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**EFFECT OF PHOSPHORYLATION ON THE PHYSICAL AND CHEMICAL
CHARACTERISTICS OF ARENGA STARCH**

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ABSTRACT

Chemical modification is usually carried out to overcome the unstable properties of native arenga starch and improve its physical and chemical properties during processing. In this study, phosphorylation of arenga starch was carried out. Native arenga starch was phosphorylated with sodium tripolyphosphate (STPP) at 2, 3, 4, 5% (starch basis, sb) in aqueous slurry at pH 8, 9, 10 and 11. The degree of substitution (DS), crystallinity, water and oil holding capacity (WHC and OHC), swelling power and solubility and sediment volume of the native and modified starches were characterized. The results indicated that DS of phosphorylated arenga starch prepared with STPP 4% (starch basis) at pH 10 was 0.115, which was the highest value among those prepared at any others. The X-ray diffraction revealed that increasing DS was related to decreasing crystallinity. The WHC and OHC of phosphorylated arenga starches increased with increasing DS indicating increase in both hydrophilicity and hydrophobicity of the starches. The swelling power, solubility and sediment volume of phosphorylated arenga starches tended to decrease with the increase of DS.

Keywords: Phosphorylation, Arenga starch, Degree of Substitution, Physical Characteristics, Chemical Characteristics

INTRODUCTION

Starch is widely used in many industrial products due to its functional properties and nutritional value. It has been most often used in industry as thickener, colloidal stabilizer, gelling, bulking, and water retention agents in food and non-food products [1, 2]. However, the applications of native starches are limited due to their storage and process instabilities. That was the reason for developing the techniques for starch modifications. Chemical modification involves the introduction of functional groups into the starch molecules, resulting in marked changes in starch physico-chemical properties. Phosphorylation starch is an example of chemically modified starches. Chemical modification is intended to facilitate intra- and intermolecular bonds at random locations in the starch granules for their stabilization [3, 4]. Pastes of phosphorylated starch are more resistant to shear and acidic conditions. Phosphorylation is generally performed by treatment of granular starch with multifunctional reagents capable of forming either ether or ester intermolecular linkages between the hydroxyl groups of starch molecules. The main reagents used for phosphorylation are sodium trimetaphosphate, mono-sodium phosphate, sodium tripolyphosphate, phosphoryl

chloride, a mixture of adipic acid, acetic anhydride, and vinyl chloride [3, 5].

The type of phosphorylation agent determines the changes in functional properties of a treated starch, because the molecular structures of the phosphorylated starch systems produced by different phosphorylation agents are different [5, 6]. Therefore, based on the reagent used for cross-linking, the final product is generally divided into three types: the first type is a mono-starch phosphate which is produced by esterification of starch with ortho-phosphoric acid, sodium or potassium ortho-phosphate, or sodium tripolyphosphate. The second type is a di-starch phosphate which is produced with sodium trimetaphosphate or phosphorous oxychloride. The third type of cross-linked starch is a phosphated di-starch phosphate which is produced by combined treatments of mono-starch phosphate and di-starch phosphate [6, 7]. Monostarch phosphate exhibit increased paste clarity, viscosity, water binding capacity [8, 9, 10]. On the other hand, the formation of distarch phosphates may help to maintain the granule integrity and to make starch paste more resistant to retrogradation, high temperature, and low pH than native starch [11, 12]. Phosphorylation of sago starch with 5% STPP increased the

paste clarity while viscosity decreased, but that with 4% POCl_3 decreased both properties [13]. X-ray diffraction and amylography have been used to characterise the crystalline/amorphous structure and amylograph of starches, respectively.

The two different reagents react with starch through different mechanism. At pH below 9.0 the terminal phosphate groups of STPP are protonated and produce monometa phosphates, which can react rapidly with starch hydroxyl groups to produce monostarch phosphates. At a reaction pH above 10, the ionized hydroxyls can attack the STPP central phosphate to form starch pyrophosphates, which can be further attacked by starch hydroxyl groups to give distarch phosphate [8]. Initial reaction of POCl_3 with water in starch slurry produces mostly dichlorophosphate. The dichlorophosphate then diffuse into starch granules, and react with the polymer chains. Cross-linking of starch molecules to give a distarch phosphate was favored by alkalinity above pH 10 in the presence of a neutral sodium salt. Otherwise, monostarch phosphates are formed [10, 14, 15]. The objectives of this study were to investigate the effect of STPP and reaction pH on the physical and chemical characteristics in the phosphorylated arenga starches. The results

may be useful in finding new applications for this type of phosphorylation arenga starch in food and non-food productions.

MATERIALS AND METHODS

Materials

Arenga starch (*Arenga pinnata* Merr.) used for this study was obtained from Sigi, Central Sulawesi Province Indonesia, sodium tripolyphosphate (STPP), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck. The chemicals used for analysis in this study were of analytical grade.

Phosphorylation of Arenga Starch

Phosphorylations of arenga starch were prepared according to the method of [16] with a slight modifications. Arenga starch (50 g) was dispersed in 100 ml of distilled water and stirred for 60 minutes at 27°C. STPP of 2, 3, 4 and 5% (starch basis, sb) was added drop-wise to the stirred slurry with various pH at 8, 9, 10 and 11 using 3.0% NaOH solution and the reactions were allowed to proceed for 60 minutes. The slurry was then adjusted to pH 6.5 with 0.5 N HCl. After sedimentation, the slurry was washed free of acid by washing four times with distilled water and once with 95% ethanol, and then dried at 40°C for 24 h in an oven. The dried samples were then grounded in a mortar and sieved (100 mesh).

Determination of Degree of Substitution of Arenga Starches

The phosphate content was determined according to the method of [17] with a slight modification. Phosphorylated 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°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. Phosphate (%) was determined as follows:

$$\text{Phosphate (P) \%} = \frac{[(\text{Blank} - \text{Sample}) \times \text{Molarity of HCl} \times 0.031 \times 100]}{\text{Sample weight}}$$

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

$$\text{DS} = \frac{(162 \times P)}{[3100 - (103 \times P)]}$$

with P being the colorimetric determined percentage of phosphorus content, 162 the molecular weight of the anhydroglucose unit, 3100 the atomic weight of the phosphorus multiplied by 100, 103 the molar mass of the phosphate in monostarch phosphate, and 102 the molar mass of the phosphate in distarch phosphate.

X-Ray Diffractometry Analysis

X-ray diffraction of native and phosphorylated starches was measured using the method of [18]. 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 θ /min from the diffraction angle (2θ) 3° to 70° 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 Analysis

WHO, OHC of native and phosphorylated starches was measured using the method of [19]. 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 rpm 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 Analysis

The method of [20] 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 minutes, cooled to room temperature and centrifuged at 3000 rpm for 15 minutes. 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.

Sediment Volume Analysis

The method of [21] was employed. Starch (1g) was mixed with 95 ml of distilled water. The pH of starch slurry was adjusted to pH 7.0 using 5% NaOH/HCl followed by heating in a boiling water bath for 15 min. Distilled water was added to make the total weight to 100g. The mixture was transferred to a 100 ml graduated cylinder and was sealed. The starch slurry was kept at room temperature for 24, 48 and 72 h and volume of sediment of starch granules was measured.

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

Degree of Substitution

The DS of phosphorylated arenga starches which were modified by different amounts of STPP 2, 3, 4, and 5% with at pH 8, 9, 10 and 11 (**Figure 1**). There seemed to be a proportionally increase in the DS with increasing concentration of STPP up to 4% (DS 0.115), which then leveled off from 4 to 5%. These results were similar to the report of [22] which showed that concentration of STTP up to 5% resulted highest DS of the phosphorylated sago starch.

The starch phosphorylation was conducted at pH 8 to 11, though the reaction at pH 10 gave the highest DS. This might have been due to the competition of phosphorus binding between the starch and NaOH used in the phosphorylation. At pH higher than 10 the phosphorus tended to react with NaOH. The increase in DS might be due to higher reactivity of OH groups at C6 which was dependent on the alkalinity of the reaction mixture [23].

Crystallinity

The X-ray diffraction patterns of native and phosphorylated arenga starches are shown in **Figure 2**. There was no pronounced difference between the native and modified starches. That is, the native and modified starches showed similar diffraction patterns with peaks at 15.24, 17.37 and 23.20° (2 θ), which are typical characteristics of A-type starch [24]. This result suggested that phosphorylation with STPP up to 4% did not dramatically alter the crystalline pattern of arenga starch. This observation was in agreement with the finding of [25], who reported that cross-linking mainly took place in the amorphous regions of starch granule and did not change the crystalline patterns of starches. If compared to native starch, the crystallinity of monostarch phosphate remains nearly the same, suggesting that the layer structure was not destroyed in the phosphorylation [26].

Degrees of crystallinity of the phosphorylated arenga starch granules were lower than that of the native starch (Figure 3). This indicated that the granules of phosphorylated 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 [27], that the degree of crystallinity of the phosphorylated corn starch low more than that of the native starch.

Water and Oil Holding Capacity

WHC and OHC of the phosphorylated arenga starches increased with the increased in DS (**Figure 3**). These data indicate that either hydrophilicity or hydrophobicity tend to improve after phosphorylation. Improvement in water and oil absorption was a result of introduction of phosphorus groups to the starch molecules, which facilitated a more enhanced holding capacity. Working with acetylated sweet potato starches, reported that the water and oil binding capacity increased with increasing DS (0.018 – 0.058) [28].

Swelling Power and Solubility

The swelling power of native and phosphorylated arenga starches are shown in **Figure 4**. It is evident that all phosphorylated arenga starches showed a greater swelling ability than native starch. Native starch requires water and heat to swell due to the packing and hydrogen bonding of the starch molecules within the granule [29]. After phosphorylation, the repulsive effects from incorporated phosphate groups facilitated water penetration resulting in a greater swelling capacity. The swelling power of phosphorylated arenga starches was as

decreased with increasing of the DS (**Figure 4**). This result was in agreement with the finding of [30] who reported the reduced swelling factor of cross-linked oat starch with increasing degree of cross linking. It is well known that cross-linking strengthens the bonding between starch chains, thus allowing them to resist against swelling. Therefore, the reduced swelling factor would be related to the formation of inter-molecular bridges by phosphorous residual after cross-linking reaction [31, 32].

The solubility of native and phosphorylated arenga starches was an overall tendency that the solubility decreased with increasing DS (**Figure 5**). A similar pattern was reported by [33] for potato starches modified with POCl_3 at different concentrations. It suggested that decreased solubility by cross-linking would be possibly due to increased density of cross-links in the starch structure which seemed to cause less disintegration of starch granules during gelatinization [34].

Sediment Volume

Sediment volume is an index of starch gelatinization and provides a clear distinction between various precooked products. It indicates the changes in starch molecular association during the process of phosphorylation. Also, it reflects the degree of phosphorylation in starch. There was

significant variation among the starch samples (**Figure 5**). The sediment volume of native arenga starch is significantly high compared to phosphorylated arenga starches that showed a significantly lower sediment volume. This result is in agreement with report by [35] that acetylated modification lowered the sediment volume of potato and sweet potato flours.

CONCLUSION

Introduction of phosphorus groups to starch molecule can be achieved by the reaction of starch with STTP, improving the hydrophilic and hydrophobic properties of the starch and producing phosphorylated arenga starch with potential for application in food product development.

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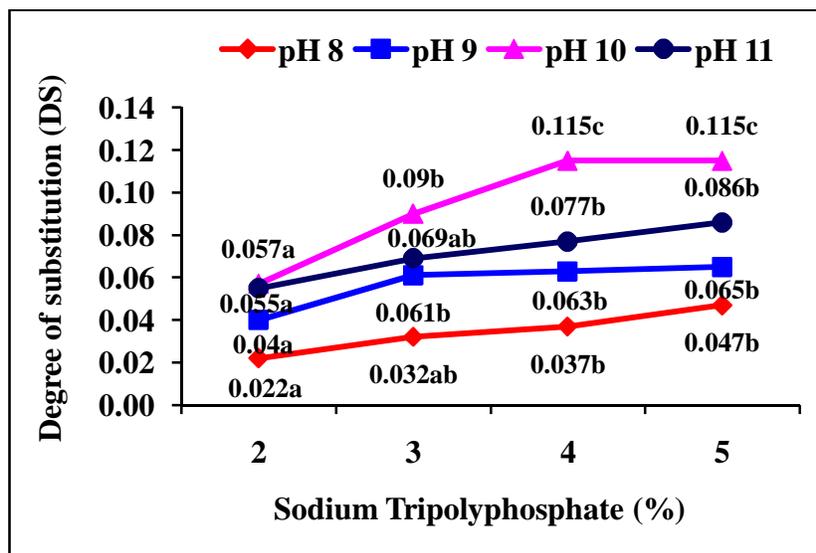


Figure 1: Effect of Sodium Tripolyphosphate with Various at pH 8, 9, 10 and 11 on the DS. Different Letters for Each pH in the Same Line are Significantly Different ($p < 0.05$)

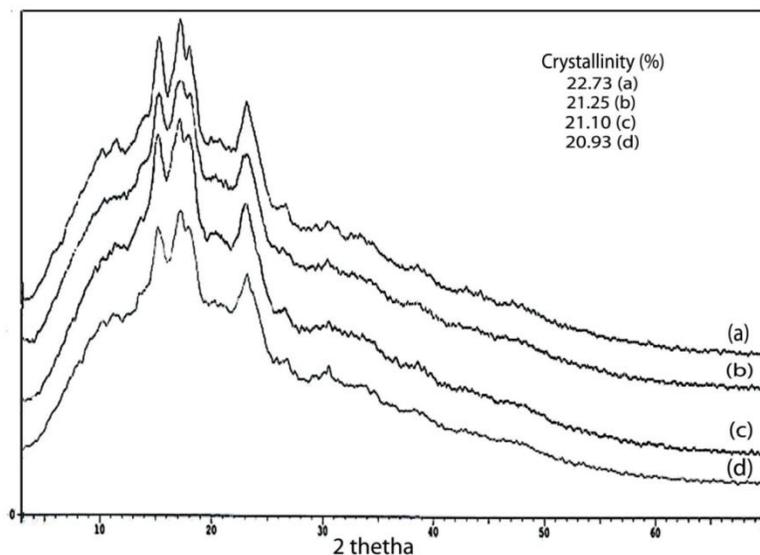


Figure 2: X-Ray Diffractograms and Crystallinity of Native (a), and Phosphorylated Arenga Starches with Different DS: 0.032 (b), 0.061 (c), and 0.115 (d)

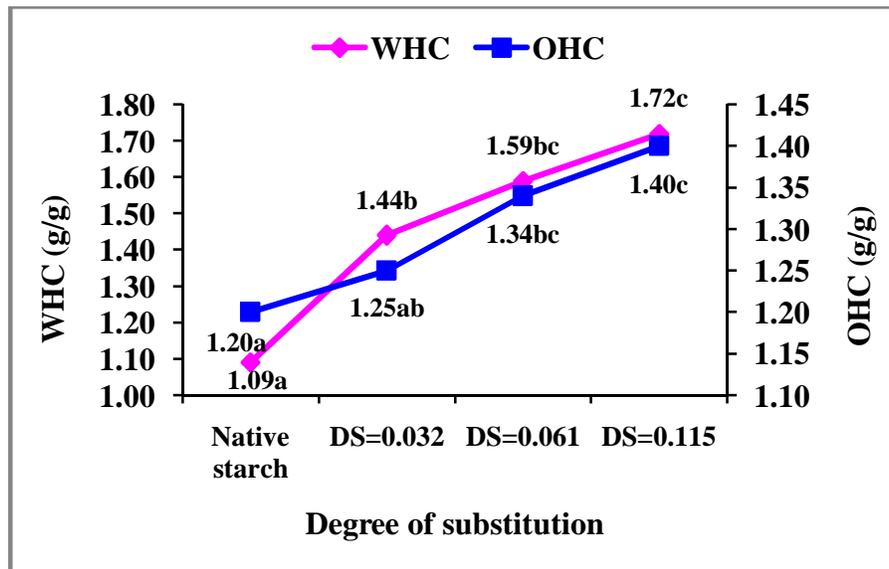


Figure 3: Effects of DS on the WHC and OHC of Phosphorylated Arenga Starches. Figures in the Graph Followed by the Same Letter Indicating no Significantly Difference at $p < 0.05$

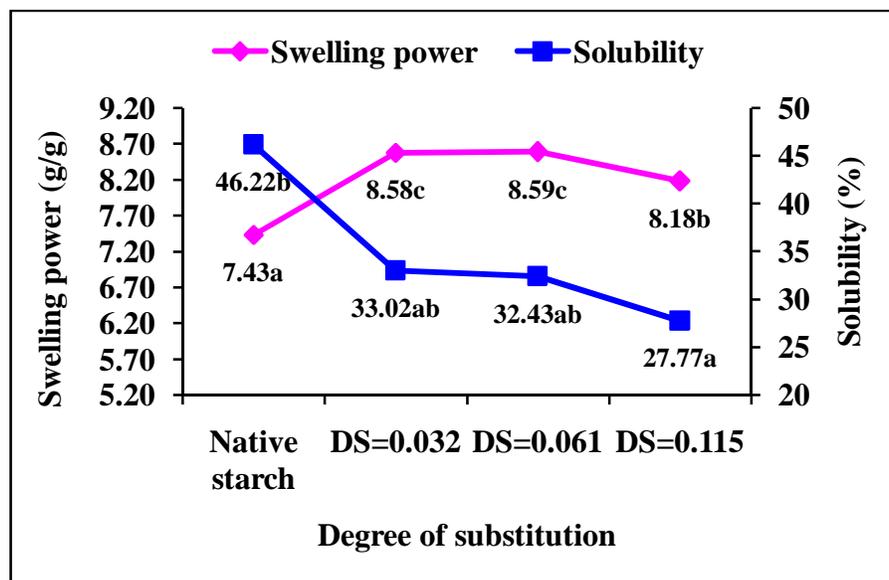


Figure 4: Effects of DS on the Swelling Power and Solubility of Phosphorylated Arenga Starches. Figures in the Graph Followed by the Same Letter Indicating no Significantly Difference at $p < 0.05$

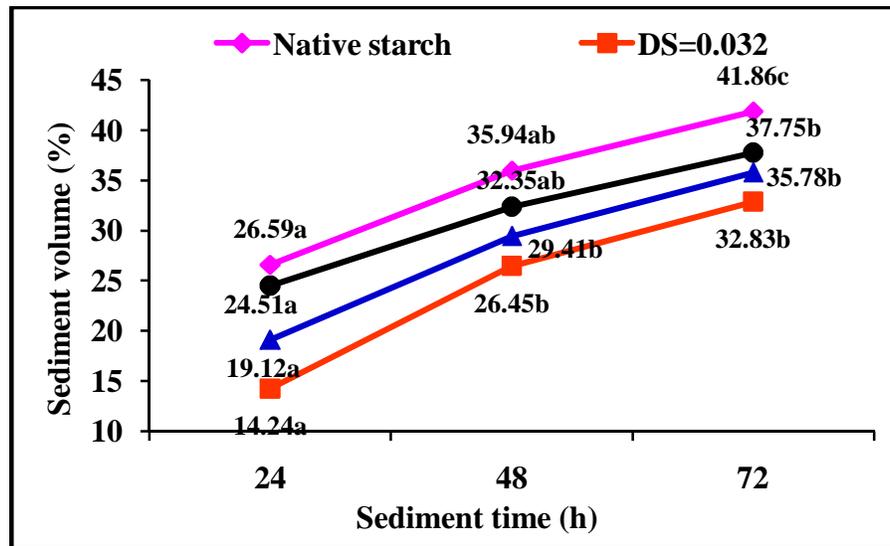


Figure 5: Effect of DS on the Sediment Volume of Phosphorylated Arenga Starches. Different Letters for Each Time in the Same Line are Significantly Different ($p < 0.05$)