Acetylation of breadfruit utarch by Using Acetic Anhydride

C F Zuhra*, S Gea, M Ginting, Marpongahtun, and S Lenny

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jl. Bioteknologi No. 1 Medan 20155, Indonesia

*E-mail: cutfatimah@usu.ac.id

Abstract. This study was aimed to synthesis starch acetate from breadfruit starch using acetic anhydride by varying the weight of acetic anhydride (10, 15 and 20%) and reaction time (30, 60 and 90 minutes). The swelling power of the starches were determined by gravimetric techniques. Evaluation of the native starch and derivatives was conducted by using Fourier transform infra-red (FTIR). The swelling power of starch acetate increased along with the length of the testing process. Acetylation of bread fruit starch using acetic anhydride by the addition of 20% acetic anhydride and reaction time 90 min resulted highest degree of substitution of 1.23.

1. Introduction
Starch is a very versatile raw material with a broad range of industrial applications such as a thickener, colloidal, stabilizer, gelling agent, bulking agent, water retention agent and adhesive. Starches were isolated from cassava, cocoyam and breadfruit by wet milling process [1].

Breadfruit (Artocarpus altilis) is tropical fruit, which belongs to the family of Moraceae. Starch obtained from breadfruit produces 18.5 g/100 g with a purity of 98.86% and the content of amylose 27.68% and amylopectin 72.32%. Native breadfruit starches have been used as a raw material to prepare different products [2].

The native starch has limited applications since it has a number of shortcomings including insolubility in cold water and tendency to easily retrograde. Modification of properties of starch and starch processing techniques have been developed at this time, the natural starch can be modified in order to have better properties. Modified of starch can be done by chemical, physical and enzymatic processes to improve the desired properties of starch. Chemical characteristic modification is to add new functional groups into the starch molecules, thereby altering the physicochemical properties such as proximate compositions, gelatinization, retrogradation, and pasting characteristics. The methods for the chemical modification of native starches include esterification (acetylation), etherification, cationization,oxidation, acid hydrolysis, and cross-linking have been reported [3].

Acetylation of starch is an important substitution method to improve the physical, chemical and funcitonal properties of starch. The acetylation process are converted from the groups CH₃COO- on to the hydroxyl groups of the glucose monomers. Acetylated starch has improved properties over its native form, it increases viscosity, solubility, swelling factor, hardness, cohesiveness, adhesiveness and translucency of the gels while it decreases. In addition, the acetylation modification process requires lower cost, making it more profitable when used in the food industry [4,5].

Colussi et al. (2015) conducted the acetylation of rice starch in aqueous medium. Acetylation increased the peak viscosity, breakdown, final viscosity, retrogradation and pasting temperature and reduced the hardness of the gels, as well as their adhesiveness and gumminess; however this did not
affect the morphology and susceptibility to hydrolysis by the α-amylase of the starch. The acetylated starches using 10 and 20 g/100 g acetic anhydride showed lower swelling power and solubility compared to native starch [6].

Dev and Prakash (2015) had carried out the acetylation reaction of isolated Bengal gram starch using acetic anhydride as reactant. Acetyl modifications of native starch increased swelling capacity, water absorption power and oil absorption capability by 17, 13 and 20% respectively and acetylation has decreased pasting temperature, pasting time, final viscosity and set back viscosity due to increase in amylose content, hydrogen bonding and porosity of starch granule [7].

Kumoro et al. (2015) performed the acetylation reaction by reacting gadung flour slurry of 20% consistency with glacial acetate acid under alkaline condition. The results show the acetylated gadung flours obtained were having higher swelling power and solubility than the native flour. Acetylation of gadung flour using glacial acetate acid with 1:3 mass ratio and pH 8.0 at ambient temperature for 30 min resulted gadung flour with swelling power and solubility similar to that of Korean wheat flour [8].

The objective of this study was to synthesis starch acetate from breadfruit starch using acetic anhydride by varying the weight of acetic anhydride and reaction time to determine the characteristics of starch acetate.

2. Materials and Method

2.1. Materials
The materials used for this study as procured from E. Merck included sodium hydroxide (NaOH), acetic acid (CH₃COOH, 30%), hydrochloric acid (HCl, 37%), ethanol 95%, dipotassium sulphate (K₂SO₄), potassium chloride (KCl) and phenolphthalein powder (PP). The surface morphologies of acetylated starchs were analyzed by scanning electron microscope (SEM EDX EVO MA 10 carl Zeiss Bruker operating at 20kV). Samples were viewed at magnification between 1000 and 10000x their original sizes. Fourier Transform Infra Red (FTIR) spectra were recorded on Shimazu IR Prestige-21 spectrometer with a disc of KBr. All FTIR spectra were recorded in the transmittance mode in the range of 4000-400 cm⁻¹.

2.2. Fabrication of starch from breadfruit
The breadfruit were purchased from the local market, the skin was peeled and the breadfruit were cut into several small piece, and washed several time until the sap is lost. The pieces of breadfruit were mashed into a paste in a home blender. Then add water to dissolve the starch and separating it from the dregs. Filtering is done repeatedly until all the starch completely dissolves. Starch allowed to settle by paying attention to the top layer of water. The more clear the water means the deposition, the better. After the water sediment is removed, starch dried at 45ºC for 24 hours. The dried starch then mashed and sieved using a 100 mesh and analyzed with FT-IR and SEM

2.3. Preparation of acetylated starch
Acetylated starch was prepared using anhydrous acetic anhydride as reactant. The breadfruit starch 150 g was dispersed in 450 ml of distilled water with constant shaking for 1 hour. The pH of the suspension was adjusted to 8.0 with an NaOH 3% solution. Slurry was reacted with acetic anhydride w/v (10, 15 and 20%) and heated into a water bath at 45 °C with a soaking time (30, 60, 90 minutes). The reaction product was filtered, then the precipitate was washed to a neutral pH. The precipitate obtained the oven at a temperature of 60 °C to constant levels. Starch acetate which is dried and then mashed and sieved using a 100 mesh and analyzed the water content, swelling power, solubility, FT-IR, SEM and determination of the degree of substitution.

2.4. Determination of the acetyl percentage and degree of substitution
The acetyl percentage (Ac%) and degree of substitution (DS) of the acetylated starch were determined by the titration method. Acetylated starch (1 g) was mixed with 50 ml 75% ethanol in distilled water. The flask containing the slurry was covered with aluminium foil and heated in water bath at 50 °C for
30 min. The samples was cooled and 40 ml of 0.5 M was added. The excess alkali in the slurry was titrated with 0.5 M HCl using phenolphthalein as an indicator until pink color disappeared. A blank using native starch was also carried out. The acetyl % and degree of substitution calculated according to Eqs. (1) and (2) respectively.

\[
\text{Acetyl}\% = \frac{(V_{\text{Blank}} - V_{\text{Samples}}) \times \text{Molarity of HCl} \times 0.043 \times 100}{\text{Sample Weight}} \quad (1)
\]

\[
\text{Degree Substitution} = \frac{162 \times \text{Acetyl}\%}{4300 - [42 \times \text{Acetyl}\%]} \quad (2)
\]

2.5. Swelling power

Swelling power were determined using methods developed by Thontowi [9], acetylated starch was weighed by 2-3 g and then place it on a dry cup of known weight, then stored in a desiccator in which already treated with a solution of saturated K₂SO₄ or KCl and weight gain was observed with the sample weighed during a period of 6, 12, 24, 48, 72 h and was calculated by the eqs. (3),

\[
\text{W}_{\text{absorption}}(\%) = \frac{\text{Weight after } \text{hour} - \text{Weight cup and sample}}{\text{Sample Weight}} \times 100 \quad (3)
\]

3. Results and Discussion

Starch acetate is the result of an esterification reaction breadfruit starch using acetic anhydride with a variation of the weight of acetic anhydride 10%, 15% and 20% with a reaction time of 30 min. Starch acetate was tested degree of substitution. Starch acetate has the highest degree of substitution then forwarded to look for the optimum time, as for the variation of time 30, 60 and 90 min.

The infrared spectra for native starch and starch acetate with different weight of acetic anhydride (Figure 1) and reaction time (Figure 2). FTIR analysis also confirmed that the addition of acetic anhydride in the acetylation reaction favors the insertion of acetyl groups to the starch molecule. FTIR spectra of different DS of acetylated starches showed some new absorption bands at 1712.19 – 1720.50 cm⁻¹ assigned to carbonyl C=O, these new absorptions suggest that the acetylated starch products were formed during the esterification process [10, 11].

![Figure 1 FT-IR spectra of(a) native starch, (b) starch acetate with 10% acetic anhydride,(c)starch acetate with 15% acetic anhydride, and (d)starch acetate with 20% acetic anhydride](image-url)
Figure 2 FT-IR spectra of: (a) native starch, (b) starch acetate with 30 minute, (c) starch acetate with 60 minute, and (d) starch acetate with 90 minute reaction time.

3.1. Acetyl percentage (Ac%) and degree of substitution (DS)
The effect of the addition of acetic anhydride at different levels (10, 15 and 20%) and reaction time (30, 60 and 90 minutes), on acetyl (%) and degree of substitution (DS) of breadfruit starch is shown in Table 1 and 2. Test of acetyl (%) and the degree of substitution were used to determine how many acetyl groups were substituted into the breadfruit starch. The degree of substitution (DS) is the average count of sight per glucose unit with substituted starch [7].

Table 1 Degree of substitution (DS) and acetyl percentage (Ac %) and of acetylated breadfruit starch at different concentration of acetic anhydride

| Starch   | Acetic anhydride (%) | DS  | Ac (%) |
|----------|----------------------|-----|--------|
| Acetylated | 10                   | 0.74| 16.45  |
|          | 15                   | 1.01| 21.1   |
|          | 20                   | 1.03| 21.61  |

Table 2 Degree of substitution (DS) and percentage of acetyl content (Ac %) and of acetylated breadfruit starch at different reaction time

| Starch   | Reaction Time (minutes) | DS  | Ac (%) |
|----------|-------------------------|-----|--------|
| Acetylated | 30                     | 1.03| 21.61  |
|          | 60                     | 1.15| 23.48  |
|          | 90                     | 1.23| 24.73  |

The DS and Ac% of acetylated starches ranged from 0.74 to 1.23, and 16.45- 24.73, respectively. Highest degree of substitution of 1.23 is derived from starch acetate made by the addition of 20% acetic anhydride and 90 minutes. In the manufacture of starch acetate substituents are influenced by
many agents are used. This is because more and more acetic anhydride used and the longer the reaction time, the more the acetyl group which can substitute the OH group, because the longer the contact time between acetic anhydride with breadfruit starch will weaken the hydrogen bonds in the starch. Differences in DS and Ac% can be attributed to the type of reagent, concentration, pH, presence of catalyst, reaction time, botanical origin and characteristics of size and structure of starch granules [6].

3.2. Swelling power
The swelling power of starch acetate was conducted to determine how much water can be absorbed by the starch acetate synthesis product. Swelling power obtained from the results of this study are presented in Figure 3 and 4. From the results of tests performed, the weight percent starch acetate absorption increased along with the length of the testing process. It is caused when the starch is heated in excess water, the starch granules absorb water so that over time it becomes fluffy starch (swelling power increased). The amount of water entering the starch granules is reduced when it becomes starch acetate due to fewer hydroxyl groups of starch are substituted by the acyl group of acetic. So that the number of hydrogen bonds of starch was reduced.

![Figure 3](image1.png)

**Figure 3** Swelling power of: (a) starch acetate with 10% acetic anhydride, (b) starch acetate with 15% acetic anhydride, and (c) starch acetate with 20% acetic anhydride.

![Figure 4](image2.png)

**Figure 4** Swelling power of: (a) starch acetate with 30 minutes, (b) starch acetate with 60 minutes, and (c) starch acetate with 90 minutes reaction time

3.3. The surface morphology
The surface morphology was analyzed by SEM. The results obtained can be seen in Figure 5 for variation weight acetic anhydride and Figure 6 for reaction time. The surface morphology of breadfruit
starch showed starch granules intact. Starch granule size larger cause the mixture slightly homogeneous. Starch modification process causes changes in the form and structure granula, where the irregular shape and structure becomes amorphous (birefringent properties was lost). This change lead to the results of morphological analysis of starch acetate showing no homogeneous properties compared to starch breadfruit.

![Figure 5](image_url) SEM images of (a) native starch, (b) starch acetate with 10% acetic anhydride, (c) starch acetate with 15% acetic anhydride, and (d) starch acetate with 20% acetic anhydride
4. Conclusion
Starch acetate was synthesized from breadfruit starch with acetic acid anhydride and the results of the IR spectroscopy test showed the formation of starch acetate was supported by the emergence vibration of $C = O$ at the wave number of 1700 cm$^{-1}$. The highest result in degree of substitution was as many as 1.23, which was obtained from starch acetate made by the addition of 20% acetic anhydride with the reaction time of 90 min.

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References
[1] Gbadamosi S O and Oladeji B S 2013 International Food Research Journal 20 2273
[2] Rincom A M and Fanny CP 2004 Archivos Latinoa mericanos de Nutricion, 54 449
[3] Ashogbon A O and Akintayo E T 2014 Starch/Stärke 66 41
[4] Mirmoghtadaie L et al 2009 Food Chemistry 116 709
[5] Singh N et al 2004 Food Chemistry 86 601
[6] Colussi R et al 2015 Journal of Food Science and Technology 62 1076
[7] Dev K Y and Prakash E P 2015 Journal of Food Science and Technology 52 4176
[8] Kumoro A C et al 2015 Journal of Food Science and Technology 52 6615
[9] Thontowi A 2014 Karakteristik Sifat Fisik Pati Tapioka Modifikasi Ganda dengan Hidroksipropilasi dan Ikat Silang (Bogor: Institut Pertanian Bogor)
[10] Chi H et al 2008 Food Chemistry 106 923
[11] Kalita D et al 2014 Journal of Food Science and Technology 51 2790