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Characteristics of butyrylated arenga starch prepared at different reaction time and butyric anhydride concentration

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Abstract

Butyrylation of starch leads to products with an increased hydrophobicity. Depending on the degree of substitution (DS), some of the highly hydrophilic hydroxyl groups in the anhydroglucose units of starch are converted to more hydrophobic butyryl groups which change the physical and chemical properties. Arenga starch was reacted with butyric anhydride at 5 - 15% (starch basis, sb) in aqueous slurry for 10 - 60 min. The modified starches were characterized for DS, crystallinity, water and oil holding capacity (WHC and OHC), swelling power and solubility and resistant starch (RS) after hydroxylation with α-amylase and amyloglucosidase. The FTIR results indicated that the modification resulted in the butyryl group incorporation with the starch as shown by absorption of the ester carbonyl group at band 1728 cm^{-1}. Increasing butyric anhydride concentration (5-15%) resulted in increase in DS (0.057-0.226), however, butyrylation with 5% butyric anhydride for 40 min resulted in the maximal DS. X-ray diffraction revealed that increasing DS was related to decreasing crystallinity. The WHC and OHC of butyrylated arenga starches increased with increasing DS indicating increase in both hydrophilicity and hydrophobicity of the starches. Swelling power increased and solubility decreased with the increasing DS. The RS derived from butyrylated arenga starches tended to increase with the increase of DS. The data obtained in this study indicated the possibility of the application of the butyrylated arenga starch with potential health properties in food product development.

Introduction

Starch is the most important digestible complex carbohydrate in human diets and is a major constituent of many processed and traditional foods. It also finds industrial applications through a wide range of modifications which alter its technological properties. Apart from physical modification of starch, the reactivity of its glucose residues allows for a number of chemical modifications providing this macromolecule with a great versatility (Sajilata *et al*., 2006). Chemical modifications of starch including esterification are efficacious methods to improve the properties of starch. The chemical and functional properties achieved depends on chemical substitution, starch source, reaction conditions, type of substituent, extent of degree of substitution (DS), and the distribution of the substituent in the starch molecule (Hirsch and Kokini, 2002).

Acylated starch is a starch ester that has been extensively studied over the last two decades (Gonzalez and Perez, 2002; Singh *et al*., 2007). In modified starch, part of hydroxyl groups in anhydroglucose units have been converted to acyl groups. Acylated starch with low DS is commonly obtained by esterification of native starch in an aqueous medium in the presence of an alkaline catalyst. A low DS with (0.01-0.20) acylated starch has been applied in many areas, such as film forming, binding, adhesion, thickening, stabilizing, texturing (Boutboul *et al*., 2002; Matti *et al*., 2004; Singh *et al*., 2004; Sodhi and Singh, 2005). Recently, acylated starch with high DS (2.0-3.0) has received attention for a number of non-food applications such as tablet binders, hot melt adhesives, cigarette filters and coating materials.

Acylated starches with low DS are also being exploited for their potential nutritional and health properties. While starch can be digested to completion by human small intestinal digestive enzymes, it appears that a fraction of resistant starch (RS) escapes into the large bowel where it is fermented by the resident microflora leading to the production of short-chain fatty acids (SCFA). Specific delivery of

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SCFA to the large bowel offers considerable benefits in clinical and non-clinical applications. Acylation of starch with DS 0.2–0.3 level of substitution with acetic, propionic or butyric anhydride has been shown to be an effective means of achieving this aim. Animal studies have shown that they pass into the large bowel where the esterified acids are released by bacterial enzyme activity (Annison *et al*., 2003). It has been suggested that RS promotes a higher proportion of butyric acid than other indigestible carbohydrates. Butyrate constitute a major energy substrate for the colonocytes and is associated with benefits in relation to colonic health (Brouns *et al*., 2002).

In the current study, butyrylated starches were prepared by reacting arenga starch with butyric anhydride in aqueous slurry to determine the optimum reaction condition, and to give the characteristics such as fourier transform infrared (FTIR) spectra, cristallinity, water and oil holding capacity, swelling power and solubility, and RS content.

Materials and Methods

Materials

Arenga starch (*Arenga pinnata* Merr.) used for this study was obtained from Klaten, Central Java Provinsi, Indonesia, having the following approximate composition: 69% amylopectin, 31% amylose, 0.82% crude protein, 0.13% crude fat, and 11% moisture content. High-purity butyric anhydride (98%) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The chemicals used for analysis in this study were of analytical grade.

Butyrylation of arenga starch

Butyrylated starch was prepared by a modified procedure of Phillips *et al*. (1999). Starch (100 g) was dispersed in 225 ml of distilled water and stirred for 60 min at 25^{o}C. Butyric anhydride of 5, 10, 15% (starch basis, sb) was added drop-wise to the stirred slurry, while maintaining the mixture at pH10 using 3.0% NaOH solution and the reactions were allowed to proceed for 10, 20, 30, 40, 50, 60 min. The slurry was then adjusted to pH 4.5 with 0.5 N HCl. After sedimentation, the slurry was washed free of acid by washing twice with distilled water and once with 95% ethanol, and then oven-dried at 40^{o}C.

FTIR spectra analysis

FTIR spectra of native and butyrylated starches was measured using KBr's method (Pushpamalar *et al*., 2006). The dry starch sample was blended with KBr in a 1:4 ratio of starch/KBr . The mixture was pressed to obtain a pellet and introduced in the spectrometer (MIDAC, prospect 269, Costa Mesa, CA, USA). Each spectrum was analyzed in the range of resolution from 500 to 4000 cm^{-1}.

Determination of butyryl (%) and degree of substitution

Butyryl (%) was determined titrimetrically based on the method of Wurzburg (1978) with a slight modification. Butyrylated starch (1.0 g) was placed in a 250 ml flask and 50 ml of 75% ethanol was added. The loosely stoppered flask was agitated, warmed to 50^{o}C for 30 min, cooled and 40 ml of 0.5 M KOH was added. The excess alkali was titrated with 0.5 M HCl using phenolphthalein as an indicator. A blank containing only the original unmodified starch was used as control. Butyryl (%) was determined as follows:

$$\text{Butyryl \%} = \frac{[(\text{Blank-Sample}) \times \text{Molarity of HCl} \times 0.071 \times 100]}{\text{Sample weight}}$$

DS is defined as the average number of sites per glucose unit that possess a substituent group.

$$DS = \frac{(162 \times \text{Butyryl \%})}{[71000 - (70 \times \text{Butyryl \%})}$$

X-ray diffractometry analysis

X-ray diffraction of native and butyrylated starches was measured using the 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 Kα radiation (λ = 1.5406 Å). 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^{o}/min from the diffraction angle (2θ) 3^{o} to 70^{o} 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 butyrylated starches was measured using the method of Larrauri *et al*. (1996). Twenty-five millilitres 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 at 2000 r.p.m. for 10 min, the residue was weighed and the 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 used to determine the swelling power and solubility of the starch. Five hundred 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, heated in a water bath at 80°C for 30 min, cooled to room temperature and centrifuged at 3000 r.p.m. for 15 min. The supernatant was decanted carefully into a new tube and the residue was weighed for swelling power determination. The weight of the centrifuge tube and the residue and the water retained was taken as W2.

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

Resistant starch

RS from the starch sample was determined according to the enzymatic-gravimetric method (AOAC, 1990) as RS behave as a dietary fiber. To determine the level of amylase-resistant starch, 4 g of samples was suspended in 160 ml of 0.08 M phosphate buffer (pH 5.5) followed by the addition of 40 µl of heat-stable α-amylase. The solution was incubated at 95°C for 60 min and then allowed to cool. After cooling, the pH was adjusted to 4.5 and the samples were further incubated with 80 µL amyloglucosidase at 60°C for 45 min. The amount of glucose in the hydrolysate was enzymatically determined the addition 10 ml of glucose oxidase reagent and the mixture was incubated at 20°C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. The concentration of RS was calculated as follow:

$$RS\ (g/100g) = (1 - G \times 0.9 / wt.\ sample) \times 100$$

where wt. sample is the initial weight (g), and G is the weight of glucose (g).

Statistical analysis

The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range tests. ANOVA data with a $P < 0.05$ were classified as statistically significant. SPSS 18.0 software, Origin 75 and Microsoft Excel 2007 program were used to analyze and report the data. Mean values from the experiments done in triplicates were reported.

Results and Discussion

FTIR spectra

The FTIR spectra of native and butyrylated arenga starches with different reaction conditions are shown in Figure 1. Butyrylation lead to the substitution of hydroxyl groups in the starch molecules with carbonyl containing groups. The characteristic peaks at 3426 cm^{-1} and 2932 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 1728 cm^{-1} appeared in butyrylated arenga starches curves (Figure 1b; 1c; 1d). The band at 1728 cm^{-1} is C=O stretching vibration of an ester group.

The results were in agreement with the report by Fang *et al*. (2002) that showed the characteristic vibration of the ester carbonyl groups in butyrylated potato starch at band 1746 cm^{-1}. The structures of butyrylated corn starch were characterized and the results indicated a new absorption peak at 1749 cm^{-1} (Garg and Jana, 2011) and butyrylated high amylose maize starch at band of 1740 cm^{-1} (Lopez *et al*., 2009) which were assigned to the C=O stretching vibration. These new absorptions suggested that the butyrylated starch products were formed during the esterification process. In addition, the spectra of butyrylated starches showed that the anhydroglucose unit moved towards a higher wave number.

Degree of substitution

Increasing the reaction time from 10 - 40 min resulted in increase in DS (Figure 2), but longer reaction time (50 – 60 min) resulted in a decrease in DS. An explanation was that, as the reaction progressed, the concentration of anhydride depleted, due to esterification and hydrolysis reactions similar to the observation reported by Ruan *et al*. (2009).

Reaction of the arenga starch with butyric anhydride at 5-15% resulted in DS 0.057-0.226 of the modified starch (Figure 3). These results were similar to the report of Garg and Jana (2011) which showed that increase in concentration of butyric anhydride (0 – 5.5%) resulted in increase in DS of the butyrylated corn starch (0 – 1.75). Bai and Shi (2011) found that as octenyl succinic anhydride increased (3 - 50%), DS of octenyl succinylated waxy maize starches increased.

Cristallinity

The X-ray diffraction patterns of native and butyrylated arenga starch samples are shown in

Figure 1. FTIR spectra of native starch (a) and butyrylated starches prepared by reaction with 15% (sb) butyric anhydride for 40 min at pH 9 (b), with 10% (sb) butyric anhydride at pH 10 (c), and with 15% (sb) butyric anhydride at pH 10 (d)

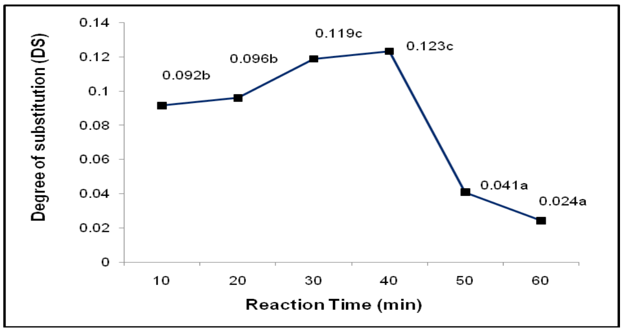

Figure 2. Effect of reaction time with 5% butyric anhydride on DS. Figures in the graph followed by the same letter indicating no significant difference at $p < 0.05$

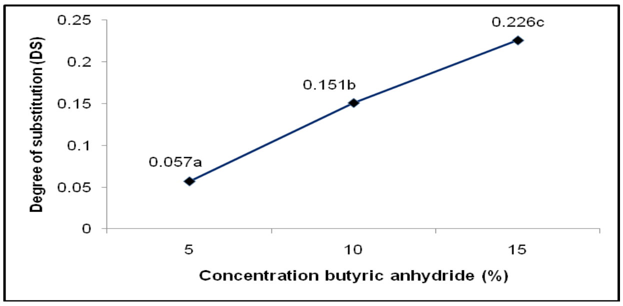

Figure 3. Effect of butyric anhydride concentration in for reaction time of 40 min on the DS. Figures in the graph followed by the same letter indicating no significant difference at $p < 0.05$

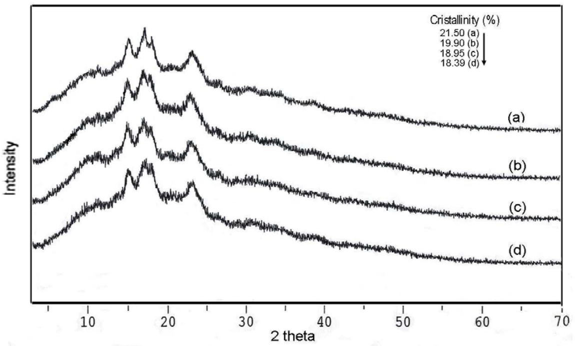

Figure 4. X-ray diffractograms and cristallinity of native (a), and butyrylated starches with different DS: 0.074 (b), 0.151 (c), and 0.226 (d)

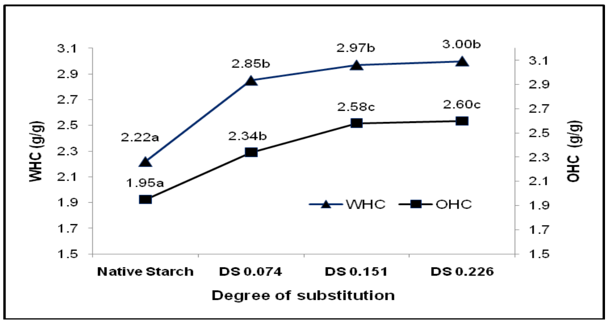

Figure 5. Effects of DS on the water and oil holding capacities of butyrylated arenga starches. Figures in the graph followed by the same letter indicating no significant difference at $p < 0.05$

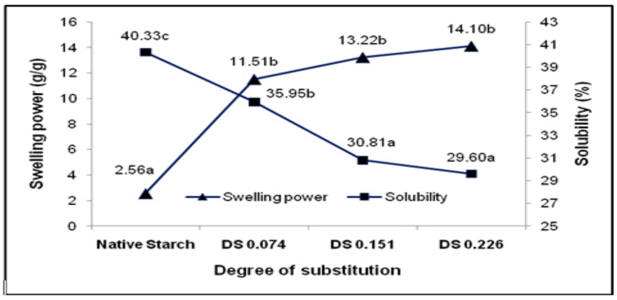

Figure 6. Effects of DS on the swelling power and solubility of butyrylated arenga starches. Figures in the graph followed by the same letter indicating no significantly difference at $p < 0.05$

Figure 4. The peaks observed showed that native and butyrylated arenga starches displayed typical A-type pattern with main peaks at $2\theta = 15^o, 17^o, 18^o$ and 23^o. Elsewhere, other groups of researchers described that A-type pattern of starches exhibited sharp peaks at $2\theta = 15^o, 17^o, 18^o$ and 23^o (Chen *et al*., 2007; Chi *et al*., 2008; Miao *et al*., 2011). Taken together, these results suggested that esterification did not change the crystalline pattern of butyrylated arenga starches up to DS 0.226. These were supported 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.

Degrees of crystallinity of the butyrylated arenga starch granules were lower than that of the native starch. This indicated that the granules of butyrylated 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 the degree of crystallinity of the acetylated corn starch granules was lower than that of the native starch.

Water and oil holding capacity

WHC and OHC of the butyrylated arenga starches increased with the increased in DS (Figure 5). These data indicate that either hydrophilicity or hydrophobicity tend to improve after butyrylation.

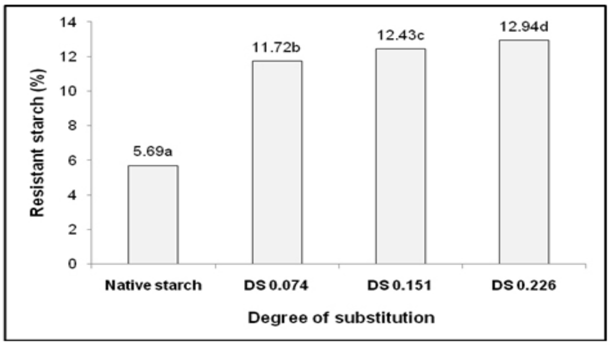

Figure 7. Effects of DS on the RS content of butyrylated arenga starches. Figures in the histograms followed by the same letter indicating no significant difference at $p < 0.05$

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 butyryl groups were not sufficient to change the behavior of hydroxyl groups. There was weakening of intermolecular hydrogen bonds in starch with the introduction of butyryl 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 Garg and Jana (2011) reported that the WHC of butyrylated corn increased with increasing DS at low level. Working with acetylated sweet potato starches, Das *et al*. (2010) reported that the water and oil binding capacity increased with increasing DS (0.018 – 0.058).

Swelling power and solubility

Swelling power of the butyrylated arenga starches tended to increase with increasing DS (Figure 6). This phenomenon was probably caused by a weakening of intermolecular association force due to introduction of butyryl groups that reduced the hydroxyl groups. These observation were similar to the results of Wang *et al*. (2011), who reported that swelling power of the octenyl succinylated corn starch increased with increasing DS values up to 0.81. This behaviour of the octenyl succinylated corn starch was ascribed to its particular structure with both hydrophobicity of octenyl groups and hydrophilicity of carboxyl or sodium carboxylate groups. When the DS was low, hydrophilic carboxyl groups played the main role. Thus, starch granules showed a higher tendency to bind water and swelling. 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 butyrylated arenga starches tended to decrease along with the increase in DS. The results were similar to the earlier report of Lawal (2004) on the solubility of the acetylated starch from new cocoyam starch, which was lower than that of the native starch. Other researchers working on different sources of starch reported similar tendency with acetylated potato starch (Singh *et al*., 2004), acetylated sword bean starch Adebowale *et al*. (2006) and succinylated red and white sorghum starches (Olayinka *et al*., 2011).

Resistant starch

RS of the butyrylated arenga starch tended to increase with increasing DS (Figure 7). This was likely due to the introduction of ester groups to the starch molecules that contribute to the partial retardation of the enzyme-substrate complex formation, causing the RS content of the chemically modified starches to increased. Earlier workers reported that RS content of octenyl succinylated corn starches (Wang *et al*., 2011) and that of octenyl succinylated high amylose maize starch (Zhang *et al*., 2011), increased with increasing the DS.

Conclusion

Introduction of butyryl groups to starch molecule can achieved by the reaction of starch with butyrate anhydride, improving the hydrophilic and hydrophobic properties of the starch and producing butyrylated arenga starch with potential for application in health food product development.

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