

## Arrowroot Starch Nanoparticles

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**Abstract.** Acetylation is one of prominent starch modification for applications of drug delivery, paper sizing, and film forming. The aim of the study was to enhance the characteristics starch nanoparticle through acetylation of arrowroot starch fractionated by butanol complex. Treatments of this research including level of acid hydrolysis prior to butanol complexation (2 and 24 h) and acetylation duration (30 and 60 min). Parameters observed including: Acetyl content and Degree of Substitution (DS), morphology, particle size distribution, crystallinity, Water and Oil Absorption Capacity (WAC) and (OAC), and FTIR. Result showed that the morphology of acetylated regenerated arrowroot starch exhibit fine, porous and narrow in size as also showed by the particle size distribution which the average diameter ranged from 152.9 to 607.2 nm and PDI from 0.298 – 0.808. The crystallinity pattern and relatif crystallinity was changed. The % acetyl and DS ranged from 3.49 - 6.34 and 0.14 – 0.26 respectively. WAC was about 425%, higher than OAC (about 380%). The FTIR spectra showed increasing intensities of peaks suggested the formation of acetylated products.

**Keywords.** Lintnerized arrowroot starch, regenerated nanoparticle, acetylation, porous starch

### Introduction

Starch, an abundant, non-toxic, biodegradable, edible, and relatively inexpensive material has been widely used in various industries such as food, textile, and pharmacy. Native starches however, have limitation in application because of the hydrophilic in nature, thus having little affinity for hydrophobic ingredients; as well as limited solubility, poor processability (high viscosity), and incompatibility with some hydrophobic polymers [1]. Chemical modification is an effort to enhance starch characteristics.

Preparation of starch by precipitating with butanol could produce nanosized particle as reported by some researcher [2-4]. Production of nanosize particle by butanol complex require preparation of starch into shorter and more crystalline amylose, such as acid hydrolysis (lintnerization) or acid-alcohol hydrolysis [3]. The extend of hydrolysis will affect on the characteristics of the starch butanol complex. The limitations of producing

nanosized particle by butanol complex is low yield and long duration of incubation. Increasing the initial starch concentration to enhance yield might produce particle beyond nanosized and tend to agglomerated. Some researchers successfully modify starch nanoparticle/nanocrystal to enhance their characteristics using acetylation [5]; citric acid [6], or fatty acid [7].

Acetylated starch is one of popular starch modification because of the significant improvement in starch properties. In acetylated starch, part of hydroxyl groups in anhydroglucose units have been converted to acetyl groups. Starch acetylation can be performed to improve significantly the physicochemical and functional properties of the starch, even with a low degree of substitution [8]. Moreover, acetylation of starch nanocrystal increase solubility in organic solvent [5]. The high solubility of the acetylated starch nanocrystals might more compatible with hydrophobic polymers. Acetylation of starch has received much attention for varied drug delivery applications, such as in the preparation of coatings for sustained release of drugs [9].

Precipitation of lintnerized arrowroot starch by butanol have produced starch nanoparticles. The product had nano meter size (about 300 nm) as measured by Particle Size Analyser. However, the formation of the product still agglomerated as shown by SEM with heterogenous particle size. Acetylation of starch nanoparticles produced by butanol complex might improve their characteristics by incorporating acetyl group to enhance the usage especially as carrier matrix. The aim of the study was to enhance the characteristics starch nanoparticle through acetylation of arrowroot starch fractionated by butanol complex.

## **Experimental**

### *Materials*

The raw materials used were Creole varieties of arrowroot tubers originated from the area around Bogor, Indonesia. Arrowroot tubers were then extracted to produce arrowroot starch by wet extraction method in accordance the general methods of extracting starch. The physicochemical characteristics of starch were as follows: moisture content 11.29%, ash 0.19%, lipid 0.46%, protein 0.12%, amylose 37.23% and reducing sugar 55.78 ppm. Chemicals used in the process include HCl, 1-butanol, methanol, HCl, NaOH, all were analytical grade from Merck and used as received.

### *Preparation of acetylated starch nanoparticles*

Starch with two different degrees of linterization (2 and 24 hours) acid hydrolysis prepared according to Jayakody and Hoover [10] were used as material for starch-butanol complex preparation according to Kim and Lim [4] with minor modifications. Linterized starch (40 g) was dispersed with hot distilled water (400 mL) and the suspension was gelatinized and autoclaved at 121°C for 20 min. The solution was cooled to 70°C and about 20% of n-butanol was slowly added to the solution to form a separated butanol phase from the starch solution. The solution was then stirred gently (100 rpm) at 50°C for 3 d. The solution was centrifuged at 5,000 rpm for 20 min, and then the precipitates were washed with ethanol and dried at 40°C overnight. The dried starch nanoparticles were acetylated according to Diop *et al* [11] with slight modification. Starch nanoparticles (10 g) were mixed with glacial acetic acid 30 ml and stirred 30 min and then added with acetic anhydride 10 ml. The temperature was raised to 40°C for 30 and 60 min (B30 and B60). The slurry was added with distilled water, drained and washed several times with distilled water. Lastly the deposit was washed with ethanol twice and dried by freeze dryer.

The parameters observed for arrowroot starch nanoparticles including the % acetyl and degree of substitution (DS), morphology by SEM, particle size distribution, crystalline pattern (XRD), water binding capacity and oil binding capacity, and the changes of chemical structure by FTIR.

### *Acetylation percentage and Degree of Substitution (DS)*

The acetylation percentage (% acetyl) and (DS) were determined titrimetrically, following the method of [12] with minor modification. Acetylated starch (0.5 g) was placed in a 250 ml flask and 25 ml of 75% ethanol in distilled water were added. The flask was agitated, and the temperature raised 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. A blank used the original unmodified starch.

### *Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)*

The WAC and OAC were measured according to methods by Medcalf and Gilles [13].

### *Observation of Morphological Surface by Scanning Electron Microscopy (SEM)*

Scanning electron microscopy (SEM) observations were performed using a JSM/6510LA Analytical Scanning Electron Microscope (JEOL) with a 20 kV voltage and micrographs. The samples (0.3 g) were fixed onto sample holders with conducting glue and then sputtered with a layer of gold. Magnification of 500, 1000 and 5000 X was used.

### *Particle Size Analysis of acetylated starch nanoparticles*

The particle size distribution of the complex particles was determined using a dynamic light scattering detector (NanoSald). The dried particles were re-dispersed in distilled water and were analyzed the size distribution and polydispersion index. The refractive index and the viscosity of water were 1.333 and 1.00 cP respectively.

#### *Crystalline type of acetylated starch nanoparticles*

The pattern of X-ray diffraction (XRD) of the samples was obtained by Shimadzu diffractometer (MaximaX-XRD-7000) with Cu-K $\alpha$  radiation ( $\lambda = 1,5406 \text{ \AA}$ ) at 40 kV in the scan range of theta degree ( $2\theta$ ) was from 5–35° and the scan rate was 2.0 deg/min.

#### *FTIR Spectra of acetylated starch nanoparticles*

The change in chemical structure of the starch was qualitatively analyzed by using FT-IR (ABB MB 3000). Samples were prepared by grinding the finely powdered starch with KBr. The spectrum was recorded over the wave number range between 400 and 4000  $\text{cm}^{-1}$ .

## **Result and Discussion**

### *Degree of Substitution (DS)*

The DS of acetylated starch nanoparticle produced from 60 min of acetylation was higher than 30 min as shown at Table 1. The duration of lintnerization starch showed different value of DS, in which starch from longer lintnerization duration revealed lower DS. The lower DS of 24 h lintnerization might related to higher crystallinity.

Table 1. Acetyl content and DS of acetylated arrowroot starch nanoparticle.

Sample	Acetyl content (%)	Degree of substitution (DS)
H 2B30	3.49±0.28	0.14±0.01
H 2B60	6.34±1.17	0.26±0.09
H 24 B30	3.76±0.17	0.15±0.01
H24 B60	5.60±1.27	0.23±0.02

Note: H2 and H24: 2 and 24 h lintnerized starch; B30 and B60: acetylation for 30 and 60 minutes

The differences DS might be related to different content of amylose and amylopectin, since the complexation with n-butanol increase amylose content. The amylose and amylopectin content will influence the substitution efficiency [14]. The strong self-association of amylose molecules might reduce their accessibility to reagents, while the

less ordered amylopectin molecules were more receptive to modification, leading to higher reaction efficiency.

#### *Morphology and particle size distribution*

Native arrowroot starch has spherical (oval) shape granule with the size ranging from 10 – 30  $\mu\text{m}$  in diameter. The acid hydrolyzed starch (lintnerized starch) maintained the granule form with slight cracks in the surface. Further treatment by butanol complexation produced starch nanoparticles in agglomerated forms (Fig. 1 a and b). The agglomerated particles were formed maybe because of the higher concentration of lintnerized starch used in this research is 5-10% (w/v) of slurry. Other research used 1-3 % (w/v) dextrin to produce nano-sized particle [4]. However, the acetylation process of regenerated starch produced from butanol complex reduces the size and structure significantly especially for 24 hour lintnerized starch. The resulting acetylated regenerated arrowroot starch was observed to form highly porous starch having lower in size with spherical and more homogenous form (Fig. 2 and 3). The distribution of particle size could be seen at Table 2.

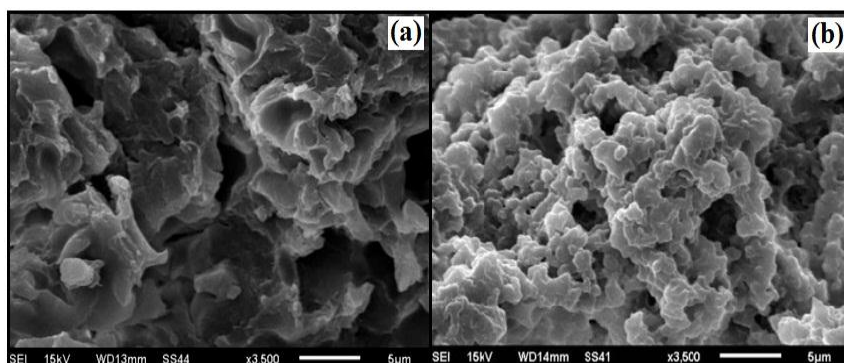


Figure 1. Morphology starch nanoparticle by butanol complex precipitation from 2 and (a) 24 h (b) lintnerized starch.

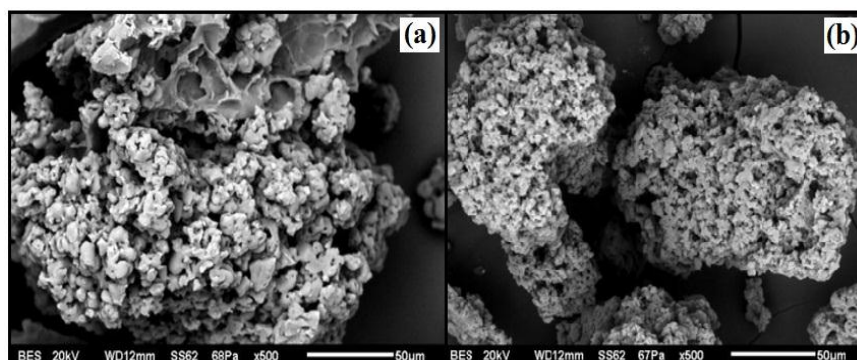


Figure 2. Morphology of acetylated starch nanoparticles (500x) from 2 and (a) 24 h (b) lintnerized starch.

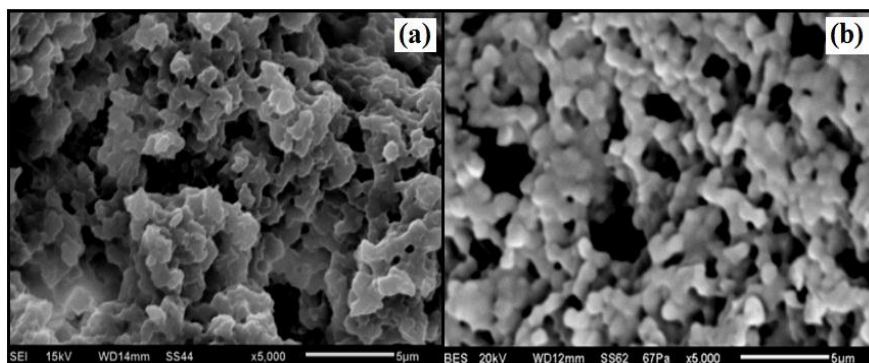


Figure 3. Morphology of acetylated starch nanoparticles derived from 2 h (a) and 24 h (b) lintnerization (5000x).

The particle size distribution of acetylated arrowroot starch nanoparticle using Particle Size Analyzer (PSA) was smaller than that of un-acetylated starch nanoparticles, with the size about 300 nm and ranged from 152.9 nm – 607.2 (Table 2). The Polydispersity Index (PDI) that indicated the heterogeneity of the particles ranged from 0.298 – 0.808. The smaller PDI indicated more homogenous. The result of PSA analysis showed particles distribution between 2 and 24 hour lintnerized starch were not so different although the longer lintnerization seem to have smaller average particle size (Table 2). The higher level of crystallinity and lower Degree of Polymerization (DP) might affect the particle size distribution.

Table 2. Average particle size and polydispersity index (PDI) of acetylated arrowroot starch nanoparticle.

Sampel	Average diameter (nm)	Range particle size (nm)	PDI
H2B60	437.6 ± 96.5	301.4 – 379.6	0.45
	373.8 ± 99.9	287.1 – 466.8	0.62
	607.2 ± 130.2	413.7 – 1290.2	0.57
H24B60	434.3 ± 127.4	340.3 – 550.6	0.54
	152.9 ± 39.0	125.9 – 487.9	0.29
	533.6 ± 153.2	44.7 – 621.4	0.41

#### *Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)*

The OAC of native starch is around 0.64 g/g. Lintnerization and acetylation will increase the capability to absorb oil (OAC) and WAC as well to about 200% as shown at Fig. 4. Further starch modification such as butanol precipitation followed by acetylation improve more the water and oil absorption. Butanol precipitation affected more to increasing WAC

and OAC than lintnerization. This might due to the formation of porous and fine structure resulted from butanol precipitation.

Unlike the previous treatments, WAC tend lower than OAC. The much higher WBC and OBC of acetylated regenerated starch occurred due to introduction of functional group to the starch molecules which facilitated more holding capacity. The higher holding capacity related to the particular structure to both hidrophilicity of carboxyl chain and hidrophobicity of acetic chain.

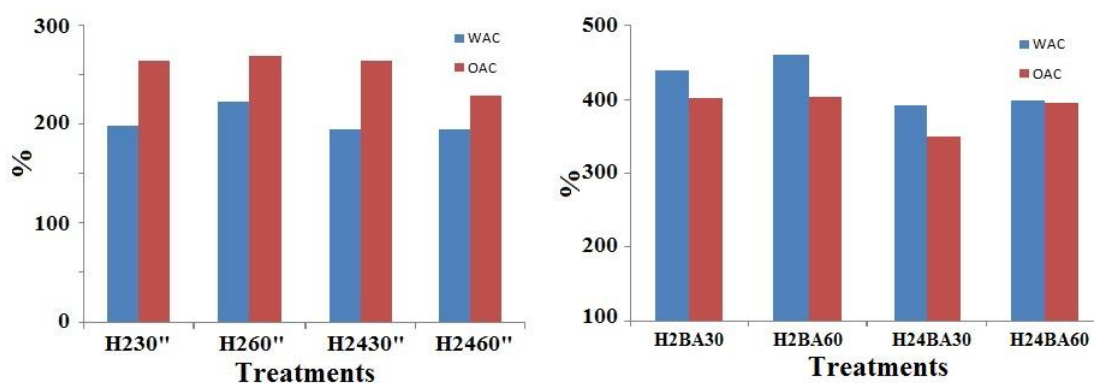


Figure 4. WAC and OAC acetylated lintnerized starch and acetylated starch nanoparticle.

The level of WAC tend to lower than OAC. The results are in agreement with other research [15]. The lower WAC and OAC at long duration of lintnerization may related to its higher crystallinity level. Das *et al* [15] mentioned that the decrease of OAC occur because of the reduction of the amorphous region in starch granule. That lead to reduction the number of available binding sites for oil. Moreover, the lower WAC for 24 h lintnerization may because part of hydroxyl groups in anhydroglucose units have been converted to acetyl groups [16]. Engagement of hydroxyl group to form hydrogen and covalent bonds between starch chain, lowers WAC [17]. The level of WAC and OAC also affected by DS level in which the higher the DS the higher the WAC and OAC level [15].

#### *X-ray Diffraction (XRD)*

The native arrowroot starch showed the typical A-type X-ray pattern. This type of crystal was characterized by peaks at (2 $\theta$ ) at 15°, 17°, 18°, 20° and 23°. The X-ray diffraction indicated that with esterification processing, crystalline structures of native starch were destroyed, and new peaks of acetylated starch was formed. For corn starch, acetylated starch showed a similar profile of native one, but it had a new peak at 9° (2 $\theta$ ), which appeared diffusion peaks of acetylated starch. Acetylated starches represented

typical peaks of acetylated starches, which had wide peaks at  $9^\circ$  and  $20^\circ$  ( $2\theta$ ) [16]. The XRD pattern of the research showed different pattern, in which the acetylated starch nanoparticle of arrowroot show peaks at  $16.9^\circ$ ,  $19.5^\circ$  and  $22^\circ$  ( $2\theta$ ) (Fig. 5). According to Osella [18] the peak at  $20^\circ$  is attributed to a well-formed “V”-structure.

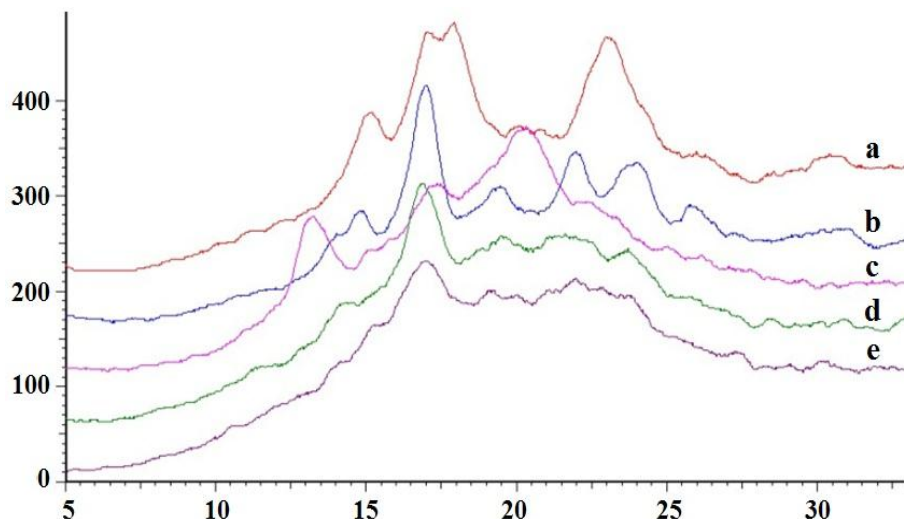


Figure 5. Crystalline pattern of native starch (a), starch nanoparticle: 2h (b); 24h (c) and acetylated starch nanoparticle: H2B60 (d) and H2B60 (e) respectively.

From the figure it could be inferred that acetylation decreased relative crystallinity and tended to form amorphous structure. The value of relative crystallinity of acetylated starch nanoparticle was 41.9% and 41.8% for 2 h and 24 h lintnerized starch respectively; meanwhile, sample without acetylation had relative crystallinity 42.3% and 48.1% for 2 and 24 h lintnerization. Compared to native arrowroot starch (23.3%) the relative crystallinity increased significantly.

#### *FTIR Spectra*

The introduction of the carbonyl groups could be evidenced by FTIR spectroscopy. In the native starch, there are several absorbancies bands at  $1159$ ,  $1082$ , and  $1014\text{ cm}^{-1}$ , which are attributed to C-O bond stretching [19]. Additional absorption bands at  $992$ ,  $929$ ,  $861$ ,  $765$  and  $575\text{ cm}^{-1}$  are due to the entire anhydroglucose ring stretching vibrations. The extremely broad band between  $3000 - 3600\text{ cm}^{-1}$  and the peak at  $2950\text{ cm}^{-1}$  corresponded to OH and CH stretching respectively [20].

FTIR spectra of starch nanoparticles resulted from butanol precipitation revealed almost similar pattern (Fig. 6). However, the acetylated starch nanoparticles showed quite different spectra with some new absorption bands at  $1375$ ,  $1240\text{ cm}^{-1}$  assigned to  $\text{CH}_3$  symmetry deformation vibration and carbonyl C-O stretch vibration, respectively [20]. These new



absorptions suggested that the acetylated starch products were formed during the esterification process. Compared to native starch and un-acetylated starch nanoparticles, the intensity peaks at 3414 and 1647 drastically weaken which indicated that acetyl groups replaced by hydroxyl groups in the starch molecules. This led to the decrease crystallinity which was in agreement with the XRD results (Fig 6. ).

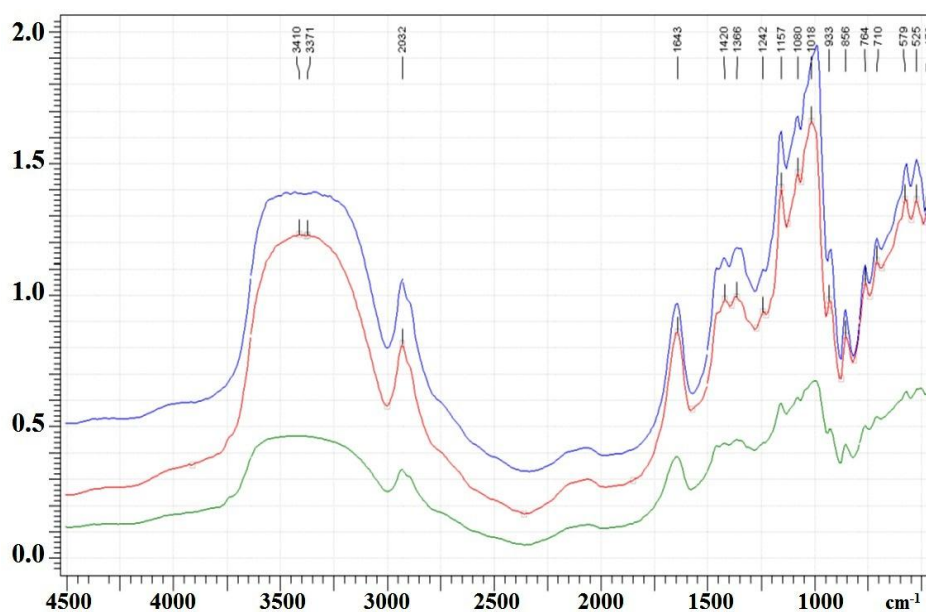


Figure 6. Spectra FTIR of native starch (a), starch nanoparticles (b), acetylated starch nanoparticles (c).

## Conclusion

Acetylation of arrowroot starch nanoparticle enhances the starch characteristics especially the morphology, particle size as well as water and oil absorption. The morphology of acetylated arrowroot starch nanoparticle exhibits fine, porous and narrow in size. Furthermore, the crystallinity pattern and relative crystallinity changes. The acetyl content and DS range 3.49 – 6.34% and 0.14 – 0.26% respectively. Acetylation improved starch properties especially porosity, size and absorption capacity. This result indicated that this complex modified arrowroot starch is potential as delivery carrier for bioactive food and pharmaceutical components.

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