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8DOI 10.1002/star. 201100169 **RESEARCH ARTICLE**

8**Facile preparation of sago starch esters using full factorial design of experiment**

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161 Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei, Taiwan 2 Department of Chemical Engineering,

Widya Mandala Surabaya Catholic University, Kalijudan, Surabaya, Indonesia A facile solvent-free method to acetylate sago starch (Metroxylon sago) is reported. Microwave (100 W) was used as the heating source and the heating time was varied from 2 to 10 min with the temperature of acetylation maintained at 100°C under continuous stirring. Using a 24 full factorial design of experiment, it was found that the

15 degree of substitution (DS) of acetylated sago starch was strongly affected by the ratio of starch

to acylating reagents, the ratio of acetic anhydride to acetic acid as the acylating reagents, the concentration of iodine as catalyst and reaction time. The physicochemical characteristics of the acetylated sago starch were assessed based on the FTIR spectra, the XRD spectra,

5 the water absorption index (WAI), and the water solubility index (WSI).

SEM was used to study the surface morphology of the acetylated sago starch at different DS. Keywords: Acetylation / Microwave / Sago Starch Received: October 31, 2011 Revised: January 29, 2012 Accepted: February 2, 2012 1 Introduction Palms (such as sago palm (Metroxylon sago)) constitute one of the oldest plant families on Earth. Sago palm grows well in tropical peat swamp rainforests, where other crop plants do not. It has great potential for starch production. Sago starch is extracted from sago pith and a single sago palm yields about 150–300 kg starch [1]. Grain of sago starch prepared from palm pith is

14 small, whitish, pinkish, or brownish. Sago starch is exported to Europe and America

for thickening soup and making puddings.

14 In Indonesia and India, it is boiled with sugar to make jelly

and porridge. Sago starch support the production of other types of food such as noodles, mono- Correspondence: Professor Yi-Hsu Ju,

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Fax: þ886-2-2737-6644 Abbreviations: DOE, design of experiment; DS, degree of substitution;

5 WAI, water absorption index; WSI, water solubility index

sodium glutamate, and soft drinks. In addition to being used as foodstuff,

24 **sago starch is also utilized to produce adhesive for paper, textiles, and plywood; as a stabilizer in pharmaceuticals;**

as raw material for producing biodegradable plastics, fuel alcohol, and ethanol [2]. In order to enhance and extend applications the mechanical properties of natural sago starch can be modified by altering its structure (e.g., affecting the hydrogen bonding of AM and AP in a controllable manner). Most common starch modifications in industry involve cross-linking, esterification, and etherification. Acetylated starch is the most typical starch ester in the market. Acetylation can be performed with relative ease to significantly improve the physicochemical and functional properties of the starch, such as lower gelatinization and pasting temperatures; improved freeze–thaw stability of pastes and gels; improved paste stability; higher redispersibility when pre-gelatinized; and greater clarity of paste and gel [3–5]. Acetylated starch (degree of substitution, DS<0.09) is used in many bakery, frozen and canned foods products and is also present in white salted noodles to improve Colour online: See the article online to view Fig. 1 in colour. texture, stability, and appearance [6]. Acetylated starch with DS higher than 0.09 has been used for non-food applications such as tablet binders, hot melt adhesives, coatings, cigarette filters, biodegradable plastics, and metal ion absorbents [7–11]. Acetylation is accomplished by treating starch slurry with acetic anhydride and sodium hydroxide [3, 5, 12], acetic anhydride and pyridine [13], and acetic anhydride and vinyl acetate [14]. However, this process uses a lot of chemically treated water to maintain pH and can cause wastewater handling problems and is time consuming. In this process certain reagents may be needed to rupture or weaken intermolecular bonds of starch to obtain high DS [15]. A simple solvent-free 10 min method was proposed to acetylate starch and other polysaccharides such as cellulose, corn starch, and potato starch [4, 7, 12, 15, 16]. No similar produce for sago starch has been published. Many factors are considered in selecting a reagent and preparative method. The reactivity of the reagent used is important to obtain high DS.

22 **Acetic anhydride in the presence of iodine as the catalyst**

9 **is an excellent acylating reagent for both starch and cellulose**

[7, 15–17]. However the use of acetic anhydride is permitted within specified limitation for foodstuffs by The United State Food and Drug Administration [18]. Acetic acid is a safe reagent for starch acetate intended for foods products. Iodine as a catalyst

9 **activates the carbonyl group of acetic anhydride, which**

9 **works only in the absence of a solvent.**

For both solvent-free and aqueous based reaction systems, micro-wave radiation is used as the energy source to reduce the reaction time. The objective of this study is to investigate the influence of acylating reagent (acetic anhydride and acetic acid, iodine), the ratio of starch-acylating reagent and heating time on the DS of acetylated sago starch using the solvent-free method as well as to evaluate the properties of the resulting products. 2 Materials and methods 2.1 Materials Commercial sago starch from Riau Indonesia was sieved passing 60 mesh and dried at 50°C for 24 h before processing (moisture content about 3.36%). Acetic acid Table 1. Variables considered for DOE and their levels (99%) and acetic anhydride (98%) were analytical grade from Sigma–Aldrich (St. Louis, USA). Sodium thiosulfate, ethanol, iodine, and potassium hydroxide were supplied by Merck (Darmstadt, Germany). Hydrochloric acid was obtained from Fluka (Steinheim, Germany). All chemical used in this work were analytical grade. 2.2 Synthesis of starch acetate A 4 g sago starch sample (24.5 mmol of anhydroglucose unit or AGU [15]) was put into a 50 mL round-

bottom glass flask with a magnetic stirrer, followed by a mixture of acylating reagents (acetic acid and acetic anhydride) and iodine (as the catalyst). Then, the round-bottom glass flask was placed in a microwave reactor (Microwave CEM Atmospheric Pressure, Microwave Synthesis/Extraction System, Matthews NC, USA), equipped with a reflux condenser. The acetylation was performed at 100°C under continuous stirring and 100 W of microwave radiation. After a certain reaction time, the flask was cooled to <50°C, then was removed from the microwave oven and cooled to room temperature. Variables such as ratio of starch to acylating reagent, ratio of acetic acid to acetic anhydride, amount of iodine and reaction time, that affect the reaction were studied using a 24 full factorial design of experiment (DOE; Table 1).

20 A saturated solution of sodium thiosulfate was added to the

mixture and stirred until

21 the mixture color changed from dark brown to colorless, indicating the transformation of iodine to iodide [16]. Then, ethanol was

20 added to precipitate the mixture. The mixture was filtered, washed with

ethanol and distilled water to remove the unreacted acetic acid and byproducts. The acetylated sago starch was dried in a vacuum oven (Lab-Line Duo-Vac Oven, Lab-Line Instrument Inc., Melrose Park, ILL, USA) at 60°C for 3 h. The dried acetylated starches obtained under different conditions were ground to fine powder before being analyzed further.

23 2.3 Determination of the degree of substitution (DS) Degree of substitution was determined by the complete basic hydrolysis of

15 the ester linkages and titration of the excess alkali,

following the method used by [16]. Parameters Mole ratio of starch to acylation reagents (A) Mole ratio of acetic anhydride to acetic acid (B) Iodine catalyst (%mole of starch) (C) Reaction time (min) (D) Low level (1) 1:2 1:0 0.15 2 Center point (0) 1:3 1:1 2.58 6 High level (p1) 1:4 0:1 5 10 F. Edi Soetaredjo et al. Acetyl content (%A) was calculated based on the following equations: $\%A = \frac{V_0 - V_n}{V_0} \times \frac{N}{M} \times 100$ (1) where V_0 and V_n are volume

6 (mL) of HCl used to titrate blank and sample, respectively; N is the normality of used HCl, M is sample amount as dry substance (g),

43 is the MW of acetyl group.

6 Acetyl content (%A) was used to calculate the DS:

DS $\frac{1}{2} \times \frac{\%A}{162} \times 100$ (2) where 162 is the MW of the anhydroglucose unit, 1 is the hydrogen mass. 2.4

17 Fourier transform-infra red (FT-IR) analysis FT-IR spectra of

native and acetylated sago starch were acquired on a Shimadzu 8400S FT-IR (Shimadzu Corporation, Kyoto, Japan) using potassium bromide (KBr) method. 2.5 XRD analysis Powder XRD patterns of native and acetylated sago starch (DS $\frac{1}{4}$ 1.2)

12 were recorded on a Rigaku Miniflex Goniometer at 30 kV and 15 mA, using Cu Ka radiation at a step size of 0.018. 2.

6 SEM analysis The morphological features of native and acetylated sago starch with different DS were observed with a Cambridge scanning electron microscope (S-360) at an accelerating voltage of 20 kV. Dried starch granules were mounted on a double-sided tape attached to a metal stub and sputtered with gold in order to make the sample conductive. 2.7 Water absorbance

18 index (WAI) and water solubility index (WSI) Water absorption index (WAI) and water solubility index (WSI) of

native and acetylated sago starch were measured using a modified method of AACC method 56–20. The experimental detail was described by [16]. WAI (g/g) and WSI (%) were calculated by the following equations: $WAI = \frac{W_2 - W_1}{W_3 - W_1} \times 100$

7(3) where W_s is weight of the tube with the sediment, W_t is weight of the empty tube,

and W_1 is weight of the dry sample and $WSI = \frac{W_3 - W_2}{W_3 - W_1} \times 100$ (4) where W_3 is weight of the dish and with dry solute, W_2 is

8 weight of the empty dish and W_1 is weight of the dry sample.

2.8 Statistical analysis The statistical DOE involved the simultaneous study of several process variables in the acetylation of sago starch. The significance of each factor and their interaction effects were evaluated by using two-level full factorial DOEs [19]. The potential variables were classified as controlled variables (Table 1) and constant variables. Constant variables such as reaction temperature, agitation speed, and microwave power were held at 100°C, medium stirring (equipment setting), and 100 W, respectively. Each controlled variables were kept at three levels, high level (p1), center point (0), and lower level (1) as listed in Table 1. After conducting all trials, results were analyzed by analysis of variance (ANOVA) technique using Minitab 14 software. 3 Results and discussion 3.1 Effect of variables on DS Facile preparation of starch acetate was performed by the reaction of starch with acylating reagents (acetate anhydride and acetic acid) using iodine as the catalyst and with the assistance of microwaves. The matrix for four variables varied at two levels (p,) and the corresponding DS obtained are shown in Table 2 according to DOE. In the range of variables studied, the highest DS obtained is 1.202. The determination of the significant factors affecting DS was done by performing two-way ANOVA (Table 3). After the effects of individual factors were assessed using factorial design; it turned out that the ratio of starch to acylating agent, the ratio of acetic anhydride to acetic acid and the iodine concentration all are significant factors ($p < 0.05$) while the reaction time was not ($p > 0.05$). For the assessment of two variables interaction, all binary interactions were showed to have significant effect on DS ($p < 0.05$). The ANOVA result also showed that three level interactions were insignificant when reaction time was excluded from the assessment. This demonstrated that individual factor interaction in the synthesis of starch acetate was not significant but the interaction between variables determines the DS of starch acetate. The effect of four variables used in this study is also shown in Table 3. The ratio of acylating agent (acetic anhydride and acetic acid) and reaction time have a negative effect on the

et al. Table 3. DS ANOVA table for sago starch acetylation Factors/Interactions

**3Constant A B C D A B A C A D B C B D C D A B C A B D A C D B C D A B C
D**

Centre point S $\frac{1}{4}$ 0.0431929 R-Sq $\frac{1}{4}$ 99.35% R-Sq (adj) $\frac{1}{4}$ 98.70% Effect 0.1690 0.3930 0.4632 0.0158
0.0760 0.1502 0.0458 0.3313 0.0452 0.0870 0.0063 0.0398 0.0805 0.0465 0.0550 Coef 0.3810 0.0845
0.1965 0.2316 0.0079 0.0380 0.0751 0.0229 0.1656 0.0226 0.0435 0.0031 0.0199 0.0403 0.0232 0.0275
0.2500 SE Coef 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635
0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.007635 0.043863 T 49.90 11.07 25.74 30.34
1.03 4.98 9.84 3.00 21.69 2.96 5.70 0.41 2.60 5.27 3.04 3.60 5.70 P 0.000 0.000 0.000 0.000 0.318 0.000
0.000 0.009 0.000 0.009 0.000 0.688 0.019 0.000 0.008 0.002 0.000 relative intensity become stronger with
increasing DS (Table 4) [5, 16, 22]. The new peaks for the CH₃ anti- symmetry deformation vibration (1435
cm⁻¹) and the CH₃ symmetry deformation vibration (1370 cm⁻¹) show clearly at high DS indicating
acetylation cause the starch molecule deformation. For native starch, there are two peaks at 1428 and 1454
cm⁻¹ and then after acetylation become one peak with weak relative intensity (Table 4). With increasing DS,
the peak intensity of O–H stretch for carboxylic acids at 3400–3450 cm⁻¹ is weakened, which indicates that
the hydroxyl group was participated in the reaction. In Fig. 1, 100 80 % Transmittance 60 40 20 0 4000 3500
3000 2500 2000 1500 1000 500 Wavelength (cm⁻¹) Figure 1. FTIR

4spectra of native sago starch and starch acetate.

Spectra from top to bottom corresponding to (1) Native starch, (2) DS $\frac{1}{4}$ 0.1, (3) DS $\frac{1}{4}$ 0.2, (4) DS $\frac{1}{4}$ 0.3, (5)
DS $\frac{1}{4}$ 0.6, (6) DS $\frac{1}{4}$ 0.8, and (7) DS $\frac{1}{4}$ 1.2. the peak in the region 1850–1760 cm⁻¹ is not available, which
indicates the product is free of unreacted acetic anhydride. The absence of spectra

22at 1700 cm⁻¹ for the carboxylic group implies that the

starch acetate obtained in this work is also free of acetic acid [23]. 3.3 XRD analysis Figure 2 shows the
results of XRD analysis for native and acetylated sago starch. The spectrum of native sago starch shows an
A-type crystal pattern typically for cereal starches [24–26], that are characterized by strong reflec- tion at
around 2 θ of 17.2 and 18.8 and a single peak at 23.18. For acetylated starch, there is a doublet at around
17 and 18.18 for A-type and a doublet at 21.2 and 26.68 for B-type. This combination indicates that
acetylated starch is typically a C-type starch. As discussed by Tester et al. [25], the

**11A-type crystalline structure is relatively compact with a low water content,
whereas the B-type crystal has a more open structure containing a hydrated
helical core.**

Therefore, acetylation causes the transition from A- to B-type polymorphic forms, which shows that the
ability of water absorption of acetylated sago starch is higher than that of native starch. 3

5.3.1 Water absorption index (WAI) and water solubility index (WSI)

Acetyl content shows a significant correlation with absorp- tion and solubility of starch acetate (Fig. 2).
Starch Table 4. The characteristic infrared frequencies for sago starch Sago Starch A Native starch DS $\frac{1}{4}$
0.1 1 DS $\frac{1}{4}$ 0.2 p1 DS $\frac{1}{4}$ 0.3 p1 DS $\frac{1}{4}$ 0.6 p1 DS $\frac{1}{4}$ 0.8 1 DS $\frac{1}{4}$ 1.2 p1 Experiment Condition B C D p1 p1 p1
1 1 p1 1 1 1 p1 p1 p1 1 p1 1 1 p1 1 Frequency with relative intensity for C–C stretch aromatics (1500–1400

cm 1) 1428 m 1454 m 1427 s 1423 w 1429 m 1422 w 1429 m 1433 w Frequency with relative intensity for C–O stretching vibrations of saturated aliphatic esters (1750–1735 cm⁻¹) – 1735 vw 1736 m 1747 s 1735 s 1749 s 1750 vs vw, very weak; w, weak; m, medium; s, strong; vs, very strong. acetates with different DS showed higher WAI and WSI at room temperature in comparison to the native sago starch. WAI of starch acetate increases with increasing DS. However, this behavior is slightly different for WSI, which reaches maximum at a DS of 0.8 and subsequently diminishes with increasing DS. Similar observation was reported for corn and potato starches [4, 5, 16]. However, the value of DS maximum is lower than that of cornstarch and potato starch. This might be due to larger granule size of sago starch than that of corn and potato starches. The introduction of Figure 2. XRD diagram for native (top) and acetylated starch (DS ¼ 1.2) (bottom). bulky acetyl into starch molecule by acetylation

10 prevents the inter-chain association in the starch acetate, and enhances the access of water to the amorphous area. This could increase the hydration and swelling behavior of starch granule

[27]. However, as DS increases, the amount of hydrophilic hydroxyl groups and the movement of water molecules within the starch molecules are reduced and as a result starch acetate becomes more hydrophobic [4]. In the acetylation, AM is the preferential site compared to AP, hence the swelling in water is mainly due to AP [14]. Therefore, for low DS, the AP is still substantial and is able to raise the water uptake in the granule. Surface erosion (Fig. 3) due to acetylation reaction indirectly affects the absorption and the solubility of starch acetate since this may promote the penetration of the water molecules, but this faculty could be reduced by the aggregation observed in high DS starch acetate. 3.4 Morphological properties The granular structure of sago starches shows significant variations in size and shape with changing DS (Fig. 4). Native sago starch granules are smooth and irregularly oval. After acetylation, the structure of granule surface was changed. Aggregations and porosity on the surface of the acetylated starch granules increase with increasing DS. The acetylation treatment brought granule fusion that

7 could be attributed to the introduction of hydrophilic group to the starch molecules and resulted in increasing hydrogen bonding.

At the highest DS tried in this study (1.2), the structure of granules was significantly changed compared to that of native sago starch. The amount of acylation reagent used in acetylation affected granule fusion, especially when acetic anhydride was used [5, 16]. F. Edi Soetaredjo et al. 4.5 4.0 WAI (g/g) 3.5 3.0 2.5 2.0 1.5 0.0 0.2 0.4 0.6 0.8 1.0 1.2 DS 14 12 10 WSI (%) 8 6 4 2 0 0.0 0.2 0.4 0.6 0.8 1.0 1.2 DS Figure 3. Relationship of DS and WAI (left) and WSI (right). Figure 4. Surface morphology of

4 native sago starch and starch acetate using

SEM (1000). 4 Conclusions Sago starch acetate was synthesized under microwave radiation using iodine as the catalyst. Full factorial DOE was applied and it was shown that four variables measured in this experiment were significant in determining the DS. The physicochemical characteristics of sago starch were observed using FTIR, WAI, and WSI. The starch acetate morphology was examined using SEM. The product obtained from this reaction is free of the acylation reagents, which is ready to be used for further applications. We thank to Prof Hwang Bing Joe from National Taiwan University of Science and Technology for the access of Microwave CEM Atmospheric Pressure. We also thank to Dr. David S. Barkley (Managing Editor, Virtlab) for the proof-reading.

4 The authors have declared no conflict of interest.

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