Study on Esterification Reaction of Starch Isolated from Cassava (*Manihot esculenta*) with Acetic Acid and Isopropyl Myristate Using Ultrasonicator

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Abstract. Cassava starch is a polysaccharide consists of amylose and amylopectin. This research was purposed to modify the starch isolated from local cassava (*Manihot esculenta*). Modification was undertaken to study the esterification reaction of cassava starch with acetic acid and with isopropyl myristate. Moreover, morphology observation was also conducted both for original starch and its modification yields. It was found that cassava's starch was isolated in 16.4% yield as a white powder. Esterification on the starch provided DS value 0.549 for ratio 1:2 of starch – acetic acid. It gave DS value 0.356 for ratio 1:3 of starch – isopropyl myristate. Treatment by ultrasonication from 0 to 60 minutes was significantly improved the DS value to 0.549 for starch – acetic acid. But it gave DS value to 0.413 for 30 minute ultrasonication of starch – isopropyl myristate. In addition, morphology of the starch observed by microscope gave different features with starch ester acetate and starch ester myristate. The original starch consists of granules, but starch ester acetate indicates a non-granules shape (amorf solid). Moreover for starch ester myristate shows a rather bigger size of granules, and all of the granules afforded were round and oval.

1. Introduction
Cassava plant is one of Indonesia's main agricultural crops [1,2]. In 2015, Indonesia produced 21,801,415 tons of cassava and production in East Java reached up to 3,161,573 tons [3]. Fresh cassava is consists of 35% starch [1] even dried cassava can contain up to 80% of starch [4]. Starch is a polysaccharide that has a high molecular weight and is composed of a polymer of glucose that binds to glycoside bonds [5].

Modification of starch is a process to change the structural of the amylose and amylopectin. Modification or functionalization is to produce better properties of starch. One of procedure for functionalization of starch is esterification [6]. Regiec and coworkers esterified potato starch with fatty acid of oleic acid called by starch ester oleate that could be a thickening agent owing to its hydrophilic and hydrophobic properties [7]. In 2012, Luo et al. [8] synthesized starch ester palmitate which improved the hydrophobicity performance exhibiting good applied prospect. In 2013 Putri et al. [9] reported the pre-gelatinization and phthalazation process of cassava starch resulting pre-gelatinized of cassava starch phthalate (PCSPh) that had a good characteristic as film-forming excipient for
transdermal film for ketoprofen. Moreover, starch ester laurate was produced by esterification reaction between cassava starch and lauric acid. High fatty acid starch esters (chain length of fatty substituent ≥ C8), which have special hydrophobicity, thermo-plasticity, and biodegradability properties, are one kind of the most important starch derivatives [10]. Tang et al. [11] synthesized esterified cellulose nanocrystal (E-CNC) by ultrasonication method. Esterification reaction without ultrasonication had a low DS value (degree of substitution) of 0.22. Using ultrasonication method, the DS value of E-CNC increased to 0.46. This paper will be reported recent finding on the esterification reaction of the cassava starch using acetic acid and isopropyl myristate assisted by ultrasonication. Visual and microscopy morphology, infrared spectra profile, and their DS value will be reported as well.

2. Experimental

2.1. Chemicals and reagent.
Raw cassava was purchased from Dinoyo traditional market, Malang, East Java, Indonesia. Chemical reagents were used such as glacial acetic acid (Smart Lab), isopropyl mirystate (Bratachem), concentrated sulfonic acid (Merck), acetone (Smart Lab), and ethanol (LIPI).

2.2. Instrumentation
We used instrumentation such as infrared spectrophotometer (Shimadzu FTIR-8400S, sample was analyzed using KBr plate (5 mg sample + 95 mg KBr)), ultrasonicator (Branson 2210), microscope (Olympus BX51 with camera DP20), vacuum pump, a set of glasses, stirrer, a sieve with 100 mesh size, oven, grater, Buchner funnel, and analytical balance (Ohaus Pioneer PA214).

2.3. Isolation starch from cassava
The fresh cassava was cleaned and grated into a bowl of water. The grated cassava was squeezed out to get starchy water. It needs to stand starchy water for around 30 minutes. The starch which has white color will settle at the bottom of bowl. The water was separated with the starch. Then the starch was dried and sifted with 100 mesh size. Then we calculated the starch percentage (weight of starch is divided by weight of fresh cassava and multiplied by 100%).

2.4. Esterification reaction between starch-acetic acid and starch-isopropyl myristate
The esterification procedure was conducted based on the research of Tang et al. [11] and Xu et al. [12] with some modification which was published in 2014 [13]. Esterification reaction was firstly conducted by ratio variation between starch and acetic acid (g/mL): 1:1, 1:2, 1:3, and 1:4. Then the mixture of starch-acetic acid was stirred for 60 minutes and then stood for 15 hours at room temperature. The mixture was added 1 drop of concentrated sulfuric acid as catalyst and stirred at 80°C for 60 minutes. The resulting suspension was kept at 69°C and stirred for 60 minutes followed by treatment in an ultrasonicator. After the reaction, the mixture was washed with cold water (10 mL) for 3 times. Then the result of esterification starch dried in an oven at a 55°C. The esterification reaction between starch and isopropyl myristate was conducted following procedure above. The solvent of ethanol:acetin (1:1) of 15 mL was used to wash the esterification yield between starch and isopropyl myristate. The esterification yields are starch ester acetate and starch ester myristate.

2.5. Esterification reaction with time variation of ultrasonication
The time variation of ultrasonication was conducted for ratio 1:2 between starch and acetic acid which has highest DS for 0, 30, 60, and 360 minutes. Moreover, we used ratio 1:3 between starch and isopropyl myristate with varying the time of ultrasonication. The results of esterification reaction are analyzed by infrared spectrophotometry. Based on research Garcia and Vidal [14], the DS value was determined by FTIR spectra with the formula:
\[ DS = \frac{0.76 \times A_{CO}}{A_{OH}} \]

Where DS is the degree of substitution, \( A_{CO} \) is the absorbance of carbonyl groups, and \( A_{OH} \) is the absorbance of hydroxyl groups in the FTIR. Determination of the morphology of starch and esterification yields was performed by a microscope with magnification of 100x, 200x, and 400x.

3. Result and Discussion

3.1. Isolation of Cassava’s Starch

Table 1 listed the isolation of starch from cassava. The cassava has different starch percentage. The highest starch percentage is at entry 3 which has 25.5% of starch at 500 gr of fresh cassava. The starch percentage is affected by age, location, climate and cassava farming area [25]. The average percentage of cassava starch is 16.4%.

| Entry | The weight of fresh cassava (g) | The weight of starch (g) | Starch percentage (%) |
|-------|--------------------------------|-------------------------|-----------------------|
| 1     | 500                            | 54.49                   | 10.9                  |
| 2     | 500                            | 65.59                   | 13.1                  |
| 3     | 500                            | 126.5                   | 25.3                  |

Figure 1 Starch (A) and esterification starch: starch ester acetate (B), starch ester myristate (C)

3.2. Esterification of Starch

Starch and esterification yields are presented in Figure 1. Figure 1.A shows the original starch forms white powder. Esterification reaction causes the characteristics of starch turns into solids (B) and white powder agglomerated (C). In addition, the success of starch esterification can be demonstrated by the changes of absorption area and absorption intensity between starch and starch esterification yields. Starch spectra (Figure 2 black line) at 3357, 2931, and 1155 cm\(^{-1}\) show the starch-containing O-H, C-H and C-O-C asymmetric groups, respectively. Esterification of starch produce new absorption at 1740 cm\(^{-1}\) which indicates the C=O ester group. In addition, it is observed that there is reduction of hydroxyl group peak at 3357 cm\(^{-1}\) region of esterification yields spectra in relative to that of its original starch. It is seen that the esterification of starch by acetic acid/isopropyl myristate was successful. The esterification reaction carried out in accordance Figure 3.
Figure 2 Infrared spectra of starch and esterification yields (black: native starch, blue: starch ester acetate, and red: starch ester myristate)

Figure 3 The esterification reaction of starch (esterification reaction between starch and acetic acid: (1) or isopropyl myristate (2))

3.3. Esterification reaction between starch-acetic acid and starch-isopropyl myristate

Figure 4 presented the DS value of esterification yields with ratio variation. The DS of starch ester acetate increase from ratio 1:1 to 1:2. However, the DS of starch ester acetate decrease from ratio 1:2 to 1:4. On the other hand, the DS of starch ester myristate slightly increase with increasing the ratio of isopropyl myristate acid from ratio 1:1 to 1:3 and turn to decrease for ratio 1:4. Theoretically, increasing the amount of reagent can increase the DS of starch because it will increase the rate of collision between reactant molecules and starch. Immobility or limited movement of the hydroxyl groups of starch also causes the esterification reaction depends on the availability of reagents in close to starch molecule [12]. However, starch acetate ester product declined in the ratio of 1:3 and 1:4, while the starch ester product myristate in a ratio of 1:4. It may be accused by equilibrium reactions that lead to backlash reaction.
3.4. Esterification reaction with variation of ultrasonication time
Ratio 1: 2 for starch-acetic acid and 1: 3 for starch-isopropyl myristate was used to investigate the effect of variation of ultrasonication time. Figure 5 showed the correlation between ultrasonication time and DS value. The treatment without ultrasonication (0 min) yields lowest value of DS 0.462 for starch ester acetate and 0.278 for starch ester myristate. Ultrasonication treatment of esterification reaction generally increased the DS. It is seen that the highest DS value of starch ester acetate is at 60 minutes. Moreover, starch ester myristate has highest value of DS at 30 minutes. However, the increasing the ultrasonication time at 360 minutes for the reaction of the starch-acetic acid and after 30 minutes of reaction starch-isopropyl myristate give decreasing DS value. The longer ultrasonication time could damage to the interior of starch [11]. Thus, only few hydroxyl groups may be substituted by acetyl and myristyl groups.

3.5. Morphology of starch and esterification yields
Morphology of starch and esterification yields were observed by a microscope which were presented in Figure 6. Figure 6 shows the starch granules are composed of visible dots in black and white. This is because the granules have birefringence properties [15] that can reflect light. The starch granule (Figure 6.A) is round and oval. While starch esterification reaction products with acetic acid (starch ester acetate) composed of granulated and non-granular (amorphous solids) (Figure 6.B-C). Starch ester myristate granule is composed of round and oval shapes (Figure 6.D-F).
Figure 5 Correlation of ultrasonication time with the DS value (solid line: acetic acid modified starch, dash line: isopropyl myristate modified starch)

Figure 6 The image was captured with magnification of 400x on a sample of starch (A), starch ester acetate (B. ratio 1:2, C. without ultrasonication treatment), and starch ester myristate (D. ratio 1: 3, E. without ultrasonication treatment, F. 30 minutes of ultrasonication).
4. Conclusion
The esterification yields of starch are starch ester acetate and starch ester myristate. Ratio variation of reactants and ultrasonication time affect the DS value. The highest DS value for starch ester acetate is 0.549 at ratio 1:2 for 60 minutes of ultrasonication. On the other hand, the highest DS value of starch ester myristate is 0.413 at ratio 1:3 for 30 minutes of ultrasonication. Starch consists of granules with round and oval shapes. Starch ester acetate is mostly composed of nongranules (amorphous solid) while starch ester myristate composed of granules.

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