Modification of Mung Bean Starch by Annealing Treatment and Acetylation

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Keywords: Mung Bean, Mung Bean Starch, Dual Modification, Annealing, Acetylation, Modified Starch, Physicochemical Characteristics of Modified Starch.

Abstract: Mung beans are mainly composed of starch (25-30%). Mung bean starch which found naturally has low stability during processing and heat sensitive. The purpose of this research was to produce mung bean starch with higher resistance to heat and higher resistance to enzyme digestion, with combination of annealing and acetylation modification. Modified starch with 60ºC of annealing temperature resulted in highest crystallinity. Additionally, with 20% of acetic acid anhydride concentration and 15 minutes acetylation reaction time resulted in acetyl percentage and substitution degree that met FDA requirement. Dual modification of annealing and acetylation with chosen treatments as mentioned, was analysed further to determine starch content, moisture content, amylose and amylopectin content, swelling power and solubility properties, colour test, resistant starch content, XRD profile, FTIR profile, pasting properties, and starch granule morphology.

1 INTRODUCTION

Mung bean belongs to Fabaceae family. This bean can be found easily in Asia, Australia, New Zealand, and Africa (Yang et al., 2018). Mung bean has ±7.91% water content, ±24.08% protein content, ±1.55% fat content, ±2.87% ash content, ±2.20% fiber content, and ±25.73% starch content (Moongngarm, 2013).

Mung bean has bioactive compound like tannin, phytic acid, flavonoid, phenolic acid, and other organic acids. These bioactive compounds in mung bean has positive effects on health, such as free radical scavenger, detoxification, anti-bacterial, prevent diabetes, and prevent cancer (Ganesan & Xu, 2018). Mung bean production in Indonesia reach 370,000 tons, this amount was not balanced by its production which reach 303,000 tons (Kementerian Pertanian, 2018). Therefore, mung bean was used as ingredient in starch production to increase its economic value.

Mung beans mainly composed of starch (25-30%). However, mung bean starch which found naturally has low stability and low heat resistance (Phrukwiwattanakul et al., 2014). Therefore, modification was needed to alter functional properties of starch and make it applicable to certain food industries.

Starch modification can change its polymer, structure, and functional properties, to increase its function in food industries or non-food industries (Lopez et al., 2010). However, study about starch modification using combination of annealing and acetylation, and its effect on physicochemical properties on mung beans starch has not been done.

The purpose of this research was to produce mung beans starch with higher resistance to heat and higher resistance to enzyme digestion, with combination of annealing and acetylation modification. Moreover, this research was done to determine the effects of selected treatment to mung beans starch physicochemical properties, with annealing heating temperature, acetic acid anhydride concentration, and acetylation reaction time as factors.

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2 MATERIALS AND METHODS

2.1 Materials and Equipment

The materials used in this research were mung beans obtained from Plaza Baru Ciledug market. Distilled water (Amidis), acetic acid anhydride, pure glucose, HCl, NaOH, anthrone reagent, iodine, KI, KOH, H2SO4 (Merck, EMD Millipore Corp.), pure amylose, phosphate buffer, α-amylase enzyme, pepsin enzyme, β-amylase enzyme (Sigma-Aldrich, EMD Millipore Corp.), ethanol (Smart-Lab, PT Smart Lab), aquadest, and phenolphthalein indicator were also used in this research.

The equipment used in this research were beaker glass, Erlenmeyer flask, volumetric flask, Mohr pipette, graduated cylinder (Iwaki), blender (HR 2071/20, PT Philips), thermometer (ASTM 12C, BRAND, Ltd.), oven (UNB 500, Memmert, Ltd.), cabinet dryer (Rekayasa Wangdi), heater (Cimarec), analytical balance (Pioneer), refrigerator (Tipe SDC 1000, PT Sanden), vortex (Tipe 37600 Mixer, Barnstead Thermolyne Corp.), spectrophotometer UV-Vis (Genesys 10S UV-VIS, Thermo Fisher Scientific Inc.), pH meter (Tipe 744, Metrohm, Ltd.), waterbath (WB 14, Memmert, Ltd.), burette "BRAND", centrifuge (Z 206 A, Hermle Labor Technik, Inc.), chromameter (CR-400, Konica Minolta, Inc.), X-Ray Diffraction (MiniFlex, Rigaku Corp.), Scanning Electron Microscope (Quanta 650 FEG, Thermo Fisher Sientific, Inc.), Fourier Transform Infrared Spectroscopy (IRPrestige-21, Shimadzu Corp.), Rapid Visco Analyzer (RVA-4, Newport Corp.), spoon, glass rod, spatula, filter cloth, evaporating dish, test tube, test tube clamp, tray, desiccator, filter paper, magnetic stirrer, stopwatch, reflux, quartz cuvette, dropping pipette, bulb pump, micropipette, metalized plastic, silica gel, aluminium foil, and centrifuge tube.

2.2 Starch Production

Starch production was done based on Abdel-Rahman *et al*. (2008) research. Decorticated mung beans were rinsed with streaming water to get rid of impurities. Then, cleaned mung beans was soaked for 2 hours, and the water was discarded. Afterward, distilled water was added to mung beans with 1:3 (mung beans: water) ratio, and crushed with blender within 3 minutes. Next, obtained mushed mung beans was filtered used 60 mesh filter cloth. Filtration process produced residue and filtrate. Distilled water was added to the residue and filtrated again two times, while the filtrate was settled for 2 hours. Precipitation process produced precipitate and supernatant. The supernatant was discarded and the precipitate was dried at 40ºC for 15-20 hours using cabinet dryer. Dried precipitate was crushed using dry blender within 1 minute, then sieved with 60 mesh sieve. This powder is mung beans starch that had to be stored in refrigerator in ± 5ºC until further analysis.

2.3 Preparation of Annealed Starch

Annealing treatment was done by mixing mung beans starch with distilled water in 1:2 (mung beans starch: water) ratio. Then, the slurry transferred to 250 ml beaker glass and covered with aluminium foil. The beaker and its content was soaked in water bath at 40°C/ 50°C /60°C for 6 hours. After cooling down, the slurry was centrifuged at 5,000 rpm for 10 minutes. Centrifugation process produced precipitate and supernatant. The supernatant was discarded, while the precipitate was washed with distilled water and filtered with filter paper. The precipitate was washed to dissolve impurities and help filtering process. Next, the precipitate was dried with oven at 40°C for 15-20 hours.

2.4 Preparation of Annealed Starch

Mung beans starch produced from selected annealing treatment was mixed with distilled water with 4:9 (annealed mung beans starch: water) ratio. Then the slurry was stirred using magnetic stirrer at 25°C for 60 minutes. pH of the slurry was set to 8 with 3% NaOH solution. Then, acetic acid anhydride (density = 1,082g/ml) with 10%, 15%, or 20% concentration based on sample weight (g) was added slowly to the slurry, while still keeping pH range between 8-8.4 with 3% NaOH solution. Reaction was settled at 25°C for 5, 15, or 25 minutes after adding acetic acid anhydride. After that, pH was set to 4.5 with 1 N HCl. Then, the slurry was filtered with filter cloth to obtain precipitate and supernatant. The supernatant was discarded, while the precipitate was washed two times with distilled water, to get rid of impurities and HCl residue. Next, the precipitate washed once with 95% ethanol to get rid of acetic acid anhydride residue in starch. Washed precipitate then dried at 45°C in oven for 24 hours.

2.5 Experimental Design

Experimental design was applied to annealed and acetylated starch. Obtained data was tested using SPSS on acetylated starch. Experimental design used in annealed starch was completely randomized design with one factor, three replication, and three level of heating temperature which is 40°C, 50°C, and 60°C. Experimental design used in acetylated starch was completely randomized design with two factor, two replication, and three level of acetic acid anhydride concentration which is 10%, 15%, and 20% along with three level of acetylation reaction time which is 5, 15, and 25 minutes.

2.6 Analysis Methods

Analysis done in this research were starch yield (Ratnayake *et al*., 2007), starch content (Ezeigbo *et al*., 2015), moisture content (AOAC, 2005), acetyl percentage and degree of substitution (Colussi *et al*., 2015), amylose and amylopectin content (Abeysundara *et al*., 2015), solubility properties and swelling power (Zaman *et al*., 2015), colour test (Nadir *et al*., 2015), pasting properties, resistant starch content (AOAC, 2000), starch granule morphology (SEM), XRD profile, and FTIR profile.

3 RESULTS AND DISCUSSION

3.1 Mung Bean Starch Characteristics

Based on identification test, it was confirmed that mung bean used as main ingredient to produce starch is *Vigna radiata* (L.) R.Wilczek. This mung bean was processed to produce starch that will be analyzed further. Results of starch analysis can be found in Table 1.

Table 1: Analysis results of native mung bean starch.

Analysis parameters	Analysis results
Starch yield	58.44 ± 12.29%
Starch content (from isolated starch)	88.31 ± 2.68%
Starch content (from mung bean flour)	29.46 ± 2.30%
Moisture content	6.39% ± 0.29%
Acetyl percentage	0.00%
Degree of substitution	0.00
Amylose	26.79 ± 0.74%
Amylopectin	73.21 ± 0.76%
Solubility	16.48 ± 0.55%
Swelling power	8.83 ± 0.52%
L* value	75.38 ± 0.18
Chroma	7.34 ± 1.18
Resistant starch content	23.96%
Crystallinity	57.94%

Mung bean starch consist of 26,79 ± 0.74% amylose content and 73.21 ± 0.76% amylopectin content. According to Kaur *et al*. (2012), amylose content of mung bean starch ranged between 29.9 – 33.6%, while amylopectin content reach ±70%. Value of amylose and amylopectin content can be different, because of different variety of mung bean plant. However, same amylose and amylopectin content can be found even in plants with same variety. Geographic and environment condition when plants were planted can affect their amylose and amylopectin content (Gao *et al*., 2014).

Amylose and amylopectin content affect physicochemical properties of starch. High amylose content (>30%) can increase possibility of starch retrogradation (Alcazar-Alay & Meireles, 2015). Moreover, increased amylose content can increase pasting properties, viscosity, and solubility properties of starch granules (Colussi *et al*., 2015). On the other hand, starch with high amylopectin content (>70%) has low capacity to absorb water, more resistant to enzyme digestion and chemical reaction, compared to starch with high amylose content (Gunaratne & Corke, 2016).

Starch content obtained from this research is 29.46 ± 2.30%. This result is higher than Moongngarm *et al*. (2013) analysis result which is 25.73%. Different sample preparation and isolation method can affect starch content obtained. Decorticated mung beans produce more starch yield and content, compared to whole mung beans (Abdel-Rahman *et al*., 2008). Moreover, soaking temperature (up to 30^{o}C) and soaking time (up to 18 hours) of mung bean in preparation process can increase starch extraction efficiency. However, soaking in room temperature for two hours is enough to produce starch for smaller scale (Usman *et al*., 2014).

Native starch acetyl percentage and degree of substitution resulted zero value. Those value was obtained because there is no acetyl group or glucose unit bound to acetyl group was found (Colussi *et al*., 2105). Resistant starch content obtained was 23.96%. This value is not much different than analysis result in Shi *et al*. (2016) research, which is ranged between 16.1-22.3%. There are some factors affecting starch digestibility, such as starch structure characteristics (amylose and amylopectin ratio, gelatinization degree, retrogradation rate, and formation of amylose complex), food characteristics, and existence of other components (Conde-Petit *et al*., 2001).

Table 2: Crystallinity percentage of annealed starch.

Annealing temperature (°C)	Crystallinity percentage (%)
Native	57.94
40°	63.59
50°	70.73
60°	88.89

3.2 Effect of Annealing Temperature to Crystallinity Percentage of Mung Bean Starch

Starch modification with annealing method makes starch granules structure become more stable, because of polymer chains re-organization in crystalline and amorph side that cause increased crystallinity percentage (Lan *et al*., 2008). Increasing temperature between glass transition and starch gelatinization temperature, can increase hydration rate and glucan chain mobility (Jayakody & Hoover, 2008). Increased crystallinity percentage of starch can be detected with XRD in Table 2.

Crystal type of starch can be detected from diffraction pattern with XRD analysis. XRD analysis results can be found in Figure 1, and can be concluded that native starch intensity as well as treated sample has diffraction angle at 2θ = 15°, 17°, 18°, and 23° (Colussi *et al*., 2014). This result showed that mung bean starch has A-type crystal, in accorandce with theories that showed peak at 2θ = 23°, but didn't show peak at 2θ = 5.6° in A-type crystal graph (Correia *et al*., 2012). Phrukwiwattanakul *et al.* (2014) research of mung bean starch resulted in similar outcome, with peak at 2θ = 15°, 17°, 18°, and 23°on the graph. Peak at same diffraction angle has been found in Colussi *et al*. (2014) research too, in rice starch with 20-32% amylose content. There is no change in starch crystal type with increased temperature up to 60°C.

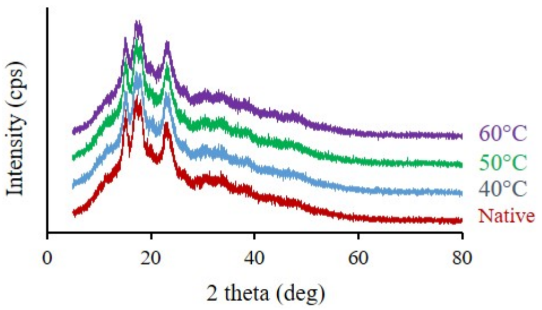

Figure 1: XRD analysis results of annealed mung bean starch.

3.3 Effect of Acetic Acid Anhydride Concentration and Acetylation Reaction Time to Mung Bean Starch

Acetyl percentage is acetyl group amount in every gram starch sample (wet basis) (Rahim *et al*., 2017). Acetyl percentage in annealed and acetylated mung bean starch can be found in Figure 2. Statistical results showed that interaction between acetic acid anhydride concentration and acetylation reaction time, affect ($p<0.05$) acetyl percentage of modified mung bean starch. Difference in acetic acid anhydride concentration or acetylation reaction time separately, also affect ($p<0.05$) acetyl percentage of modified mung bean starch as well.

According to Figure 2, the highest acetyl percentage found from acetylated starch with 20% acetic acid anhydride concentration and 25 minutes reaction time. Increasing reagent concentration and reaction time can increase the chance for substitution group to bind with starch. Increased bonds can increase acetyl percentage and acetylation reaction efficiency (Ackar *et al*., 2015).

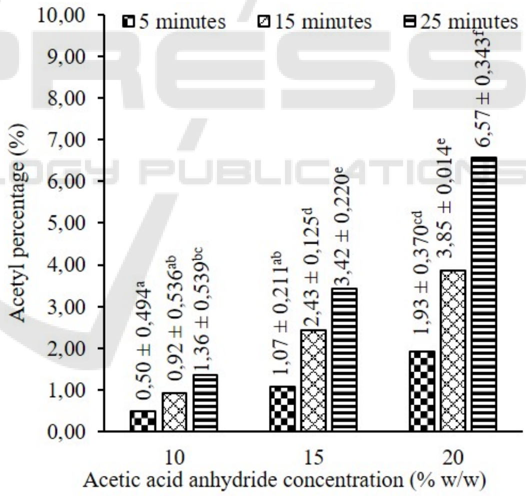

Figure 2: Effect of acetylation condition to acetyl percentage of modified mung bean starch. Different superscripts indicate significant difference ($p<0.05$).

Degree of substitution (DS) is average amount of unit glucose side which bound to substitution group (acetyl group) (Rahim *et al*., 2017). DS of annealed and acetylated mung bean starch can be found on Figure 3.

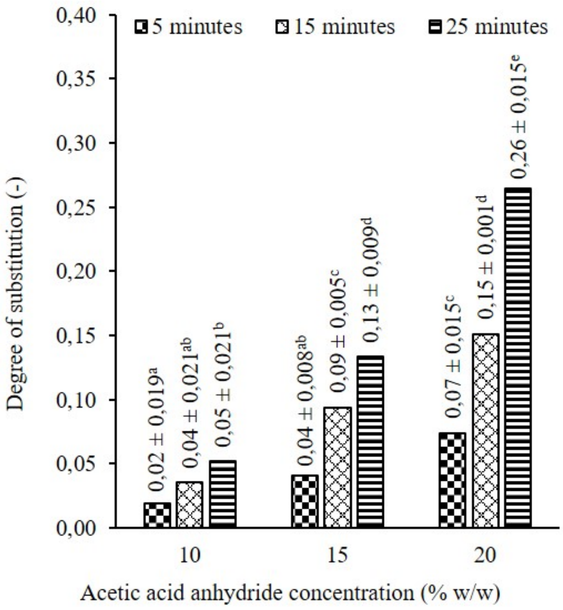

Figure 3: Effect of acetylation condition to degree of substitution of modified mung bean starch.

Statistical results showed that interaction between acetic acid anhydride concentration and acetylation reaction time affect ($p<0.05$) DS in modified mung bean starch. Difference in acetic acid anhydride concentration or acetylation reaction time separately also affect ($p<0.05$) DS in modified mung bean starch. The highest DS found from acetylated starch with 20% acetic acid anhydride concentration and 25 minutes reaction time. DS is related to acetyl percentage, more acetyl group found in starch will also increase the chance of acetyl group to bind with hydroxyl group in starch (Ackar *et al*., 2015).

Acetylated starch with 20% acetic acid anhydride and 15 minutes reaction time, meets FDA requirement which stated that DS found in starch can't exceed 0.2. However, there is no significant difference ($p>0.05$) in DS between acetylated starch with 20% acetic acid anhydride concentration and 15 minutes reaction time, compared to acetylated starch with 15% acetic acid anhydride and 25 minutes reaction time. Considering time efficiency and DS value proximity to FDA requirement, then acetylation treatment with 20% acetic acid anhydride and 15 minutes reaction time became selected treatment.

Incorporation of acetyl group in starch can be confirmed with FTIR (Colussi *et al*., 2014). Result of FTIR analysis of annealed and acetylated starch with series of acetic acid anhydride concentration and acetylation reaction time can be found on Figure 4.

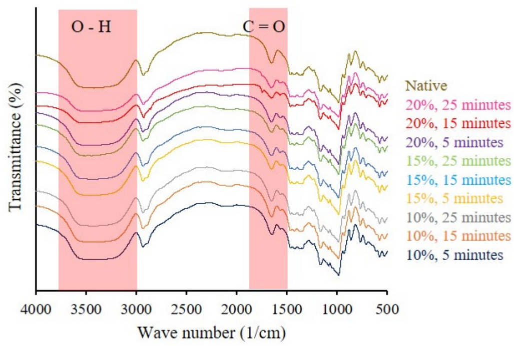

Figure 4: FTIR analysis results of annealed and acetylated mung bean starch.

Acetylation reaction can subtitute hydroxyl group in starch molecule to carbonyl contained group. This occurrence resulted in decreased hydroxyl group intensity along with increased carbonyl group intensity. Hydroxyl group intensity in starch can be found at 3700–3000 cm^{-1}. While C=O intensity in acetyl group can be found at 1700-1500 cm^{-1} (Rahim *et al*., 2017).

Selected annealing treatment was chosen based on highest crystallinity percentage. Increased crystallinity percentage cause decrease in swelling and solubility, increase in heat stability, and increase in starch resistance of alpha amylase enzyme (Song et al., 2011; Siswoyo & Morita, 2010). Therefore 60°C became selected temperature for annealing, before acetylation treatment. Additionally, selected acetylation treatment was based on highest acetyl percentage and DS that meet FDA requirement. FDA limit DS of modified starch to maximum 0.2 if the starch was going to be used as food ingredient (Xu et al., 2004).

Selected acetylation treatment was combined with selected annealing treatment, which is 60°C annealing temperature, 20% acetic acid anhydride concentration, and 15 minutes acetylation reaction time. Modified starch with selected treatments was analyzed with starch content, water content, solubility properties and swelling power, pasting properties, XRD profile, resistant starch content, amylose and amylopectin content, colour test, and starch granule morphology as parameters.

3.4 Physicohemical Properties of Selected Starch

Solubility properties and swelling power of mung bean starch decreased after dual modification. Swelling power of native starch was decreasing from 18.11% to 11.62%, while solubility properties of native starch was decreasing drom 16.48% to 8.83%. This decreasing value was different significantly ($p<0.05$) and can be found in Table 3. Native starch referred to starch without any treatments, while selected starch has been annealed and acetylated by selected treatment. Decreased value of swelling power and solubility properties can be caused by annealing treatment, which re-organized starch crystal to become more compact. Interaction between amylose and amylopectin from annealing also decrease hydration of amorph side (Zavareze & Dias, 2011). Moreover, incorporation of acetyl group in acetylation reaction, makes starch molecule becomes more hydrophobic (Luo & Shi, 2012).

Basic rheology properties is viscosity, which affected by temperature, concentration, and shear stress (Alcazar-Alay & Meireles, 2015). Rheology properties results can be found in Table 3. Pasting properties is a term used to explain transformation in starch after gelatinization. Rapid Visco Analyzer (RVA) can be used to explain viscosity parameter as a function to temperature and time.

Starch suspension was given shear forces when analysis was conducted. Suspension will show peak viscosity, which started after gelatinization and the value will increase along with expansion of starch granule (Alcazar-Alay & Meireles, 2015). Decreased peak viscosity can be caused by decreased absorption capacity of starch (solubility properties) and decreased starch ability to form a paste (swelling power) (Marta & Tensiska, 2017).

Table 3: Psycochemical properties of native and selected starch.

Psycochemical properties	Native starch	Selected starch
Solubility (%)	16.48±0.55[a]	8.83±0.52[b]
Swelling power (% w/w)	18.11±0.44[a]	11.62±0.63[b]
Pasting temperature (°C)	75.8	81.6
Peak viscosity (cP)	5384	3372
Hot paste viscosity (cP)	3397	2939
Cold paste viscosity (cP)	5516	5075
Breakdown (cP)	1987	433
Setback (cP)	132	1703
Resistant starch (% w/w)	23.96	24.68
Amylose (%)	26.79±0.74[a]	24.09±0.72[b]
Amylopectin (%)	73.21±0.76[a]	75.91±0.68[b]
L* value	75.38±0.18[a]	75.77±0.10[b]
Chroma value	7.34±1.18[a]	7.74±0.19[a]

Breakdown describes a difference value between peak viscosity and hot paste viscosity. Breakdown shows starch stability when exposed to heat, while hot paste viscosity related to heat resistance of starch or weakness of starch granule chains (Marta & Tensiska, 2017). Decreased value of breakdown will increase starch stability when heated. This occurrence can be caused by increased crystallinity and more compact structure of starch chain when annealing and acetylation treatment was conducted (Ariyantoro, A.R. et al., 2018; Mendoza et al., 2016).

Pasting temperature after acetylation treatment supposed to be lower than native starch, because incorporation of acetyl group that weakens starch granule structure, can break the compact structure of starch granule. However, annealing treatment before acetylation can increase compactness or crystallinity of starch structure (Simsek et al., 2012). This treatment combination can increase pasting temperature of starch.

Setback is a difference value between cold paste viscosity and hot paste viscosity. Increasing value of setback, will increase the chance of retrogradation to occur (Wang et al., 2015). In cooling period, amylose leaching will form a three-dimensional gel network. This gel formation can increase viscosity, which is cold paste viscosity (Alcazar-Alay & Meireles, 2015). In this research, annealing and acetylation treatment increase setback value. When annealing occurred, granule starch texture changed to become more compact. This occurrence can cause increasing viscosity when starch cools down and increase retrogradation rate. Starch with high setback value is a good gelling agent, which is desirable in certain food industry (Marta & Tensiska, 2017).

Comparison of XRD analysis results between native and selected starch can be found in Figure 5. Mung bean starch crystal type remain unchanged which is A-type crystal, even after acetylation with 20% acetic acid concentration and 15 minutes reaction time. Native or treated starch intensity was found at same diffraction angle which is $2\theta = 15°$, 17°, 18°, and 23° (Colussi *et al*., 2014). However, crystallinity percentage of annealed starch in 60°C decrease from 88.89% to 87.80% after acetylation. According to Colussi *et al*. (2015), decreased crystallinity percentage after acetylation also decrease hydrogen bond in starch, resulted in decreased crystalline structure.

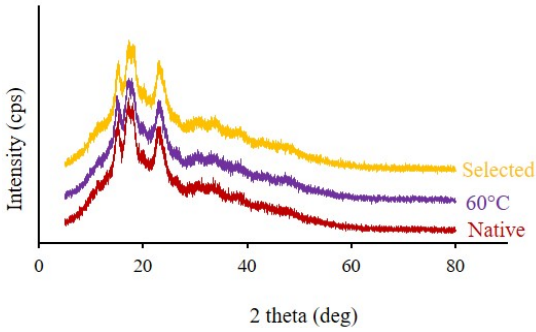

Figure 5: XRD analysis results of native, annealed (60°C), and selected starch.

Resistant starch content increased from 23.96% to 24.68% according to Table 3. Heating step in annealing form more compact structure of starch granule, because of hydrogen bond between amylose or amylopectin (Sajilata et al., 2006). Acetylation also has important role in increasing resistant starch content. Acetyl group that bind with starch chain, can hinder active side of α-amylase enzyme that breaks starch bonds (Sahnoun et al., 2015). α-amylase is an enzyme that capable of hydrolyze starch randomly at α-(1→4) D-glucosidic bond, to produce glucose and oligosaccharide. Starch hydrolysis with α-amylase can be hindered by acetylation treatment (Chen et al., 2004).

Colussi et al., (2015) research has used same acetic acid anhydride concentration, and same reaction time as selected treatment in this research. They found no significant difference in resistant starch between native and acetylated rice starch. Meanwhile, in Song et al., 2011 research, annealing before cross-linking treatment can increase resistant starch content to 31.5%. However, they used 50°C annealing temperature for 12 hours and cross-linking reaction time for 4 hours. This founding concluded that annealing temperature, reagent concentration, and starch modification reaction timecan affect hydrolysis rate of starch (Song et al., 2011).

Amylose content of native and modified starch can be compared in Table 3. Amylose content decreased from 26.79% to 24.09% after acetylation. Decreased amylose content resulted in increased amylopectin content from 73.21% to 75.91%. These decreasing number was significantly different ($p<0.05$).

Acetyl group found in starch granule can hinder helix structure of amylose to bind with iodine because of steric hindrance (Gonzales & Perez, 2002). Another possibility that can cause decreasing amylose content is depolymerization of amylose chain. Starch contains polymer chains that can be broken to monomers by increasing temperature or degree of substitution (Kapelko-Zeberska *et al.*, 2017). Decreased amylose content also happened in acetylated banana starch (Reddy *et al.*, 2014), acetylated buckwheat starch (Sarkar, 2016), and acetylated potato starch (Kapelko-Zeberska *et al.*, 2017).

Figure 6: Physical appearances of mung bean flour, starch and modified one.

Physical appearance of starch as food ingredient is important, because it can affect product acceptability. (Dahiya *et al.*, 2015). Colour difference between samples can be found in Figure 6. Decorticated mung bean flour has yellow to brown colour, while native and annealed mung bean starch has white colour. However, annealed and acetylated starch has brighter white colour. L* value and chroma analysis results can be found in Table 3. Increased L* value was significantly different ($p<0.05$), while increased chroma value was not significantly different ($p>0.05$). Acetylation treatment can increase starch L* value and decrease chroma value. Higher increase in L* value will make starch appear whiter, while chroma value describes sample colour purity. However, chroma value is not really considered to determine colour characteristic of starch (Bolade & Oni, 2015). Increasing L* value and decreasing chroma value is desirable from consumer perspetive (Ali *et al.*, 2016).

Morphology of starch granule can be observed using Scanning Electron microscope (SEM). SEM magnification used in this analysis was 350×, 750×, 1500× and 3000×. Magnification 3000× was used to observe starch granule surface and shape more clearly. While magnification 350×, 750×, and 1500× was used to ensure starch granule condition in 3000× was found on another magnification too. Mung bean starch has granule size between 7.65 - 33.15 µm (Abdel-Rahman et al., 2008). Mung bean granule starch can be found in Figure 7.

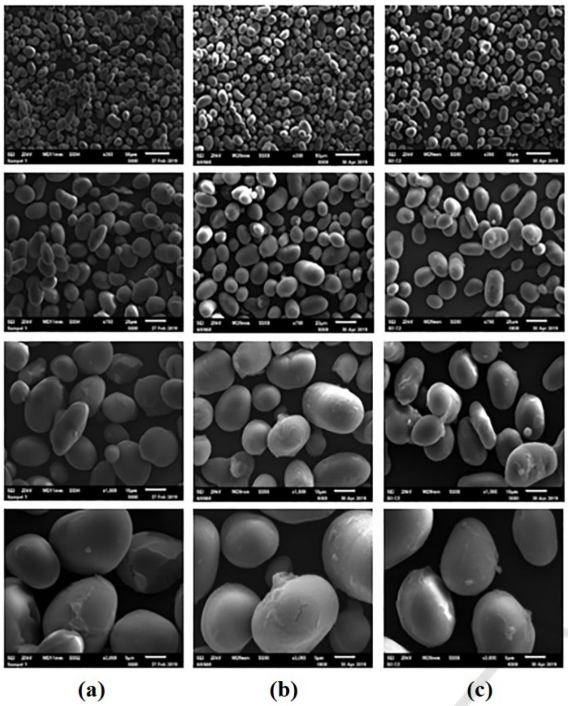

Figure 7: SEM analysis results of native starch (a), annealed starch (b), and annealed-acetylated starch (c). Magnifications used from top to bottom figure were 350×, 750×, 1500×, and 3000×.

Mung bean granule shape remains unchanged when observed with 3000× magnification. This occurrence also found in 350×, 750× and 1500× magnification. Bigger mung bean starch granule has oval shape, while smaller one has round shape (Liu & Shen, 2007).

Several native and modified starch granules have uneven surface, but the particle structure is still intact. Uneven surface can be observed clearly in 3000× magnification, but there's no deformed starch granule found even in 350×, 750× and 1500× magnification. Uneven starch granules can be caused by drying temperature starts from ± 40°C (Lewicka et al., 2015). Heating process can break hydrogen bonding between starch polymer chains, resulting weaker granule structure and rough granule starch surface (Nadiah et al., 2015). Deformed starch granule has higher capacity to absorb water, making it more susceptible to enzyme hydrolysis and high temperature (Ali et al., 2014). Therefore, intact granule starch shape is desirable, especially if added into food product that used high temperature in the process.

4 CONCLUSIONS

Increasing annealing temperature up to 60°C can increase crystallinity percentage. Other than that, increasing acetic acid anhydride concentration and acetylation reaction time, can cause increased starch acetyl percentage and degree of substitution. Selected treatment for starch analysis was annealing with 60°C temperature, and acetylation with 20% acetic acid anhydride concentration for 15 minutes reaction time. Starch produced from combination of annealing and acetylation with selected treatment, has higher resistance and stability when heated, along with higher resistant starch content compared to native starch.

Combination of annealing and acetylation treatment to mung bean starch, caused decreased amylose content and increased amylopectin content. Solubility properties and swelling power value decreased, correspond with decreased peak viscosity in pasting properties analysis. Decreased peak viscosity was followed by decreased breakdown value, increased pasting temperature, and increased setback value. Modified starch has brighter colour, and its granule shape remains unchanged compared to native starch. Starch with these physicochemical characteristics were suitable to be used as food ingredient in cookies or cereal industries.

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