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Effect of Esterification on Cassava Starch: Physicochemical Properties and Expansion Ability

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Abstract

In this study, the characteristic of cassava starch has been developed through esterification using ethyl lactate. Commercial cassava starch was modified by using (0.5; 0.75; 1.00; 1.25; 1.50 % v/v) ethyl lactate solution with pH 6, 7, 8, 9 for 15, 25, 35, 45, 55 minutes and the cassava starch was dried with sun drying. The cassava starch has been esterified with 1.5% ethyl lactate for 15 minutes pH 6 gave highest volume expansion of 2,438.9 %. For swelling power, the highest value was 19.32 swelling power at variable concentrations of 1.5% ester, esterification period of 15 minutes, and pH 6. The lowest amylose content was 15.68 % in ester concentration of 1.5 %. From the scanning electron micrograph, shows that the cassava starch after and before modification has not changed since the modifications performed under the gelatinization temperature. FTIR spectra of native cassava starch and ester cassava starch has shown shift of the carboxyl group of starch from 1,072.37 to 1,087.80 cm^{-1} and a decrease in the OH group is from 3,452.53 to 3,417.81 cm^{-1} . Bond formed indicated that the esterification reaction occurs between cassava starch with ethyl lactate ester.

Keywords: expansion, cassava starch, ester starch, ethyl lactate, modified starch

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INTRODUCTION

Starch is an important natural resources which can be obtained from may renewable sources such as corn, potato, rice, wheat, peas or cassava. It's field of application is very broad from food, paper, and pharmaceutical to plastic industries (Barrios *et al.*,

2013). The main reason starch is chemically treated before commercially use is to split the long glucose chains of the polymer molecule to increase and obtain the maximum possible amounts of starch in technical applications (Dias *et al.*, 2011). Food grade starches are chemically modified mainly to increase paste

consistency, smoothness, and clarity, and to impart freeze-thaw and cold storage stabilities (Jane, 1997; Liu *et al.*, 1999a, 1999b; Perera *et al.*, 1997; Shi & Bemiller, 2000; Woo and Seib, 1997).

Modification of cassava starch has been carried out, among others, chemical, physical, enzymatic, and genetic (Kaur *et al.*, 2012). According Demiate *et al.* (2000) modification of cassava with natural fermentation followed by drying in the sun can improve expansion ability of cassava flour. Modified cassava starch by addition of lactic acid has been developed to speed up the fermentation process (Vatanasuchart *et al.*, 2005). Cassava starch modification history begins with sun dried and fermented cassava starch, known as *Polvilho azedo* or sour cassava starch. *Polvilho azedo* is a traditional product originating from Brazil and known in some countries in South America. Cassava starch extracted from cassava and soaked in a tank for natural fermentation for 30 days with an excess of water 5 cm above the surface of cassava starch. The process was dominated by lactic fermentation (Cereda, 1994). Wet starch is dried in the sun for 1-2 days depending on the weather and season (Cardenas and Buckle, 1980).

The treatment of starch by UV light, with or without a photosensitizer (Bertolini 2001), induces changes in its functional properties; it increase water buding capacity and solubility (Gholap *et al.*, 1993) and decrease hot paste viscosity (Fiedorowicz *et al.*, 1999). UV photodegradation of starch produced glycosidic bond cleavages, with a shortening oh amylose chain and debranching of amylopectin chain, through free radical formation (Bertolini *et al.*, 2001; Sumardiono *et al.*, 2018). Starch shows various changes in characteristics after modifying such as solubility, gelatinization temperature, viscosity, and stability of the paste, moisture, water retention, and film property (Rajan *et al.*, 2006).

Many research groups have tried to understand what kind of modification is responsible for the baking properties of cassava sour starch, but until now there is no conclusive answer. Esterification is one of modifications, which can impart hydrophobicity to

starch products by substitution of free hydroxyl group (Hujibrechts *et al.*, 2008) and has been carried out by a variety pathway (Barrios *et al.*, 2013). Esterification of starch using long-chain fatty acids is going to produce thermoplastic starch, so it can be used further to the needs of the pharmaceutical and plastics industries (Rajan *et al.*, 2006). This research will develop a modified starch using esters in the field of food.

Cassava starch (*Manihot esculenta* Crantz) is very important for the food industry, but the utilization is still limited. Cassava starch has no expansion ability compared with wheat flour. Wheat formed viscoelastic dough, caused by of gluten and gliadin proteins, capable of retaining gas during fermentation and at the early stage of the cooking process (He and Hoseney, 1991). This present study was undertaken to examine effects of esterification cassava starch using ethyl lactate on changes in molecular properties responsible for volume expansion behavior.

MATERIALS AND METHODS

Materials: Commercial cassava starch was purchased from Margoyoso, Pati (Indonesia). Other chemical agents were analytical grade from Merck (Germany).

Preparation of ethyl lactate: In this study, ester was obtained by mixing lactic acid and ethanol with various concentrations (0.5, 0.75, 1.0, 1.25, 1.5% v/v). Ratio of lactic acid and ethanol is 1:1. The mixture was allowed to stand for 24 hours. After 24 hours ester solution was diluted in 1000 ml of distilled water.

Experimental prosedure: 500 g of cassava starch was dissolved in ester solution. pH was measured on variable 6, 7, 8, 9. The solution was stirred using a magnetic stirrer with a stirring speed of 8 scales for variations in prescribed time 15, 25, 35, 45, 55 minutes. The product was filtered with a filter cloth, then placed on a portable solar dryer and dried for 9 hours. The scheme of experimental can be seen in Figure 1.

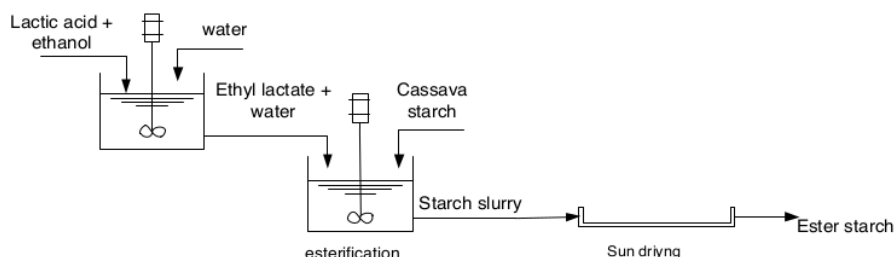


Figure 1. Esterification process of cassava starch

Swelling Property: Starch (0.1 g) was mixed with 10 ml distilled water in centrifuge tube and heated using a water bath with a temperature of 60°C for 30 minutes. The supernatant was separated using a centrifuge at a speed of 2500 rpm for 15 minutes. Swelling power is calculated by Eqs 1.

$$\text{Swelling Power (gr/gr)} = \frac{\text{supernatant weight}}{\text{dry starch weight}} \quad (1)$$

Determination of amylose content: Colorimetric method was used for determining the changes in amylose contents of starch samples after treatments (AACC, 2000).

FTIR Analysis: Results of esterification can be demonstrated using FTIR analysis (Fourier Transform Infra-Red). FTIR analysis is used to determine the change of carbonyl and carboxyl groups with starch ester carbonyl groups. Samples in the form of granules mixed with dry KBr. The mixture was made into a movie that will be analyzed by the light beam FT-IR spectrophotometer.

SEM Analysis: Scanning electron microscopy (SEM) was used to determine the surface starch esterified photography, cracks, and surface starch films. Starch samples mounted on aluminum specimen stubs with double-sided tape, and then coated with a 20 nm gold.

Frying Properties: Volume expansion is calculated by measuring the volume of dough before and after frying. Dough was made 20 times in order to obtain diverse and representative data of the sample. Dough diameter was measured with ruler, before frying (D1) and after frying (D2), the calculated each volume. Dough volume is calculated as the assumption that product perfectly spherical. Volume expansion level measured by Eqs 2.

$$\text{Volume expansion (\%)} = \frac{V_1}{V_2} \times 100\% \quad (2)$$

RESULTS AND DISCUSSION

Swelling Power Analysis

Swelling power of all variables on the study of modified cassava starch is shown in Table 1 and Figure 2. Esterification of starch is one of chemically method of modified starches. Modified starch using an esterification reaction aims to change the functional groups, hydroxyl groups of starch with carbonyl groups of esters (Jerachaimongkol *et al.*, 2006). Esterification process was carried out by reacting ethyl lactate esters with cassava starch, and then dried in the sun. From the results, the highest value was 19.32 swelling power at variable concentrations of 1.5% ester, esterification period of 15 minutes, and pH 6. In Figure 2 (a) shows increasing swelling power values due to increased concentrations of esters. Swelling power is strongly influenced by the network bonds between starch molecules. With addition of ester molecules into starch molecules, the bonds between starch molecules will weaken thus increasing the value of swelling power. The higher ester concentration will replace more hydroxyl groups in starch.

Cassava starch esterification process using ethyle lactate is strongly influenced by concentration variables of esters. In Fig 2 (b) shows that increasing period of esterification will decrease swelling power up to 35 min. This is because of long esterification period caused starch granule swollen. When the swelling process prolonged, the starch wall will begin to split, and damaging the granule, thereby reducing the swelling power. In the processing time 45 min, the swelling power increased, as the prolong esterification process lead to the amylose content reduction and amylopectin content in reverse.

Table 1. Experimental data of ester cassava starch in esterification variable

Esterification variabls			Volume expansion (%)	Swelling Power (gr/gr)	Amylose content (%)
Time (min.)	Ester Con. (%)	pH			
15	0.50	6	1,757.60	13.82	19.80
15	0.75	6	2,057.08	17.48	18.33
15	1.00	6	2,148.49	18.35	18.04
15	1.25	6	2,339.37	18.65	17.40
15	1.50	6	2,438.90	19.32	15.68
15	1.50	6	2,438.90	19.32	15.68
25	1.50	6	2,290.63	15.27	16.57
35	1.50	6	2,195.20	10.29	17.30
45	1.50	6	2,148.49	12.28	16.78
55	1.50	6	2,012.36	13.53	16.10
15	1.50	6	2,438.90	19.32	15.68
15	1.50	7	2,242.58	11.01	15.90
15	1.50	8	2,242.58	9.36	16.40
15	1.50	9	1,717.35	8.66	17.39

Amylopectin was insoluble in water. If the content of amylopectin increased, the paste will be increased and also swelling power value will increase. Starch degradation will occurs easier at acidic pH. Esterification at neutral or alkaline pH reduces expansion of cassava starch and inhibits depolymerization process. In Figure 2 (c) shows that the higher the pH esterification the swelling power decreased.

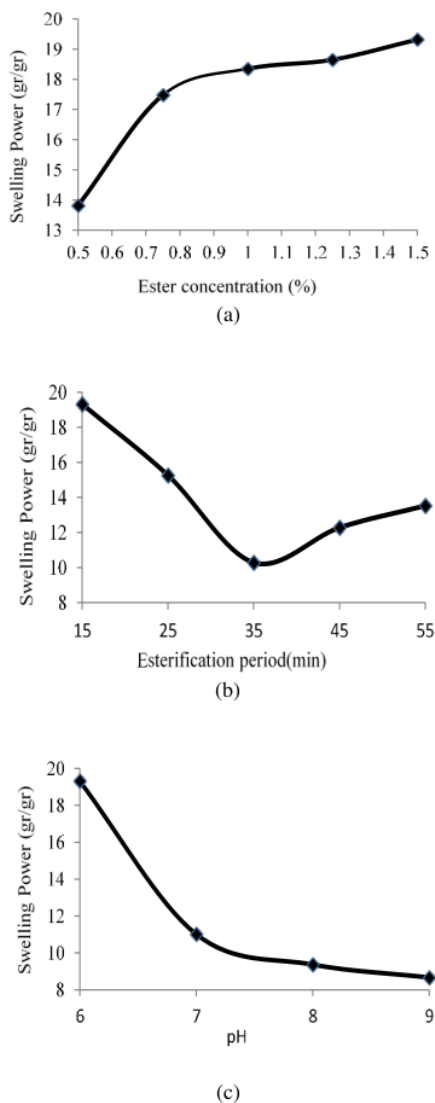


Figure 2. Swelling power of ester starch as function of ester concentration (a), esterification period (b), and pH (c).

Amylose Content Analysis

In the results showed that the amylose content in starch modified starch decreased as compared with native starch. Amylose content measurement can be seen in Table 1. Figure 3 shows the relationship between addition of ester concentration (a), time of esterification (b), and pH (c). On concentrations variable of esters showed a decrease in amylose content. The higher concentration of esters has lower amylose content. Lowest amylose content was 15.68 in ester concentration of 1.5 %. At the period esterification variables showed an increase up to 35 minutes and then decreased until 55 minutes.

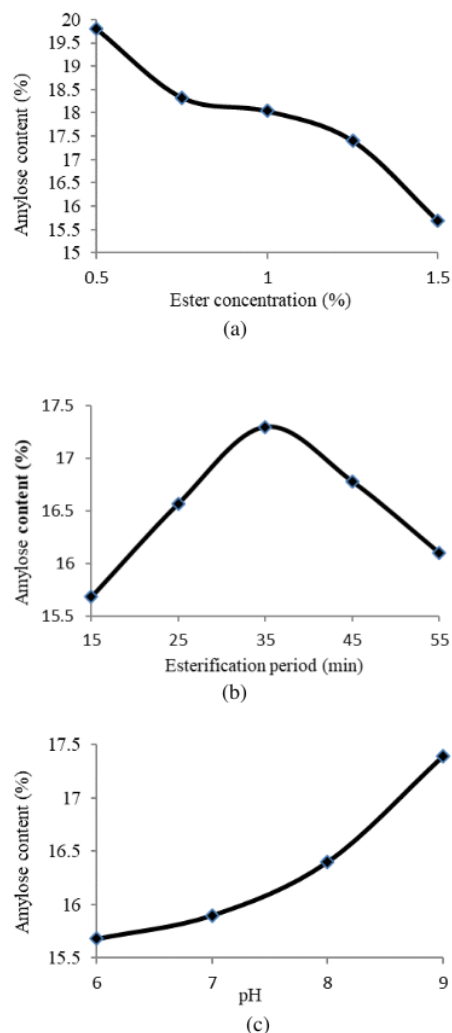


Figure 3. Amylose content of ester starch as function of ester concentration (a), esterification period (b), and pH (c).

Amylose content in native starches decreased after modified using ethyl lactate esterification methods. Reduced amylose was caused by partial depolymerization of amylose by ester and sunlight irradiation. Depolymerization of amylose led to the formation network structure of starch that affects the expansion ability. Lower amylose content caused better starch expansion properties. In a previous study were also explained that the amylose and amylopectin simultaneously degraded by acid hydrolysis with or without UV radiation (Sumardiono *et al.*, 2018; Bertolini *et al.*, 2000).

In addition, pH variable can be seen that the amylose content increased with increasing pH Fig 3 (c). At pH 6 amylose content in starch was 15.68, whereas at pH 9 amylose content of starch was 17.39. Amylose depolymerization occurs optimally under acidic conditions than under alkaline conditions, at neutral or alkaline pH may inhibit the depolymerization of starch thereby reducing the development of cassava starch.

SEM Analysis

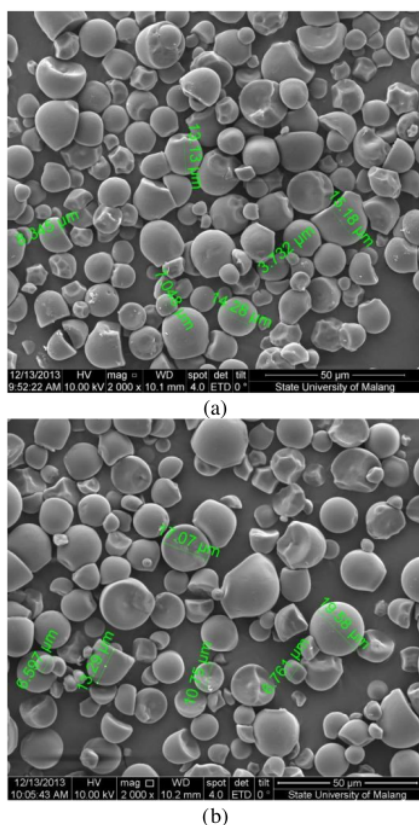


Figure 4. SEM micrograph of starch granule of native starch (a) and ester starch (b)

Surface morphology of cassava starch was observed using SEM (Scanning Electron Micrograph) with a magnification of 2000 times. The sample used in the SEM analysis is unmodified cassava starch (native starch) and starch esters with variable optimum concentration of 1.5% ester, esterification period of 25 minutes, and pH 6. Figure 4 shows the surface structure of native starch (a) and starch esters (b).

In Figure 4 shows that the cassava starch after and before modification has not changed since the modifications performed under the gelatinization temperature. Both have a structure that is round and have irregular pieces at the ends. The size of both types of particles varies. Native starch has a particle size averaging between 3.73 to 15.18 μm , while the starch ester having an average particle size of the larger of between 6.59 to 19.58 μm .

FTIR Analysis

FTIR spectra of native starch and esters starch are shown in Fig 5. In both spectra can be seen a few peaks of functional groups that are characteristics of starch include CO stretch with a wavelength of 1000-1,260 cm^{-1} , CH bend between 1,290-1,430 cm^{-1} , C=C between 1,630-1,690 cm^{-1} , CH stretch between 2,800-2,950 cm^{-1} , and OH stretch 3,300-3,400 cm^{-1} .

In Figure 5, it can be seen that the spectrum of native starch showed a CO stretch spectrum at a wavelength of 1,072.37 cm^{-1} , CH bend at a wavelength of 1,419.63 cm^{-1} , C=C at a wavelength of 1,643.30 cm^{-1} , CH stretch between 2,933.68 cm^{-1} , OH stretch 3,452.81 cm^{-1} . In ester starch showed CO stretch spectrum at a wavelength of 1,087.8 cm^{-1} , CH bend at a wavelength of 1,417.63 cm^{-1} , C=C at a wavelength of 1,643.30 cm^{-1} , CH stretch between 2,933.68 cm^{-1} , OH stretch 3,417.81 cm^{-1} . These results indicate a shift in the carbonyl group (CO) and hydroxyl group (OH).

From the results of the absorption peaks seen by the shift of the carboxyl group of starch from 1,072.37 to 1,087.8 cm^{-1} and a decrease in the OH group is from 3,452.53 to 3,417.81 cm^{-1} . Decreasing of OH group showed a substitution of hydroxyl groups of starch molecules with the ester carbonyl group. Increased carbonyl group due to the addition of starch on starch esters, carbonyl group substituting OH groups of starch will lead to changes in the structure of the modified starch (Perera *et al.*, 1997). Bond formed indicates that the esterification reaction occurs between tapioca starch with ethyl lactate ester.

Frying Properties

Modified cassava starch will be applied to process of coated peanut. Coated peanut processing was carried out by mixing 5 g of cassava starch and 3 ml of warm water temperature of 60 $^{\circ}\text{C}$. The dough is formed and measured initially at D1 diameter. The spheres are fried in hot oil with temperature 160 $^{\circ}\text{C}$ for 1 minute. Fried coated peanuts were measured the diameter of D2, then calculated the volume expansion.

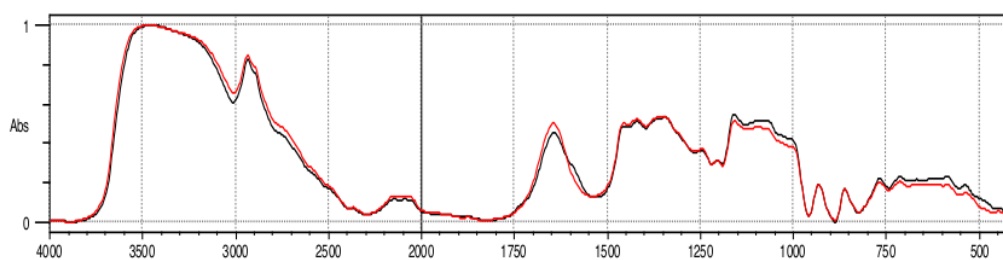
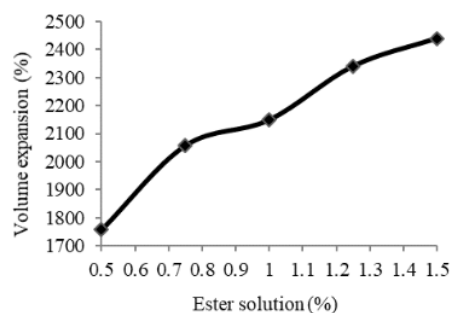
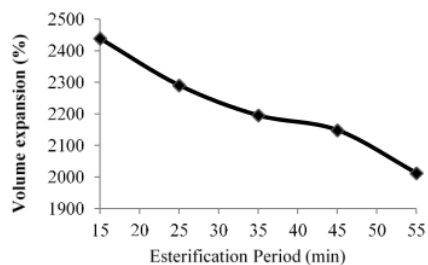


Figure 5. FTIR spectrum of native starch (red) and ester starch (black)

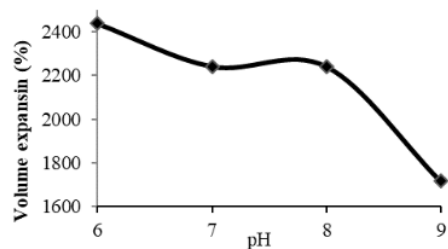
Volume expansion level data on each variable can be seen in Table 1 and Figure 6 (a), (b), (c).



(a)



(b)



(c)

Figure 6. Volume expansion of pilus as function of ester concentration (a), ester period (b), and pH (c).

Figure 6 (a) shows that the higher ester concentrations will increase volume expansion level. The largest volume expansion level is 2,438.9 % shown in ester concentration of 1.5 %. Most influential variable in esterification is starch concentration. While in the time variable esterification and pH levels decreased volume expansion level. Increasing ester concentration because the pH solution decreases. pH on starch effect on the amylose degradation process. Figure 7 shows the significant difference in volume expansion level between native starch with starch esters. The decrease in amylose content causes dough expansion ability (Vatanasuchart *et al.*, 2000).

Sufficient reaction with UV light causes partial depolymerization of amylose molecules and form hydrogen-bonded network structure. The network structure can absorb and store water during gelatinization process. UV drying enough energy help to improve starch properties development. Increasing the specific volume caused of increased amount of water lost during the frying process. At starch gelatinization due to heating, the water absorbed evaporated because of the high pressure caused by the expansion of the current driving force (Sumardiono *et al.*, 2017; Bertolini *et al.*, 2001). The process of water evaporation during frying process causes volume expansion increasing.



Figure 7. (1) coated peanut before frying, (2) fried product of native cassava starch, and (3) fried product of ester cassava starch

CONCLUSION

Cassava starch was modified to prepare starch-based rheological properties by esterification with ethyl lactate. Addition of ester molecules into starch molecules caused the bonds between starches molecules weaken thus increasing the value of swelling power. The higher ester concentration will replace more hydroxyl groups in starch. Structure of cassava starch after and before modification has not changed since the modifications performed under the gelatinization temperature. Both have a structure that is round and have irregular pieces at the ends. From the results of the absorption peaks seen by the shift of the carboxyl group of starch from 1,072.37 to 1,087.80 cm^{-1} and a decrease in the OH group is from 3,452.53 to 3,417.81 cm^{-1} . Decreasing of OH group showed a substitution of hydroxyl groups of starch molecules with the ester carbonyl group, its indicates that the esterification reaction occurs between tapioca starch with ethyl lactate esters.

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NOTATION

v_1 : volume of coated peanut before frying process

v_2 : volume of coated peanut after frying process

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