

The influence degree of substitution on the physicochemical properties of acetylated arenga starches

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Article history

Received: 24 August 2015

Received in revised form:

17 March 2016

Accepted: 23 March 2016

Abstract

Chemical modification is usually carried out to overcome the unstable properties of native arenga starch and improve its physicochemical properties during processing. In this study, acetylation of arenga starch was carried out. Native arenga starch was acetylated with acetic anhydride at 5, 10, 15 and 20% (starch basis, sb) in aqueous slurry at pH 7, 8, 9 and 10. Acetyl percentage (Ac%), degree of substitution (DS), fourier transform infrared spectroscopy (FT-IR) spectra, crystallinity, water and oil holding capacity (WHC and OHC), swelling power and solubility of the native and modified arenga starches were investigated. The results indicated that Ac% and DS of acetylated arenga starches prepared with combination of acetic anhydride 15% (sb) and pH 8 was 6.221% and 0.249, which was the highest value among those prepared at any others. The FTIR spectra of modified starches resulted in the acetyl group incorporation with the starch as shown by absorption of the ester carbonyl group at band 1720 cm⁻¹. The X-ray diffraction revealed that increasing of DS was related to decreasing of crystallinity. The WHC, OHC, swelling power and solubility of acetylated arenga starches increased with increasing of DS indicate increasing in both hydrophilicity and hydrophobicity of the starches.

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Keywords

Arenga starch

Physicochemical

Acetylation

Degree of substitution

Introduction

Starch is the major raw material in the food industry because of its good thickening and gelling properties, which make it an excellent ingredient for the manufacture of various food products (Betancur *et al.*, 2002). Native starches, irrespective of their sources are undesirable for many applications because of their inability to withstand processing conditions, so there is a need to improve desirable functional properties. In order to meet the requirements of specific industrial processes, starches are chemically modified by degradation, substitution or cross-bonding.

Chemical modifications improved the physicochemical and functional characteristics of the starch and tailored it to specific food applications. Chemical modification is generally achieved through derivatization such as etherification, esterification, cross-linking and acid hydrolysis (Santacruz *et al.*, 2002). In acetylation, hydrophilic hydroxyl groups are substituted with hydrophobic acetyl groups. It makes starch more hydrophobic and prevents the formation of hydrogen bonding between hydroxyl groups and water molecules (Owolabi *et al.*, 2014).

Acetylated starch exhibits increased stability in

food applications compared to its native counterpart; therefore, it has been used to increase the stability and resistance of food products to retrogradation (Singh *et al.*, 2004). Starch acetates are prepared commercially with a low (0.1-0.3) degree of substitution (DS) through the reaction of an aqueous suspension of starch granules with acetic anhydride (Biswas *et al.*, 2008; Shogren, 2003; Shogren and Biswas, 2006).

Arenga starch is an important source of starch in tropical countries. It is prepared from the pith of palm *Arenga* (species *Arenga pinnata*). Arenga starch obtained from *A. pinnata* is an economically important palm species in Indonesia. Chemical modifications by acetylation in arenga starch have not yet been reported in literature, although they have been applied to starch and other polymers. Starch acetylation promotes the introduction of the acetyl group in amylose and amylopectin molecules forming a granular starch ester which has superior properties when compared to its native form. In fact, it has been used to confer higher thermal stability and resistance to retrogradation (Singh *et al.*, 2004). According to Colussi *et al.* (2014), acetylation in rice starch reduces crystallinity and increases thermal stability. The objective of this study was to obtain an acetylated arenga starch with an acetylation

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percentage suitable and degree of substitution (DS) for use in food, and to evaluate the influence of the DS on the physicochemical properties of acetylated arenga starches.

Materials and Methods

Materials

Arenga starch (*Arenga pinnata* Merr.) used for this study was obtained from Sigi, Central Sulawesi Provinsi, Indonesia. High-purity acetic anhydride 98% was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck. The chemicals for analysis used in the study were of analytical grade purchased at local agent.

Acetylated of arenga starch

Acetylated starch was prepared by a modified procedure of Phillips *et al.* (1999) with slight modification. Starch (100 g) was dispersed in 225 ml of distilled water and stirred for 60 min at 25°C. The acetic anhydride of 5, 10, 15, 20 % (starch basis, sb) was added drop-wise to the stirred slurry, while to maintain the pH 7, 8, 9 and 10 using 3.0% NaOH solution. The reaction was allowed to proceed for 60 min after the completion of acetic anhydride addition. The slurry was then adjusted to pH 4.5 with 0.5 N HCl. After sedimentation, it was washed free of acid, twice with distilled water and once with 95% ethanol, and then oven-dried at 40°C.

Determination of acetyl percentage and degree of substitution

The percentage of acetyl groups (Ac%) and degree of substitution (DS) were determined titrimetrically, following the method by Sanchez *et al.* (2010). Acetylated starch (1.0 g) and 50 ml of 75% aqueous ethanol were placed in a 250 ml flask. The loosely stopper flask was agitated, warmed to 50°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. The solution was left to stand for 2 h, and then the alkali leached from the sample was titrated. A blank, using the original unmodified starch, was also used. Initially, the acetyl (%) was calculated as:

$$\text{Acetyl \%} = \frac{(\text{Blank} - \text{Sample}) \times \text{Molarity of HCl} \times 0.043 \times 100}{\text{Sample weight}}$$

Blank and sample were titration volumes in ml, sample weight was in g. DS is defined as the average number of sites per glucose unit that possess a

substituent group.

$$\text{DS} = \frac{(162 \times \text{Acetyl \%})}{[4300 - (42 \times \text{Acetyl \%})]}$$

Fourier transform infrared spectroscopy (FT-IR) spectra analysis

Sample preparation and analysis parameters were performed according to Diop *et al.* (2011). Tablets were prepared from the mixture of the sample with KBr at a ratio of 1:100 (sample: KBr). Infrared spectra of native and acetylated starches samples were obtained by a Fourier Transform Spectrometer (IR Prestige 21, Shimadzu) in the 5000–400 cm⁻¹ region.

XRD analysis

X-ray diffraction of native and acetylated starches was measured using method of Miao *et al.* (2011). X-ray diffraction analysis was performed with an X'Pert PRO X-ray powder diffractometer (PANalytical, Almelo, The Netherlands) operating at 40 kV and 30 mA with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$). The starch powders were packed tightly in a rectangular glass cell (15 x 10 mm, thickness 0.15 cm) and scanned at a rate of 2°/min from the diffraction angle (2 θ) 3° to 80° at room temperature. The crystallinity was calculated according to the equation below:

$$X_c = \frac{A_c}{(A_a + A_c)}$$

where X_c is the crystallinity, A_c is the crystalline area and A_a is the amorphous area on the X-ray diffractogram.

Water and oil holding capacity

Water and oil holding capacity (WHO, OHC) of native and acetylated starches was measured using method of Larrauri *et al.* (1996). Twenty-five ml of distilled water or commercial olive oil were added to 250 mg of dry sample, stirred and left at room temperature for 1 h. After centrifugation, the residue was weighed WHC and OHC were calculated as g water or oil per g of dry sample, respectively.

Swelling power and solubility

The method of Adebowale *et al.* (2009) was employed to determine the swelling power and solubility of the starch. Five hundred (500) mg of starch sample was weighed into a centrifuge tube and it was reweighed (W₁). The starch was then dispersed in 20 ml of water. It was then heated at temperature of 80°C for 30 min in a thermostated water bath. The mixture was cooled to room temperature and centrifuged at 3000 g for 15 min. Supernatant was

decanted carefully and residue weighed for swelling power determination. The weight of dry centrifuge tube and the residue and the water retained was taken as W_2 .

$$\text{Swelling power} = \frac{W_2 - W_1}{\text{Weight of starch}}$$

Aliquots (5 ml) of the supernatant were dried to a constant weight at 110°C. The residue obtained after drying of the supernatant represented the amount of starch solubilized in water. Solubility was calculated as g per 100 g of starch on dry weight basis.

Statistical analysis

The data reported were the means of triplicate measurements. Statistical analysis were carried out with Duncan's multiple test ($P < 0.05$) using SPSS version 18 software (SPSS Institute Inc., Cary, NC).

Results and Discussion

Acetyl percentage (Ac%) and degree of substitution (DS)

Table 1 shows rates of the percentage of acetyl groups and degree of substitution of the acetylated arenga starch at different levels with combination of acetic anhydride (5%, 10%, 15%, 20% (sb)) and pH (7, 8, 9 and 10). The acetylation of arenga starch promoted the incorporation of acetyl groups in the molecule, resulting in an acetyl percentage and degree of substitution between 0.858 to 6.221% and DS 0.033 to 0.249 respectively, allowing food application. The highest Ac% (6.221%) and DS (0.249) was reached by acetylation with combination of acetic anhydride 15% (sb) and pH 8. Some studies on acetylated starches were used to evaluate the effect of the concentration of acetic anhydride and pH, since both molecules were composed of anhydroglucose. The molecule of arenga starch had a favorable effect on diffusion and absorption of acetyl groups when compared to starch molecules. This is due to the fact that starch molecules are arranged in the form of granules which hinder access to acetic anhydride, although arenga starch showed similar levels of acetylated starches in the same reaction conditions replacement.

The effect of adding different levels of acetic anhydride (2–12%) on acetyl and the degree of substitution of potato and corn starches was studied by Singh *et al.* (2004). These authors reported that the increase of the substitution degree of acetylated starch was dependent on the acetic anhydride concentration. In the case of potato starch, the DS ranged from 0.18 to 0.24, while corn starch showed a

Table 1. Percentage of acetyl groups (Ac%) and degree of substitution (DS) at different with combination of acetic anhydride (AA) and pH.

Treatments	Ac (%)	DS
5% AA - pH 7	1.931	0.074
5% AA - pH 8	2.560	0.099
5% AA - pH 9	1.924	0.074
5% AA - pH 10	1.717	0.066
10% AA - pH 7	1.395	0.053
10% AA - pH 8	2.361	0.091
10% AA - pH 9	1.288	0.049
10% AA - pH 10	0.858	0.033
15% AA - pH 7	5.039	0.199
15% AA - pH 8	6.221	0.249
15% AA - pH 9	2.576	0.099
15% AA - pH 10	1.284	0.049
20% AA - pH 7	5.775	0.232
20% AA - pH 8	5.232	0.208
20% AA - pH 9	5.991	0.239
20% AA - pH 10	5.678	0.227

lower DS, ranging from 0.13 to 0.18.

FTIR spectra

The chemical structure of native and acetylated arenga starches samples was studied by means of Fourier transform infrared spectroscopy. The collected spectra are included in Figure 1. Acetylation lead to the substitution of hydroxyl groups in the starch molecules with carbonyl containing groups. The characteristic peaks at 3700-3000 cm^{-1} and 3000-2800 cm^{-1} are the hydroxyl groups (O-H) and methylene (C-H) stretching vibration of the glucose unit, respectively. The absorption at about 1651 cm^{-1} is due to residual bound water (H_2O). Compared to that of the native starch curve (Figure 1a), new absorption band at 1720 cm^{-1} appeared in acetylated arenga starches curves (Figure 1b; 1c; 1d). The band occurred at 1720 cm^{-1} is C=O stretching vibration of an ester group. The intensity of those bands increased with the esterification level, whereas the intensity of the band assigned to the stretching of hydroxyl groups of starch (3700–3000 cm^{-1}) gradually decreased as a consequence of the increasing number of hydroxyls which were replaced by ester groups.

Acetylated barley starches had strong absorption bands at 1735–1740 cm^{-1} (C=O stretching of acetyl group), 1368 cm^{-1} (C-H in acetyl group) and 1234 cm^{-1} (C-O stretching of acetyl group) with evidence of acetylation. On the other hand, since the intensity of the hydroxyl group peak at 3000–3600 cm^{-1} decreased, it has been suggested that the hydroxyl groups in the starch molecules were converted into acetyl groups (Halal *et al.*, 2015). The structures of butyrylated corn starch were characterized and the results indicated a new absorption peak at 1749 cm^{-1} (Garg and Jana, 2011), butyrylated high amylose maize starch at band of 1740 cm^{-1} (Lopez *et al.*, 2009)

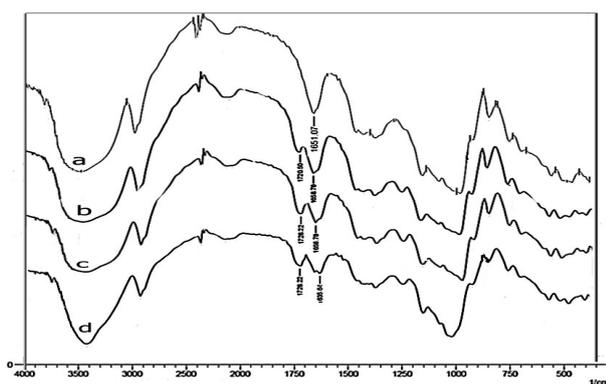


Figure 1. FTIR spectra of native arenga starch (a) and acetylated arenga starches at different of DS: 0.066 (b), 0.208 (c) and 0.249 (d)

which were assigned to the C=O stretching vibration and butyrylated arenga starch at band of 1728 cm^{-1} (Rahim *et al.*, 2012).

Crystallinity

The X-ray diffraction patterns of native and acetylated arenga starch samples are shown in Figure 2. The peaks observed in present study showed that native and butyrylated arenga starches displayed typical A-type pattern with main peaks at $2\theta = 15^\circ$, 17° , 18° and 23° . The X-ray diffraction patterns of the native, acetylated and oxidized barley starches presented strong peaks at 15° , 17° , 18° , 19° and 23° (2θ), characteristic of type A cereal starches, as reported in corn, barley and rice starches (Chavez *et al.*, 2008; Zavareze *et al.*, 2010) These results suggested that esterification did not change the crystalline pattern of acetylated arenga starches up to DS 0.249. These were agreed with the findings of Song *et al.* (2006), who mentioned that the esterification occurred primarily in the amorphous regions and did not change the crystalline pattern of starches.

Esterification reduced the relative crystallinity of acetylated arenga starches when compared to native arenga starch. Ac% and DS increase of acetylated arenga starches (Table 1) reduced the relative crystallinity. This indicated that the granules of acetylated arenga starches had been damaged to some extent by the modification processes. Intra and intermolecular hydrogen bonds were responsible for the highly ordered crystalline structure. The results were in accordance with those of the earlier report of Lopez *et al.* (2010), that degree of crystallinity of the acetylated corn starch granules was lower than that of the native starch. The results were consistent with X-ray diffraction results that showed lower crystallinity when compared to that of native barley starch (Halal *et al.*, 2015).

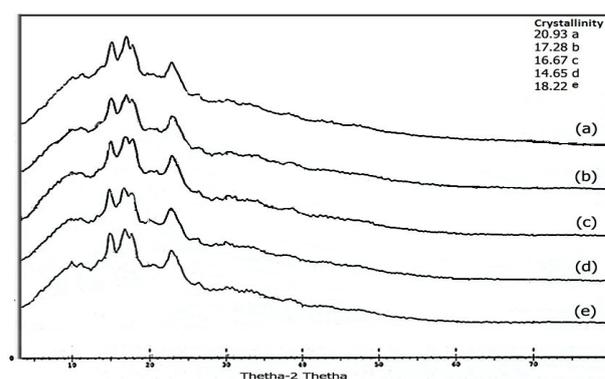


Figure 2. X-ray diffractograms and cristallinity of native arenga starch (a), and acetylated arenga starches with different DS: 0.066 (b), 0.091 (c), 0.208 (d) and 0.249 (e)

Water and oil holding capacity

WHC and OHC of the acetylated arenga starches increased with the relative increase DS (Figure 3). These data indicate that either hydrophilicity or hydrophobicity tend to improve after acetylation. Improvement in water and oil absorption was a result of introduction of functional groups to the starch molecules, which facilitated a more enhanced holding capacity.

At low level of DS, the acetyl groups were not sufficient to change the behavior of hydroxyl groups. There was weakening of intermolecular hydrogen bonds in starch with the introduction of acetyl groups. Adebowale *et al.* (2006) found that WHC of the acetylated sword bean starch at DS 0.14 was higher than that of the native starch, while Teli and Valia (2013) reported that the OHC of the acetylated banana was higher than that of the raw banana. Working with acetylated sweet potato starches by Das *et al.* (2010) reported that the water and oil binding capacity increased with increasing DS (0.018 – 0.058).

Swelling power and solubility

The swelling power and solubility of native and acetylated arenga starches are given in Figure 4. Swelling power of the acetylated arenga starches tended to increase with increasing DS (Figure 4). This phenomenon was probably caused by a weakening of intermolecular association force due to introduction of acetyl groups that reduced the hydroxyl groups. These were similar to the results of Souza *et al.* (2015) that swelling power of the acetylated oat β -glucan was higher than native starch, and increased with rise in acetic anhydride and DS. Das *et al.* (2010) showed that swelling power of acetylated sweet potato starch increased with increasing DS from 0.018 to 0.058.

Solubility of the acetylated arenga starches tended to increase along with the increase in DS. The results

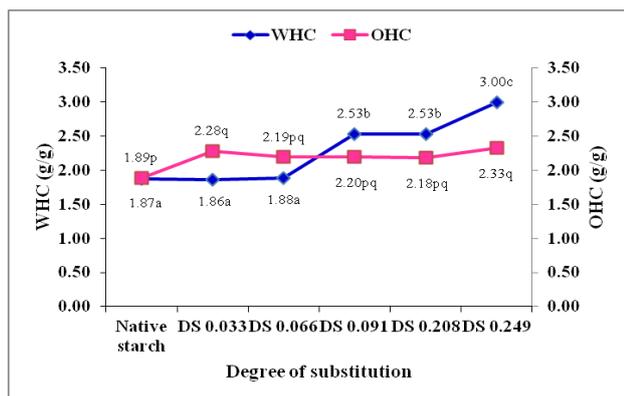


Figure 3. Effects of DS on the water and oil holding capacities of acetylated arenga starches. Numbers in the graph followed by the same letter in common are not significantly different at $p < 0.05$

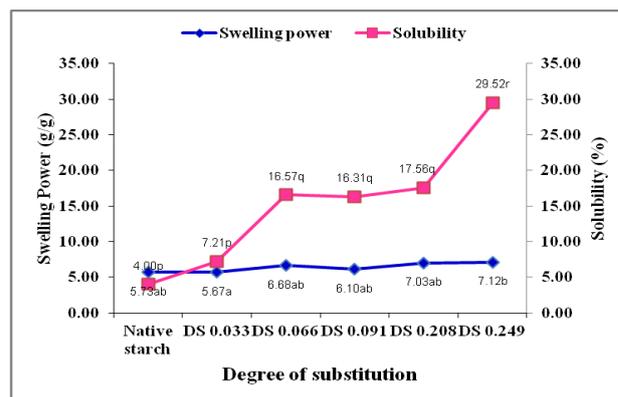


Figure 4. Effects of DS on the swelling power and solubility of acetylated arenga starches. Numbers in the graph followed by the same letter in common are not significantly different at $p < 0.05$

were similar to the earlier report of Kapelko *et al.* (2015) that solubility of the acetylation of acetylated potato starches preparations increased their solubility in water and water absorbability was than native starch.

Conclusion

The acetylation of arenga starch promoted the incorporation of acetyl groups in the molecule, resulting in a degree of substitution between 0.033 to 0.249, allowing food application. Acetylation of the arenga starch molecule decreased the of crystallinity and increased the WHC, OHC, swelling power and solubility along with the increase in DS indicating that acetylation improved physicochemical properties of the starch. This report will be useful to promote the use of the acetylated arenga starch for industrial applications in food production.

Acknowledgements

We gratefully acknowledged the Directorate of Research and Community Service, Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia, for providing financial support from the National Strategic Research Grant Program in 2015.

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