = \frac{(163 \times Acetyl(\%))}{[4300 - (42 \times Acetyl(\%))]} \qquad (2)$$

Solubility (*WS*) and swelling power (*SP*) of gadung flours were determined by modifying the method of Li and Yeh [17]. A starch sample 1.0 g (W_0) was accurately weighed and quantitatively transferred into a clear dried test tube and re-weighed as W_1.

The starch was then dispersed in 50 mL of distilled water. The resultant slurry was heated at 60 ^{o}C for 30 min, with constant mixing. The mixture was cooled to 30 ^{o}C and centrifuged at 100 × g for 15 min in a Superspeed centrifuge (Sorvall® RC-6, Kendro laboratory products, NC, USA). Aliquots (5 mL) of the supernatant were dried to a constant weight at 110 ^{o}C and denoted as W_c. The residue obtained after drying the supernatant represented the amount of starch solubilized in water. Solubility was calculated as g per 100 g (%) of starch on a dry weight basis.

$$WS = \frac{W_c}{W_0} \times \frac{50}{5} \times 100 \tag{3}$$

The supernatant was separated and swollen starch as the precipitate was weighted. The residue obtained from the above experiment (after centrifugation) with the water it retained was quantitatively transferred to the clean dried test tube used earlier and weighed (W_2). To calculate the swelling power, the weight of residue was divided by the original weight after solubility subtraction [18].

$$SP = \frac{W_2 - W_1}{W_0 \times (100 - WS)} \tag{4}$$

The microstructural analysis of the native and acetylated gadung flour was performed using a scanning electron microscope (FEI Inspect S-50 that using data analysis software server XT microscope). The sample surfaces were coated with a thin layer of gold using a Bal-Tec SCD 005 sputter coater to provide electrical conductivity. X-ray diffraction measurements were performed on a diffractometer (PanAnalytical Xpert Pro) using Cu Kα radiation. The samples were scanned over a 2θ range varying from 5° to 40°. The crystallinity percentage (*% C*) was determined from the diffractogram by calculating the area corresponding to the crystalline peaks [19]:

$$\%C = \frac{A_p}{(A_t - N)} \tag{5}$$

Where *Ap* is calculated from the difference between the area under the curve and the area of the amorphous halo; At is the total area under the curve; *N* is the instrumental noise (*N*), according to the following equation:

$$N = 149.6 cps * \deg \tag{6}$$

The amorphous halo was determined with the amorphous component of starch obtained with an extraction procedure previously reported by Bogracheva *et al.*, where a completely gelatinized sample on the Rapid Visco Analyzer (RVA-4) was used [20].

RESULTS AND DISCUSSION

The proximate analysis of native gadung flour

Proximate analysis of food materials is intended to determine macro components content, such as carbohydrate, protein, fat, crude fiber, ash and moisture. Proximate composition of gadung tubers, gadung tuber flour and their comparison with other tuber crops are presented in Table 1.

Table 1. *Chemical composition of gadung tuber and flour, and other tuber flours (%)*

Component	Gadung tuber	Gadung flour	*Dioscorea esculenta* flour [19]	*Colocasia esculenta* flour [20]
Carbohydrate	18	92.3	19.38	17.8
Protein	1.81	1.1	1.26	4.69
Fat	1.6	0.9	0.18	0.97
Fiber	1.9	2.4	0.49	2.8
Ash	0.7	0.3	1.19	1.88
Moisture	77	3	77.15	72.1

As seen in Table 1, the carbohydrate content in the gadung tuber is comparable to the other tubers, which is 18 % of the edible portion. This value is high enough and makes gadung tubers can be one of reliable food sources ensure food security, especially during drought. The protein content of gadung tuber is 1.81 %, which is slightly higher when compared with its closest relatives, the *Dioscorea esculenta* tuber [21]. However, gadung tuber protein content is less than half of the protein content in taro (*Colocasia esculenta)* tuber [22]. Therefore, protein enrichment is compulsory if gadung tubers will be used as food material. High fat content in gadung tubers is offset by its high crude fiber content. Therefore, gadung tubers are best consumed by people suffering from indigestion. Although the ash content is less than half of that of taro tubers, but the ash content in tubers of yam tuber is also almost equivalent to *Dioscorea esculenta* tuber. However, high moisture content in the gadung tuber (77 %) makes this tuber vulnerable to deterioration by fungal attacks. That is why, this research suggests gadung tubers to be stored in the form of flour with moisture content not exceeding 3 % so that tuber damage by fungi and other microfloral activities can be avoided. Table 1 also shows that attempt to transform gadung tubers into gadung tuber flour causes only a slight decrease in of protein, fat and ash content, but increased carbohydrates and crude fibre contents.

Effect of glacial acetic acid/gadung flour (GAA/GF) mass ratio

While most acetylation of starch is conducted using nucleophilic agents such as acetic anhydride of vinyl acetate, this work employ glacial acetic acid as a modifying agent. The formers are more reactive, but they are also more expensive and hazardous to human. Acetylation of gadung flours was carried out using slurry method employed distilled water as a dispersing agent and sodium hydroxide as a catalyst at ambient temperature. Effect of GAA/GF mass ratios on water solubility and swelling power of acetylated gadung flour is presented in Table 2.

As seen in Table 2, native gadung flour has low swelling power and water solubility, which are 6.9 ($g{\cdot}g^{-1}$) and 4 %, respectively. These values are slightly higher than those reported by Tatiyakul *et al*. for taro tuber flour [23], which are 2.5 ($g{\cdot}g^{-1}$) and 3.5 %. While Adebowale *et al*. reported that the swelling power and water solubility of Bambarra groundnut flour were 2.59 ($g{\cdot}g^{-1}$) and 5.5 %, respectively [24]. The low swelling power and water solubility of gadung flour is mainly related to its high amylose content. The amylose content of gadung starch and flour are 39.30 and 34.72 %, respectively [15, 23].

Table 2. *Effect of GAA/GF mass ratio on WS, SP and DS of acetylated gadung flours*

Time (min)	**GAA/GF Mass Ratio**								
	1:3			**1:4**			**1:5**		
	WS (%)	**SP ($g\cdot g^{-1}$)**	**DS (-)**	**WS (%)**	**SP ($g\cdot g^{-1}$)**	**DS (-)**	**WS (%)**	**SP ($g\cdot g^{-1}$)**	**DS (-)**
0	4.0	6.9	0	4.0	6.9	0	4.0	6.9	0
10	8.0	7.2	0.16	6.0	7.1	0.17	4.0	7.1	0.17
20	9.0	7.2	0.17	6.0	7.2	0.17	4.0	7.2	0.18
30	9.3	7.3	0.18	6.0	7.6	0.18	4.0	7.3	0.18
40	6.0	7.9	0.18	6.0	7.8	0.18	4.0	8.6	0.20
50	6.0	8.1	0.19	6.0	8.1	0.19	4.0	8.5	0.18
60	6.0	8.4	0.17	6.0	8.3	0.18	4.0	8.8	0.17
Korean wheat flour WS: 7.8-9.3 (%) SP: 7.3-8.5 ($g\cdot g^{-1}$) [25]									
American wheat flour WS: 6.8-7.9 (%) SP: 6.3-7.3 ($g\cdot g^{-1}$) [25]									

High amylose content in the starch granules causes the amylose molecules in the form of crystalline become more compact and interwoven by amylopectin. This structure form the surface of the starch granules become almost solid and hinder the diffusion of water molecules into starch granules [26]. High fat content in the gadung flour also allows the formation of complexes with amylose, which also inhibit amylose leaching and granules swelling. In addition to amylose-amylopectin ratio, swelling power and water solubility is also influenced by the molecular weight distribution of amylose and amylopectin, the degree and length of branches and conformation [27].

Table 2 also shows that the more glacial acetic acid used in acetylation as shown by larger GAA/GF mass ratio increased the water solubility of gadung flour. Similar result was also reported by Singh *et al*. on the acetylation of corn and potato starches [6], and Raina *et al*. who studied acetylation of rice starch [28]. As seen in Table 2, prolonging reaction time also improve water solubility of gadung starch in accordance with increasing degree of substitution. High amylose content in gadung flour slower the acetylation reaction speed and finally slower the increase of degree of acetylation. At low degree of substitution, the starch granules of gadung flour are predominantly in the form of crystalline or retrograded, which are insoluble in water at ambient temperature. Low acetylation reaction speed with an increase in amylose content might be due to amylose crystal or complexes. In its crystalline form, amylose usually only melts at higher temperature than amylopectin, therefore amylose is more resistant to swelling and reaction [29]. At reaction time longer than 40 minutes and GAA/GF of 1:3, a reduction in water solubility of acetylated gadung flour was observed. Shogren and Biswas reported that at low temperature, acetylation occurred at slow speed until the degree of substitution 0.3 was achieved at 40 minutes [30]. They also reported that water solubility of acetylated high amylose corn starch decrease if degree of substitution is higher than 1. This is possibly due to an increase in hydrophobicity of acetyl groups. As the degree of substitution increases, the number of substituted hydroxyl groups by acetyl groups also increased. This condition increases starch hydrophobicity and reduces hydrogen bonding in the starch molecules and finally retards hydration of starch [31]. Incorporation of acetyl groups to starch molecules reduces the interaction between starch molecules which increase water solubility and swelling power of starch granules. The introduction of acetyl groups into starch molecules also reduces the interaction between outer chains of amylopectin and interaction between amylose chains [10]. Acetylation also provides a way for water molecules to move toward the amorphous parts of starch caused by changes in the arrangement of intragranular structure as a result of steric effects and destruction of hydrogen bonds in starch grains [32]. In the starch molecule, there are three free hydroxyl groups with different levels of reactivity. Primary OH group located at position C_6 is highly reactive and will be immediately react with the acetyl group when compared with the two secondary OH groups at positions C_2 and C_3 as a result of steric hindrance. Primary OH groups are located on the outer surface of the starch molecules so that they can immediately react with the acetyl group. Meanwhile, two secondary OH groups located in the inner surface of the starch molecules form hydrogen bonds with the OH groups owned by nearby glucose units. Among the secondary OH groups, the OH group on C_2 is more reactive than the OH group at C_3. This happens because the OH group at C_2 is more acidic and closer to the hemiacetal [33].

After being modified with glacial acetic acid, gadung flour has higher swelling power than the native flour. However, acetylated gadung flour swelling power is not significantly influenced by the amount of acetic acid used (GAA/GF value) in the acetylation process. As expected, acetylated gadung flour swelling power continues to increase with increasing reaction time. As shown in Table 2, the swelling power values of acetylated gadung flour at certain reaction time (0-30 minutes) but with different mass ratio of GAA/GF are very close to each other. Similar result is also reported by Singh *et al*. on the acetylation of corn starch [6]. This phenomenon is thought to be caused by the presence of fat in the gadung flour [34]. Because acetylation on the study was carried out at low temperatures, the acetylation occured only in the amorphous starch grains, causing swelling in that section. Leaching of amylose into water almost impossible at low temperatures, but with the strong bonds between the water molecules with the outer branches of the starch molecules will form a gel [30].

Gadung flour would be modified to suit industrial applications with very low level of acetate group donors. de Graaf *et al*. suggested that acetylated starches with degree of substitution (DS) between 0.01-0.2 are approved by the FDA for used as food materials [35]. Their usage is based on properties with respect to film forming, binding, adhesivity, thickening, stabilizing and texturing. With that point of view, acetylation of gadung flour using glacial acetic acid is targeted to obtain acetylated gadung flour with DS within 0.01-0.2. The degree of substitution increases with reaction time. However, the degree of substitution did not significantly affected by mass ratio of glacial acetic acid to starch. This finding is consistent with that reported by Singh *et al*. who observed that DS increased progressively with increase in level of acetic anhydride (up to 8 %) during the acetylation of potato starch using acetic anhydride [6]. However, any further increase in level of acetic anhydride (up to 12 %) did not result in an increase of DS. With GAA/GF mass ratios of 1:3, 1:4 and 1:5, the glacial acetic acid concentrations used in this work were corresponding to between 16.67-25 %, so was higher than 12%. The longer the reaction time, the more time is available for an acetyl group to diffuse and undergo adsorption to the surface of starch molecules [36]. However, this increase is not linear. The reaction takes place very rapidly at the beginning of the reaction (first 10 min) and then slowed down after the reaction lasted for 20 minutes. This can be explained by the fact that as the acetylation reaction proceeded, the glacial acetic acid is depleted. In addition, the reactive sites on the starch molecules are also increasingly reduced as a result of structural modification of the starch molecule backbone [37]. Instead of being affected by esterification reaction, the lower the concentration of acetyl groups in the reaction medium with increasing reaction time can also be caused by hydrolysis of the ester starch (starch acetate) produced or elimination reactions as a result of excess water (from starch and by-products esterification) are not discharged from the reactor during the reaction [38]. However, the highest degree of substitution obtained from gadung tuber flour acetylation with glacial acetic acid at 40 minutes was only 0.24, or still lower when compared with the degree of substitution of corn starch, which is about 0.27 [31]. This is likely due to differences in amylose content and structure, grain size and fragility of yam tuber starch in the flour and corn starch.

Based on the value of swelling power and water solubility of acetylated gadung tuber flour obtained, the best reaction conditions chosen were the mass ratio of GAA/GF flour 1:3, *p*H 8, and the reaction time of 30 minutes. Acetylated gadung flour obtained has swelling power and water solubility 7.3 ($g \cdot g^{-1}$) and 9.3 (%), and meet the standards of water solubility and swelling power of Korean wheat flour [25].

Effect of *p*H

The *p*H of the flour slurry determines the successfulness of esterification reaction between starch and carboxylic acid molecules. The effect of *p*H on the swelling power and water solubility of acetylated gadung flours is detailed in Table 3. Table 3 shows that at *p*H 9 and 10, the water solubility of acetylated gadung flours were the same as the native one. Acetylation at moderate alkalinity did not change the water solubility of gadung flour. However, acetylation of gadung flours at low alkalinity (*p*H 8) results flours with higher water solubility. Similar phenomenon was also found by Adebowale *et al*. during their study on acetylation of Bambarra groundnut flour [24]. The increase in water solubility of flour is a result of an increase in starch hydrophobicity. In addition, the water solubility of the starch also depends on degree of substitution and polymerization [39].

Table 3. *Effect of pH on WS, SP and DS of acetylated gadung flours*

Time (min)	pH								
	8			9			10		
	WS (%)	SP ($g \cdot g^{-1}$)	DS (-)	WS (%)	SP ($g \cdot g^{-1}$)	DS (-)	WS (%)	SP ($g \cdot g^{-1}$)	DS (-)
0	4.0	6.9	0	4.0	6.9	0	4.0	6.9	0
10	8.0	7.2	0.16	4.0	6.5	0.20	4.0	8.1	0.20
20	9.0	7.2	0.17	4.0	6.7	0.21	4.0	8.7	0.23
30	9.3	7.3	0.18	4.0	6.9	0.22	4.0	7.8	0.23
40	6.0	7.9	0.18	4.0	7.4	0.23	4.0	7.4	0.24
50	6.0	8.1	0.19	4.0	7.2	0.21	4.0	7.5	0.23
60	6.0	8.4	0.17	4.0	7.6	0.20	4.0	8.9	0.22
Korean wheat flour WS: 7.8-9.3 (%) SP: 7.3-8.5 ($g \cdot g^{-1}$) [25]									
American wheat flour WS: 6.8-7.9 (%) SP: 6.3-7.3 ($g \cdot g^{-1}$) [25]									

Table 3 also reported that in general the swelling power of acetylated gadung flours were higher than that of native gadung flour. Highest increase in swelling power was

found for acetylation at *p*H 10, while the lowest was observed during acetylation at *p*H 9. Adebowale *et al*. reported that *p*H did not significantly affect the swelling power of acetylated Bambarra groundnut flour and starch [24]. Only slight changes in swelling power values were found during acetylation of Bambarra groundnut in either acidic or basic conditions. They also reported that the increase in swelling power was more pronounced under basic condition (*p*H 8-10) than acidic condition (*p*H 2-6). This phenomenon might be due to interaction between protein and starch. Under basic condition, both starch and protein bring positive charge, while under acidic condition only protein brings positive charge [40]. Therefore, swelling is more pronounced at basic condition, and being depending on the protein content in the starch.
Table 3 shows that the degree of substitution increases with *p*H of the reaction medium. As a catalyst, sodium hydroxide initiates the reaction by forming alcoholate ions along the starch polymer, resulting in activated starch molecules [41]. Song *et al*. reported that at *p*H 9.5, side reaction (acetate starch hydrolysis) will be the dominant reaction [42]. In contrast to that, at *p*H 7.5 hydroxyl groups in the starch molecules are not activated enough to perform nucleophilic substitution attack to the unsaturated electrophilic carbon of acetic acid molecules [43]. The nuclecophilic reaction involves addition and elimination mechanisms [44]. Song *et al*. [42] and Jeon *et al*. [45] found that the optimum condition for esterification of rice starch was at *p*H 8.
As seen in Table 3, *p*H 8 is the preferred esterification condition for gadung flour as at this condition the reaction yielded gadung flour with similar swelling power and water solubility with Korean wheat flour [25]. Hui *et al*. also found that *p*H 8 is the best reaction condition for esterification of potato starch with anhydride octenyl succinate [43]. In addition, Bhosale and Singhal also reported *p*H 8 as the optimum *p*H for esterification of corn and amaranth starches with anhydride octenyl succinate [46].

Morphology of the native and acetylated gadung flours

The micrographs of native and acetylated gadung flours obtained from Scanning Electron Microscopy analysis are presented in Figures 1.

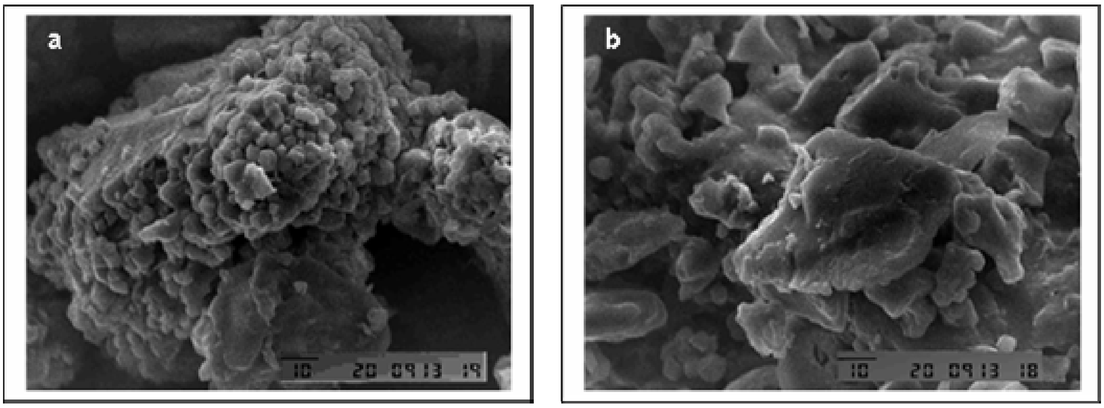

Figure 1. *SEM image of native (a) and acetylated (b) gadung flours (1000× magnification, Bar = 10µm)*

Figure 1 (a) shows that gadung starch granules are smooth-surfaced and polyhedral. The surface-average diameter of the granules is about 4.0 mm. Tattiyakul *et al*. also reported similar finding [23]. As seen in Figure 1 (a) and (b), the structure and morphology of gadung flours changed significantly in their size and shape after being modified by acetylation. Similar results were reported by Singh *et al*. on acetylated corn and potato starch [6].

Figure 1 (b) shows that the surface of acetylate gadung flours resembles flat flower petals. The wavy surface of acetylated gadung flours was a result of gelatinization of the starch surface when NaOH added to the reaction system to maintain the *p*H during the introduction of glacial acetic acid as reported by Luo and Shi on their corn starch acetylation study [31]. In addition, as the acetylation took place the starch granules developed blisters or small protuberances on their surfaces. A deep groove was also formed in the central core region of the starch granule and appeared as folded structures with their outer sides drawn inwards. The acetylated gadung flour starch granules size was within 25-70 μm, which were far bigger than the native ones. Changes in granule sizes also indicate a loss of order in the starch structure due to the chemical modification, and a fusion of starch granules was observed. Acetylation causes aggregation or cluster formation of starch granules in the gadung flours. The agglomeration takes place as a result of introduction of hydrophilic acetyl groups into starch molecules, which increases the number and strength of hydrogen bonding. Then, the starch molecules are very close to each other forming starch clusters and finally combine to form aggregates [6]. The granule fusion was observed to be more pronounced for small size granules compared to the bigger ones. Singh *et al*. also found similar phenomena for the acetic anhydride modified potato starch [6].

The X-ray diffraction patterns and intensities of the major diffraction peaks of the native and acetylated gadung flour are presented in Figure 2.

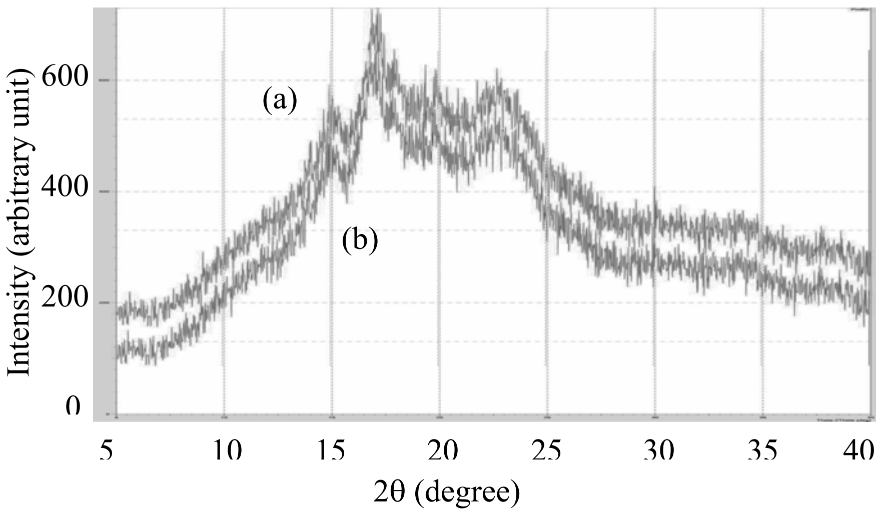

Figure 2. *Diffractogram of native (a) and acetylated (b) gadung flour*

Figure 2 shows the X-ray diffractogram spectra of native and acetylated gadung flours. We can observe that native gadung flour exhibited maximum peak intensity at Bragg angle (2θ) of 17.09º and other strong peaks were found at 15.03º, 18.11º and 22.93º (2θ). This finding is in good agreement with Tattiyakul *et al*. [23]. They reported that gadung tuber starch exhibited maximum peaks at (2θ) of 17.27º. Other significant peaks

were 5.67°, 14.47°, 22.27° and 24.7° (2θ) [23]. The results confirm that gadung tuber starch is of B-type, which is typical for most tuber starches.
On the other hand, the acetylated gadung flour exhibited maximum peak intensity at 17.03° (2θ). The other significant peaks were 14.98°, 17.95° and 21.94° (2θ). Acetylation has altered gadung starch crystallinity as indicated by the shiftings of all Bragg angles of the peaks to lower angles. Acetylation has caused the crystallinity of starch in the gadung flour decreased from 5.27 % to 2.02 %. Sha *et al*. also reported that rice starch crystallinity reduced after acetylation due to a lot of acetyl groups introduction in the rice starch molecules [47], while Bello-Perez *et al*. reported loss of crystallinity of barley starch [48]. This slight loss of crystallinity is owed to the acetylation that took placed in the crystalline region of the starch. However, due to the fact that most starch modification took placed in the amorphous region (composed by the amylase), and that does have a low degree of substitution, the crystalline region is not significantly damaged. The intra and intermolecular hydrogen bonds are responsible for highly ordered crystalline structure [49]. As more hydroxyl groups in the starch molecules are substituted by acetyl groups, the remaining hydroxyl groups have less opportunity to form intermolecular hydrogen bonds, and thereby resulted in the destruction of the ordered crystalline structure [38]. Additionally to disorganization of starch components during the acetylation, partial depolymerisation may be produced in starch components, principally in the amylopectin [48].

CONCLUSION

Based on the experimental results, a good process condition for the production of acetylated gadung flour is the acetylation process at a weight: volume ratio of gadung flour: water (1:5), the mass ratio of glacial acetic acid: gadung flour (1:3), *p*H 8.0 and reaction time 30 minutes. This process condition provide the acetylated gadung flours of 0.18 degree of substitution with similar swelling power and water solubility values of Korean wheat flour. Acetylation led to change gadung flours morphology, structure and crystallinity.

ACKNOWLEDGEMENT

The authors would like to thank the Directorate General of Higher Education, Ministry of National Education of The Republic of Indonesia for its financial support through Hibah Penelitian Strategis Nasional/ National Strategic Research Grant 2013 under contract No. 96/SP2H/PL/Dit. Litabmas/IV/2013.

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