7.10. Modification of Colocasia esculenta Starch with Acetylation Process

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Modification of *Colocasia esculenta* Starch with Acetylation Process

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Abstract

Modified starch is treated to change one or more physical or chemical properties. Modified starch is needed in food products to improve the texture of the paste, improve viscosity, and maintain starch granules at high temperatures. One chemical modification of starch is acetylation using acetic acid. Many tubers are not yet widely used, so they need to be developed through the change of acetylation-modified starches. Tubers *(Colocasia esculenta)* has a large enough starch content so that it has the potential to be processed and has high economic value. The purpose of this study was to determine the best treatment between the concentration of acetic acid and the soaking time in starch modification. This study used a completely randomized design factorial. The first factor was the concentration of acetic acid (4%, 6%, and 8%) and the second factor was the duration of soaking (30, 60, and 90 minutes). The best treatment was modified starch with 8% acetic acid and 90 minutes soaking time. This resulted to contained moisture of 5.97%, ash 1.39%, starch 63.49%, amylose 29.93%, swelling power 20.44 g / g, solubility 17.67%, yield 89.28%, substitution degree 0.66, viscosity 3589 cP.

Keywords: *Colocasia esculenta*, starch, modification, acetylation, acetic acid

Introduction

Colocasia esculenta is one type of tuber that has been widely known by the public. Nurbaya and Estiasih (2013) states that in 2011 through the implementation of alternative food area activities, the total productivity of several regions was 661 quintals/hectare in Indonesia. Tuber starch content was 66.8% with a water content of around 7.2% (Julianto, 2014).

The starch that has not been modified (native starch) has several disadvantages, namely: it requires a long cooking time, a paste that forms hard and not clear, is too sticky, can not stand the acid treatment, low viscosity, low solubility, and strength low swelling. These constraints cause natural starch to be of limited use in the food industry. The technology has been developed to modify starch to obtain a higher brightness (clearer), stable viscosity at both high and low temperatures, gels formed clearer, gel texture formed more softening, low stretch strength, starch granules break more easily, high swelling strength, high solubility, and time and temperature of starch granules to break lower (Koswara, 2009).

Natural starch can be modified so that it has the desired properties. Modification is intended as a change in molecular structure that can be done chemically, physically, or enzymatically. Natural starch can be made into modified starch with desired characteristics or according to need (Hee-Young An, 2005). Modification of starch by acetylation is one modification that is widely used. The structure of starch can be changed and the desired characteristics of starch such as being more resistant to

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retrogradation and low temperatures without changing the physical appearance of modified starch. Factors that influence the speed of chemical reactions are the reactant concentration, pH, temperature, reaction time, and type of starch. Modification with acetic acid produces starch with thinner properties if dissolved, more soluble, and lower molecular weight produces starch which has high viscosity if the solution is made and is more resistant to mechanical treatment (Hardoyono, 2007).

The desired important properties of acetylated modified starch include swelling power (soups, sauces, and broths), solubility (instant spices), and higher viscosity (pie filling) compared to natural starch. In addition, the modification process with acetylation requires lower costs, so it is more profitable if used in the food industry (Ayucitra *et al.,* 2009).

Research Method

Research Material

Materials used in the study include *Colocasia esculenta* tubers obtained from Traditional Markets, Acetic Acid, NaOH, HCL and aquadest. The ingredients used for the analysis are HCL (PA Sigma Aldrich) 3%, NaOH 30%, Luff Schrool, KI 20%, H2SO4 25%, Ethanol 95%, Acetic Acid 99% 1N, KOH 0.5 M, HCL (PA Sigma Aldrich) 0.5 N, PP indicator.

Research Procedures

- Making *Colocasia esculenta* starch

The *Colocasia esculenta* soaked in a 7.5% salt solution in a ratio of 4: 1 (salt solution: tuber tuber) for 1 hour. Then was washed. The *Colocasia esculenta* tubers were crushed and extracted in a ratio of 4: 1. That have been extracted. Then squeezed using a filter cloth. The starch milk was deposited for 6-8 hours. The starch sediment that has been formed was dried at 60 ° C for 6 hours.

- **Modification of *Colocasia esculenta* starch**)

The 75gr starch of *Colocasia esculenta* soaked in 375 ml distilled water to facilitate the process of mixing starch with acetic acid reagents. Acetic acid with concentrations of 4%, 6%, and 8% was added simultaneously to the starch solution. The pH of the solution during the reaction was maintained 8 - 8.4 with a 0.3 M NaOH solution. After a certain reaction time interval (30, 60, and 90) minutes, 0.5 N HCl solution was added to pH 6. The starch slurry was then filtered. The precipitate washed with distilled water to pH 7. Starch deposits were then dried at 40 ° C. The modified starch obtained was analyzed for its water content (AOAC 1995), ash content (AOAC, 1995), starch (AOAC 1995), amylose (Riley *et al*., 2006), swelling power, solubility (Kiatponglarp 2007) and degree of substitution (Chen dan Voregen 2004).

Result and Discussion

The Characteristic of Colocasia esculenta Starch

The resulted of swelling power analysis, solubility, yield, water content, ash content, starch content, amylose content, and the yield on unmodified *Colocasia esculenta* starch can be seen in Table 1.

Table 1. The characteristic of *Colocasia esculenta starch* (not been modified)

Compound	total			Literature
Moisture (%)	11,38	±	0,08	13,18 [b)]
Ash (%)	1,27	±	0,04	0,81 [c)]
Starch (%)	79,95	±	0,05	79,24 [a)]
Amylose (%)	34,13	±	0,06	22,82 [a)]
Swelling Power (g/g)	10,48	±	0,06	9,48 [a)]
Solubility (%)	11,38	±	0,02	10,60 [a)]
Yield (%)	17,72	±	0,01	17,44 [d)]
Viscosity (cP)	1312	±	12,72	4327 [c)]

Source: a) Widiawan et al (2012) b) Rahmawati et al (2011) c) Agustin (2011) (4) Zahruniya (2014)

Amylose content in *Colocasia esculenta* starch was equal to 34.13%. This is different from the results of the study of Widiawan et al (2012) found amylose content in tuber starch that is equal to 22.82%. Swelling power on *Colocasia esculenta* starch was 10.48 (g/g) greater than swelling power of tubers resulted by Widiawan which is 9.48 (g / g). Viscosity in tubers starch which was 7760.50 cP is different from the viscosity of cassava starch that is equal to 44327 cP (Agustin, 2011).

The Colocasia esculenta Modified Starch

Water Content

The water content in *Colocasia esculenta* modified starches ranged from 5.25% - 5.97%. The highest water content was obtained in the treatment of acetic acid concentration of 8% and soaking time of 90 minutes which was 5.97%. The water content of raw material (unmodified starch) was 11.382%. After changing into modified starch, the water content obtained was lower. The effect of acetic acid concentration and soaking time can be seen in Figure 1.

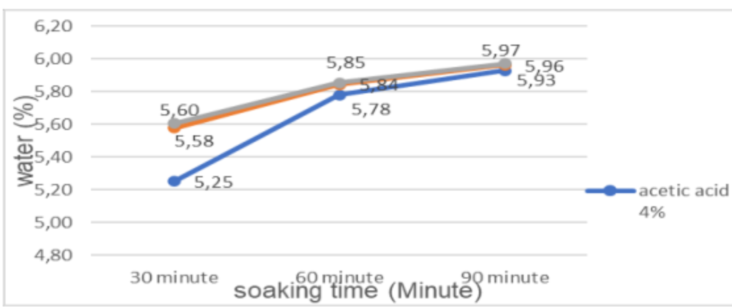

Figure 1. The water content of *Colocasia esculenta* modified starch

Figure 1 showed that the higher the concentration of acetic acid and the longer soaking time, the water content of modified starches increased. This was due to the higher concentration of acetic acid and the longer the soaking time in the acetylation process. The absorption of water by the starch granules will occur. Starch granules will expand because they absorb water in the presence of heating.

The states that the longer the reaction time, the starch granules will expand because they absorb water by heating. The more OH-starch groups substituted by the acetyl group. Pudjihastuti (2010) states that the presence of acid can hydrolyze the starch chain. The starch is reduced so that the starch chain tends to be shorter and easier to absorb water. The results of study that the higher the concentration of acetic acid used, the higher the water content produced. The acetylation process generally will increase the ability to absorb water. This is associated with an increase in the degree of substitution by the acetyl group which causes acetylated starch to have a weaker hydrogen bond than starch without modification. The more water enters the acetylated modified starch granules than unmodified starch (Bolade and Oni 2015).

Ash Content

The ash value of *Colocasia esculenta* modified starch ranged from 1.01% - 1.39%. The highest ash content was obtained from the treatment of acetic acid concentration of 8% and the soaking time of 90 minutes which was 1.39%. The relationship between the concentration of acetic acid and the soaking time on the ash content of modified starch can be seen in Figure 2.

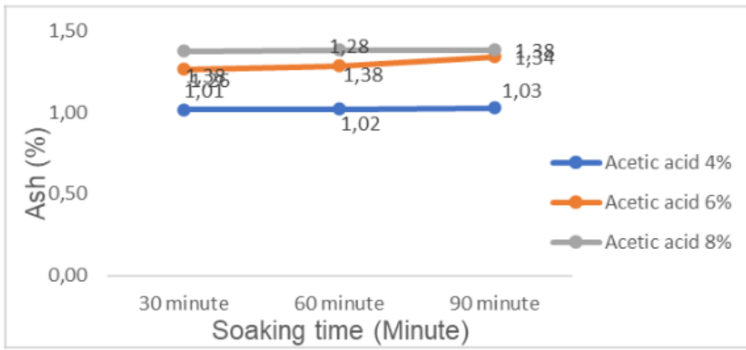

Figure 2. The ash content of *Colocasia esculenta* modified starch

In Figure 2. showed that the higher the concentration of acetic acid and the longer the soaking time, the ash content in the modified starch increased. This is due to the higher concentration of acetic acid and the longer soaking time causing more acetyl groups to bind to the starch. The acetyl group that binds to starch will not be lost during the combustion process so that the ash content increased.

The states that the longer the acetylation time, the more the acetyl groups bind to starch so that when they are burned the acetyl groups are not damaged. The state that modified starch granules have pores on the surface and internal cavities in the hilum, thus providing more open access to the inside of the granules. Thus, it will facilitate the absorption of acetate into modified starch granules thereby increasing ash content.

Starch

The average starch content of *Colocasia esculenta* modified starch with the treatment of acetic acid concentration was 63.67% - 65.38%. Based on Table 2. showed that the higher the concentration of acetic acid, the starch content of modified starches decreased. This is because the higher the concentration of acetic acid causes the degradation of starch is also greater due to the hydrolysis process.

Table 2. Starch content in *Colocasia esculenta* modified starch by treatment of acetic acid concentration

Acetic Acid (%)	Starch (%)	Solubility (%)	Substitution Degree (%)	Viscosity (cP)
4	65,38 ± 0,1509[c]	15,71 ± 0,1914[a]	0,25 ± 0,0204[a]	1397,33 ± 263,2291[a]
6	64,28 ± 0,1618[bc]	16,36 ± 0,1819[b]	0,46 ± 0,0313[b]	1960,67± 239,3006[b]
8	63,67 ± 0,1690[a]	17,51 ± 0,1943[c]	0,65 ± 0,0131[c]	3126,50± 518,1759[c]

Note: The average value followed by the same notifications showed that it is not significantly different ($p < 0.05$)

Acids can hydrolyze glycosidic bonds to produce amylose with shorter chains and lower molecular weights. In this process, two stages of attack occur in the starch granules, namely the stage of rapid attack in the amorphous region and the slower stage of attack in the amylopectin fraction contained in the crystalline region. The average value of starch content due to the soaking time treatment was 64.28% - 64.58% (Table 3).

Table 3. The *Colocasia esculenta* starch content of soaking time treatment in acetic acid

Soaking Time (Minute)	Starch (%)	Solubility (%)	Substitution Degree (%)	Viscosity (cP)
30	64,59 ± 0,8709^{c}	16,34 ± 0,9044^{a}	0,43 ± 0,2065^{a}	1835,67 ± 694,6417
60	64,48 ± 0,8359^{b}	16,55 ± 0,9252^{b}	0,45 ± 0,2012^{a}	2147,33 ± 977,2229
90	64,28 ± 0,0782^{a}	16,69 ± 0,9102^{c}	0,47 ± 0,1994^{a}	2501,50 ± 978,7141

Note: The value followed by the same notifications showed that it is not significantly different ($p < 0.05$).

Based on Table 3. showed that the longer the soaking time of acetic acid, the starch content of *Colocasia esculenta* modified starches decreased. This is due to the longer soaking time resulting in the contact time between acetic acid and starch also getting longer so that resulted in hydrolyzed starch. The resulting starch levels decreased. Hee Young An (2005) states that the longer the hydrolysis time, it reduces the starch chain. The longer contact time of starch with acetic acid causes more acetyl groups to replace the OH-groups. The acetyl group in starch weakens the hydrogen bonds so that the starch levels decrease.

Amylose

The amylose content in *Colocasia esculenta* modified starches ranged from 30.56% - 33.48%. The highest amylose content in the treatment of acetic acid concentration of 4% and soaking time of 30 minutes is equal to 33.48%. The relationship between acetic acid concentration and soaking time on amylose content in modified starch can be seen in Figure 3.

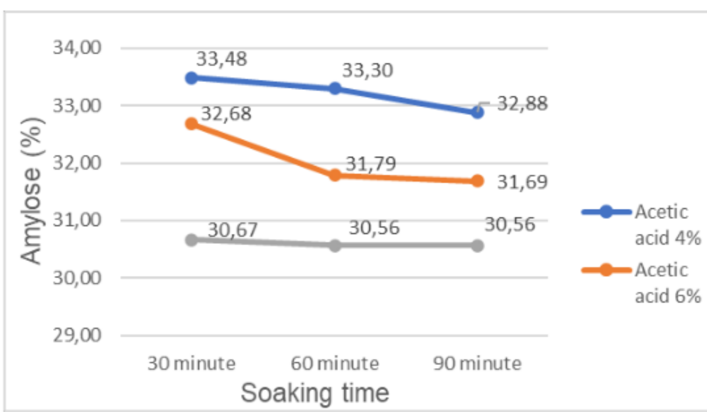

Figure 3. The amylose content of *Colocasia esculenta* modified starch

In Figure 3. showed that the higher the concentration of acetic acid and the longer the soaking time, the amylose content in modified starch decreased. This is because amylose is hydrolyzed by acetic acid along with an increase in the concentration of acetic acid and soaking time. That more simple sugars are formed from the hydrolysis process, causing amylose levels to decrease.

The found a decrease in amylose levels can be caused by acid hydrolysis also attacks some of the amylose. Specifically, that starch hydrolysis occurs in the amorphous part of the starch granules, where amylase is found in the amorphous part of the starch granules. The higher the concentration of acid, the more polysaccharides that are hydrolyzed. The amylose molecules are easily broken down compared to amylopectin molecules so that when acid hydrolysis takes place it decreased the amylose group. Ackar et al. (2015) state that modification of starch using organic acids will cause a decrease in amylose levels. The organic acids can break down starch molecules into simpler molecules. The simple is resulted through cutting reaction of α-1,4-glycosidic bonds from amylose and α-1,6-D glycosidic form amylopectin.

The retrograded starch, and particularly retrograded amylose, are the most thermally stable forms. Retrograded amylose is especially useful as a source of thermally stable RS3 for commercial food applications since it survives most food processes. Retrograded starch has been studied extensively for understanding the behavior of gels and certain staling processes in foods. Characterization of retrograded starch has been done as it interferes with the total dietary fiber (TDF) assay, with an emphasis on the negative impact of retrogradation (Haralampu, 2000).

Swelling Power

The swelling power of *Colocasia esculenta* modified starches ranged from 17.76% - 20.47%. The highest swelling power in the treatment of acetic acid concentration was 8% and the soaking time was 90 minutes which was equal to 20.47%. The relationship between the concentration of acetic acid and the soaking time on swelling power in modified starch can be seen in Figure 4.

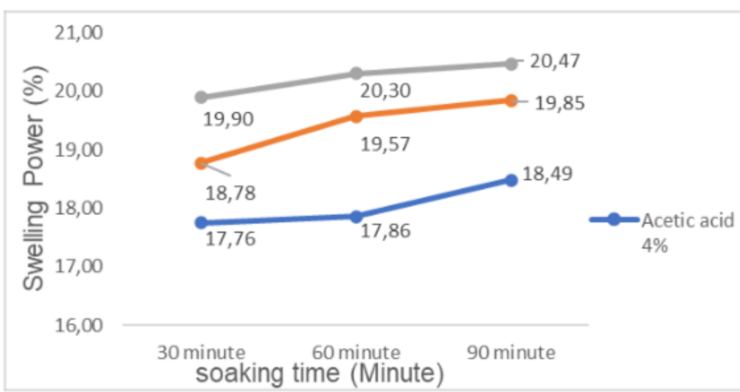

Figure 4. The swelling power of *Colocasia esculenta* modified starch

In Figure 4. showed that the higher the concentration of acetic acid and the longer the soaking time, the swelling power in the modified starch increased. This is due to the more acid added and the longer soaking time can cause the starch to be in contact. That more hydrogen bonds in the starch weaken and water easily enters the starch granules. This process makes the starch granules more expand.

Acetylation of corn and potato starches decreased the transition temperatures and enthalpy of gelatinization and increased swelling power and light transmittance. However, the change in these was greater in the potato starches with a higher percentage of small-sized granules (Singh, 2004).

The argues that acids can interfere with the hydrogen bonds found of starch, making it easier for starch granules to expand. This is supported by Teja et al., (2008), stating that the substitution of acetyl groups in sago starch weakens the hydrogen bonds of the starch so that water becomes more easily potential causes starch to become more expanded.

The argue that Swelling Power is affected by the breakdown of amylose and amylopectin starch granules which are capable of binding to water during the heating process. The stated that Swelling Power is strongly influenced by the presence of amylose. The longer the reaction time, the more amylose is reduced. The decrease in the amount of amylose causes an increase in swelling power so that the longer the operating time at the same temperature, the higher the acetylation of starch swelling power.

Solubility

The more acetic acid concentration used, the more solubility in *Colocasia esculenta* modified starch increased (Table 2). This was due to the weakening of the hydrogen bonds in the modified starch. The weakening of hydrogen in starch facilitates water penetration into starch granules.

This is supported by Teja (2007), the presence of acetyl groups in acetylated starch causes hydrogen bonds to weaken. This makes it easier for water to penetrate deeply into starch granules. As water enters more easily, the tendency to form hydrogen bonds between starch and water molecules is greater. This hydrogen bonding holds water back from the starch granules so that the starch dissolves. Bolade and Oni (2015), stated that the acetylation process has a synergistic effect on starch solubility. Increased solubility begins with bonding to the intragranular starch which weakens causing the starch chain to be broken so that water easily enters the starch granules. The acetyl group is known to have the ability to disperse starch better in a solution system. The solubility value of *Colocasia esculenta* modified starch of the treatment of acetic acid soaking time was 16.34% - 16.69% (Table 3).

Table 2 showed that the longer the soaking time of acetic acid, the solubility of the modified starch increased. The modification process because of the longer the soaking time, the more amylopectin compounds are reduced. The starch is more water-soluble due to soaking time which causes the starch to be hydrolyzed longer, thus causing an increased solubility. Pungky (2006) stated that the comparison between amylose and amylopectin will affect the soluble nature of starch. Amylopectin is water-insoluble. In the modification process, the longer the immersion time, the more amylopectin compounds are reduced. The resulting starch is more soluble in water so that the starch solubility increases.

Degree of Substitution

The degree of substitution of *Colocasia esculenta* modified starch of the treatment of acetic acid concentration was 0.25% - 0.65%. Table 2 showed that the higher the concentration of acetic acid, the degree of substitution of modified starches increases. The higher concentration of acetic acid, giving a greater opportunity for the substituted acetyl groups in the hydroxyl group to increase the degree of substitution.

The found that the degree of substitution has increased because of increasing acid concentration. Damage to acetylated starch granules increases with an increasing acid concentration in the acetylation process. This indicates that the starch molecule structure has been damaged by the addition of acid which replaces the OH group in starch. The substitution of OH groups by acetyl groups can occur in the crystalline portion of starch molecules.

The degree of substitution of modified starch in the treatment of soaking time was 0.43% - 0.47% (Table 7). The longer the soaking time of acetic acid, the degree of substitution of modified starch increased. The longer the soaking time, the more OH groups substituted by the acetyl group, resulting in an increasing degree of substitution.

Amalia said that the longer the soaking time results in a higher degree of substitution. The longer the reaction time caused the more acetyl groups that diffuse. Teja (2007) states the longer the reaction time, the more OH starch groups substituted by acetyl groups. The longer contact time between acetic acid and starch, so that over time the acetyl group in acetic acid will weaken the hydrogen bonds in the starch.

Viscosity

The viscosity of *Colocasia esculenta* modified starch of the treatment of acetic acid concentration was 1397.33 cP - 3126.50 cP (Table 2). The higher concentration of acetic acid caused higher viscosity. The breaking of amylose and amylopectin bonds which caused further changes such as an increase in water molecules.

Alsuhendra and Ridawati (2009) argue that acids can cause hydrolysis of starch chains composed of amylose and amylopectin. Amylose molecules are more easily broken than amylopectin molecules so that when acid hydrolysis takes place it will decrease the amylose group. As the amylose content decreases the viscosity is greater because of the lower amylose content. The higher consistency of starch gel was formed. While in the control of starch chain hydrolysis does not occur so that the viscosity tends to be smaller. Subagio (2006) states the higher concentration of acetic acid used, the higher the viscosity produced. The acid will degrade cell walls in such a way that the liberation of starch granules occurs. This starch granule liberation process will cause changes in the characteristics of the flour produced in the form of increased viscosity and ease of dissolving.

The viscosity of *Colocasia esculenta* modified starch with soaking time treatment was 1835.67 cP - 2501.50 cP. Table 3 showed that the longer soaking time of acetic acid, the viscosity of the modified starch increased. This is due to the longer soaking time, the amylose content decrease with the dissolution of amylose in water so that the viscosity is greater.

The argues that the longer the acid hydrolysis reaction time to modified starch, the lower amylose content. Amylose content is inversely correlated with the consistency of a starch gel. This decrease in the amylose group results in a higher gel consistency, increasing starch viscosity. Laga (2006) states the viscosity is related to the gelatinization process, water absorption rate, and the proportion of amylose and amylopectin present. The number of hydroxyl groups in starch molecules is very large so that the greater the ability to absorb water. The increased viscosity starts when the starch granules begin to swell. The water which was originally outside the granule and moved freely before the suspension was heated, is now inside the starch grains and cannot move freely anymore.

Colissi, *et al*. (2014) states physicochemical properties of amylose of rice starch at 6% anhydrous acetic acid concentration and 90 minutes soaking time add viscosity, increased swelling strength, and increased starch solubility. However, an increase in the thermal stability of modified rice starch with the acetylation process.

In Ayucitra's research (2012) about the preparation and characterization of modified corn starch with acetylation method at 8% anhydrous acetic acid concentration and soaking time of 60 minutes increased the swelling power of 15.15-27.85%, solubility 8.50% -17, 38%, substitution degree 0,008 - 0,21.

The profiles of the viscosity of the natural starch (NS) and acetylated, appears in figure 6, is observed that the peak of the viscosity of the NS is below that of the starches treated with acetates. The starch modified with AA (anhydrous acetic) shows a peak of the viscosity of 386 Pa.s to a temperature of 90 ºC, whereas the peak of the viscosity of the starch treated with VA he is in 234 Pa.s to a temperature of 82.5 ºC. This owes to itself the acetylation damages in the granules of starch (Garcia *et al*., 2012).

Conclusion

Modification of starch by acetylation is one modification that is widely used. The structure of starch can be changed to the desired characteristics of starch such as being more resistant to retrogradation.

Modified starch with 8% acetic acid concentration and soaking time 90 minutes is the best treatment with a water content of 5.9700%, ash content 1.3911, starch content 63.4968, amylose 29.9307, swelling power 20.44700 (g / g), solubility 17.6672%, yield 85.2846%, substitution degree 0.6661, viscosity 3589 cP.

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