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Production of Resistant Starch from Kepok Banana's Hump (*Musa Balbisina L*) by Autoclaving-Cooling Method

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Abstract. The research was aimed to produce starch from kepok banana's hump (*Musa balbisina L*) and determine appropriate retrograded temperature. Resistant starch was food nutrition that has healthy functional for human digestion. Processes of production were two parts which are the production of starch from kepok banana's hump and modification of starch (production of resistant starch) that used two cooling temperatures which are 4 ^{o}C and -18 ^{o}C. The methods of research were precipitation, decantation, autoclaving-cooling and drying processes. The best characteristic was shown in starch modification of 4 ^{o}C temperature that produces 25% of resistant starch content, 28% of amylose content and 5% of water content. Based on these data, the best modified has a high potential to be applied as the main ingredient of edible film that it was a functional packaging.

INTRODUCTION

Banana is one of the special plants that is grown in Indonesia country but banana has some waste which can contaminate the environment. Hump is one of the banana waste and the utilization of it can give positive value for environment and development value of waste [1]-[2]. Kepok banana's hump (*Musa balbisina L*) is one of the species from banana plant and it has starch content of about 75% with 67.5 ^{o}C of gelatinization temperature that can use in food diversification, especially food nutrition [3]-[4]. Starch is a macromolecule that has 2 constituent components that are amylose and amylopectin. Amylose is a molecule from the straight chain of starch and amylopectin from a branch chain of starch.

Resistant starch is one of the starch variances which can be obtained naturally from it and from process modified. Beside that, resistant starch was one of the nutrition food, special as nutrition for the health of human digest. Resistant starch cannot digest by digestive enzymes in small intestine because the starch will be fermented before it enters into big intestine. Fermented-resistant starch which entered into small intestine will become food and energy source for probiotics bacteria, so that metabolism in big intestine will be effective [5]. Resistant starch has V type those are RS I (encapsulated starch), II (resistant granules), III (retrograded amylose), IV (chemically modified starch) and V (amylose lipid complex) [6]. Modified of starch consists of 3 methods that were physically modified, chemical modified, and enzymatic modified. Different modified for starch will produce resistant starch with different types and levels.

Physical modification is simple, secure, and effective modified to produce and increase resistant starch. Autoclaving-Cooling is one of the physical modified methods that use high pressure heating and combine with cooling. The principal of the autoclaving-cooling method is destruction by high pressure heating and retrogradation

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of amylose (recrystallization of molecules from amylose and amylopectin granules) by cooling, so that starch will have resistant characteristic type III. Resistant starch type III until V can survive with food processing than resistant starch types I-II [7]. Therefore, the utilization of resistant starch was based on characteristic of polymers, resistance, amylose content then resistant starch will give the value added in the product that uses it, especially as the main ingredient.

The polymers and resistance characteristic was potential to use as the main ingredient for the edible film as a functional packaging, especially for health functional of the digest. Based on the research of [8] edible film has potential as health functional when it was eaten by human and the whole of edible film functional was suitable with resistant starch characteristic as the main ingredient in edible film and applied to use.

This research was conducted to produce resistant starch from kepok banana's hump by the autoclaving-cooling method and got the best-retrograded temperature for starch between 4 ^{o}C and -18 ^{o}C. Characteristic resistant starch content was the main point to value this study and characteristics amylose and water content was the support point to value this research.

MATERIALS AND METHODS

Materials

The materials used in research that were kepok banana's hump, distilled water, alpha glucosidase enzyme, gluco-amylase enzyme, pepsin enzyme, sodium bisulfate, sodium hydroxide (NaOH) 30%; sodium hydroxide 1 N; sodium hydroxide 0.375 M; sodium thiosulfate ($Na_2S_2O_3$) 0.1 N; hydrogen chloride (HCl) 3%; hydrogen chloride 2 M; hydrogen chloride 0.275 M; iodine solution, starch indicator, luff-schoorl solution, potassium iodide, ethanol 80%; sulfuric acid (H_2SO_4) 4 N.

Instruments

The instruments used in the research were sieve, basin, autoclave, refrigerator, freezer, burette, analytical balance, cutter, reflux, centrifuge, UV spectrometer water bath, Erlenmeyer flask, beaker, porcelain cup, measuring flask, thermometer, hotplate, pH meter.

Procedures

Extraction of Starch from Kepok Banana's Hump [9]

Kepok banana's hump was cleaned from banana bark and was cut with small size then it was ready milled. After that, the milled result was mixed with sodium bisulfate about 1% for 2-3 minutes then it was mixed with distilled water about 0.3%b/v then the mixed was squeezed and separated between dregs and juice with filter. The juice was precipitated for 12 hours while combined with decantation and the water was changed about 3 times. The result was sediment that was starch extract. After that, the extract was ready to be dried.

Physical Modified for Kepok Banana's Hump Starch [10]

Kepok banana's hump starch was mixed with water by the ratio between water and starch were 20:80. The starch was readily processed in autoclave that was given heating with high temperature (121 ^{o}C) and pressure for 15 minute. After that, the starch was saved at room temperature for 1 hour then it was saved each in 4 ^{o}C and -18 ^{o}C which process was used in two cycles. After that, the starch was ready to be dried by 50 ^{o}C for 4 hours.

Measurement of Water Content

Sample (starch) weighed about 2 gram in porcelain cup then it was heated in the oven for 3-5 hours until it got constant weigh. After that, water content was counted with the difference between sample weight before heated and after heated. The percentage of water content was obtained from Equation 1.

$$\text{Water Content} = \frac{b-c}{a} \text{ x } 100\% \quad (1)$$

Where a = weight of sample, b = weight of cup + sample before dried, c = weight of cup + sample after dried

Measurement of Amylose Content

Sample was weighed about 0.1 gram and it was mixed with 1 mL of ethanol 95% and 9 mL of NaOH 1 N then it was heated by 95 °C for 10 hours. After that, the mixed was poured in 100 mL of measuring flask that was fitted then it was used as a stock solution. That solution was used 5 mL and it was mixed 1 mL with acetic acid 1 N, 2 mL of iodine solution, and the mixed was fitted in 100 mL of measuring flask. After that, it was measured with spectrophotometer UV in 590 nm of wavelength to get the absorbance of sample then the value of absorbance was ready to use in the calculation of amylose content. Besides hat, this count needed standard curve of pure amylose that was obtained with the same measure of the sample. The percentage of amylose content was obtained from the Equation 2.

$$\text{Amylose content (\%)} = \frac{\text{c x v x fp x 100}}{\text{w}} \quad (2)$$

Where c = amylose concentration (mg/mL), v = volume of sample (mL), w = weight of sample (mg)

Measurement of Starch Content

Sample was weighed about 1 gram then it was hydrolysis with HCl 3% about 150 mL for 3 hours. After that, it was made that had 7 of pH with NaOH 30% and it was fitted in 250 mL of measuring flask then it was used as a stock solution. That solution was used 10 mL then it was mixed with 15 mL of distilled water and 25 mL of luff school solution. After that, it was heated until boiled and it waited for 10 minute then the solution was cooled and it was mixed with 25 mL of sulfate acid 4 N, 2 gram of potassium iodide, nd 1 mL starch indicator. The mixed solution was titrated with sodium thiosulfate until it was had a white color. After that, the starch content be calculated with mg glucose that was got from titrated of mL thiosulfate. The percentage of starch content was obtained from the Equation 3.

$$\text{Starch content (\%)} = \left(\frac{mg\ glucose\ x\ \ fp}{sample\ weights}\ x\ 100\%\right) x\ 0{,}9\ (starch\ factor) \quad (3)$$

Measurement of Resistant Starch Content

Sample was weighed about 0.5 grams then it was mixed with 25 mL of phosphate buffer 2M, pH 6. After that, it was hydrolyzed by alpha enzyme of 2 mL for 30 minutes in 60 °C then it was hydrolyzed by glucose enzyme of 2.5 mL for 30 minute in 60 °C with condition of pH 4.5 that was filled HCl 0.275 M then it was hydrolyzed by pepsin enzyme of 0.05 mL for 30 minute in 60 °C with condition of pH 7.5 that was filled NaOH 0.375 M. After that, it was separated with centrifuge about 3000 rpm for 10 minute then the filtrate was saved and the sedimentation was used to heat in 100 °C for 20 minute. After that, it was mixed with 1 mL of KOH 4 M for 30 minute and 1 mL acetate buffer, pH 4.75. Next, it was hydrolyzed again with glucose amylase for 30 minute in 60 °C with condition of pH 4.5 that was filled HCl 2 M then it was separated with centrifuge about 3500 rpm for 30 minute then the filtrate was used and the sedimentation was saved. After that, the whole the filtrate was fitted in 50 mL of measuring flask then it was used as a stock solution.

That solution was used 4 mL then it was mixed with 6 mL of distilled water and 10 mL of luff school solution. After that, it was heated until boiled and it was waited for 10 minute then the solution was cooled and it was mixed with 10 mL of sulfate acid 4 N, 0.8 gram of potassium iodine, and 1 mL starch indicator. The mixed solution was titrated with sodium thiosulfate until it was had a white color. In this case, the hydrolyzed starch content and the resistant starch were counted. The percentage of resistant starch was obtained from the Equation 4.

$$\text{Hydrolysis Starch content (\%)} = \left(\frac{mg\ glukosa\ x\ fp}{bobot\ sampel}\ x\ 100\%\right) x\ 0.9\ (faktor\ pati\ ) \quad (4)$$

$$\text{Resistant Starch} = 100 - \%\ Hydrolysis\ Starch\ content \quad (5)$$

RESULTS AND DISCUSSION

Starch Content of Hump of Kepok Banana

Kepok banana's hump extraction aimed to get starch that used water distilled as a soluble by precipitation and decantation process because starch was a macromolecule that was soluble in water. Optimization of extraction starch used repetition of water distillate about 3 times for 12 hours that aimed to get purer starch and ensure no micro waste that followed. Starch is macromolecules that would soluble in water so the starch from the source was ready to get. Besides that, the extraction process has been used sodium bisulfate that purposed to reduce browning enzyme. This research has shown starch content of kepok banana's hump that was 87% by luff school method. The content shown that extraction starch was successful and this method was competent to use.

According to [11] principle of analysis starch content with luff schoorl method that is count the mg glucose in starch by hydrolysis. Hydrolysis starch by HCl 3% has been made monosaccharides that is glucose and glucose would reduce Cu excess from luff schoorl solution. After that, Cu excess was reduced by potassium iodide and sulfuric acid as an oxidizing agent, so that iodine was removed. The last, iodine that would titrate by sodium thiosulfate and the volume titration indicates that count of glucose content in starch and it could show the starch content that multiplying by 0.9 as a factor of starch.

Amylose Content of Starch Variance

The result of amylose content shown for the variance of starch and it was served in figure 1. The variances of starch are no modified starch (kepok banana's hump) and modified starch. The best amylose content of starch could show in starch modified 4 °C that was 28% and it was followed by starch-modified -18 °C that was 26% and starch no modified that was 25%.

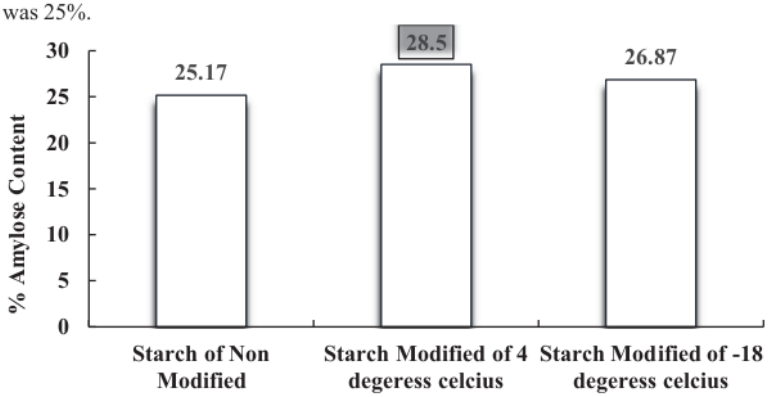

FIGURE 1. Amylose content of starch variance

Starch modified 4 °C shown that cooling temperature from refrigerator used to accelerate retrograded process from starch. The heated process from autoclave made the amylose and amylopectin granules from starch that was cracked and the granules were reunited by retrograded process in cooling method. Based on research results of [12] during the cooling process, amylose chain cut becomes a short chain of amylose and some branches chain of amylopectin chain cut become short chain of amylose. So that, the starch modified by the autoclaving-cooling process has amylose content that increases from natural starch (kepok banana's hump starch). Although, at -18 °C more levels were decreased than 4 °C but they increased from natural starch so cooling temperature was an important factor to get the best content of amylose. Decrease of starch-modified at -18 °C might be caused by the

utilization of cooling temperature that was so high, so -18 °C could not make good condition for retrograded process. Based on data of amylose content and references from some journal [13]-[14] the increase of amylose content shown that the resistant starch has a characteristic to use as a nutrition food and it was the important factor for the characteristic of edible film.

Resistant Starch Content of Starch Variance

The result of resistant starch content is shown for the variance of starch and it was served in figure 2. The resistant starch analysis was measured by hydrolysis enzyme method that combine with luff schoorl method. The best resistant content shown at 4 °C that was 25% and it was followed by starch-modified at -18 °C that was 16% and starch no modified which was 10,6%.

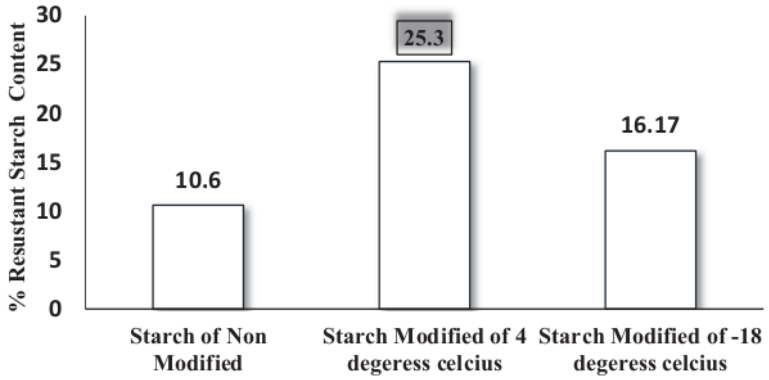

FIGURE 2. Resistant starch of starch variance

Starch modified 4 °C shown that resistant starch content was directly proportional to amylose content. That proportional condition happened because of the retrograded process that was increased the crystallinity degree of starch so starch becomes less susceptible to hydrolysis with digestive enzyme and decrease of starch digestibility [15]. The resistant starch was shown in starch no modified too that was resistant starch naturally contained in kepok banana's hump starch but it was the resistant starch type I that was more easily damaged than resistant starch from starch-modified. Based on the research results of [16] Resistant starch modified with retrograded process that was type III of starch resistant. So, the resistant starch was suitable to be processed become some product, like edible film or others, and it could save its health functional.

Water Content of Starch Variance

The result of resistant starch content shown for the variance of starch and it was served in figure 3. Analysis of water content used heated and discharge water of starch. The content of water shown is different from the starch of no modified and starch with modified that was starch modified of 5% and starch no modified of 11%.

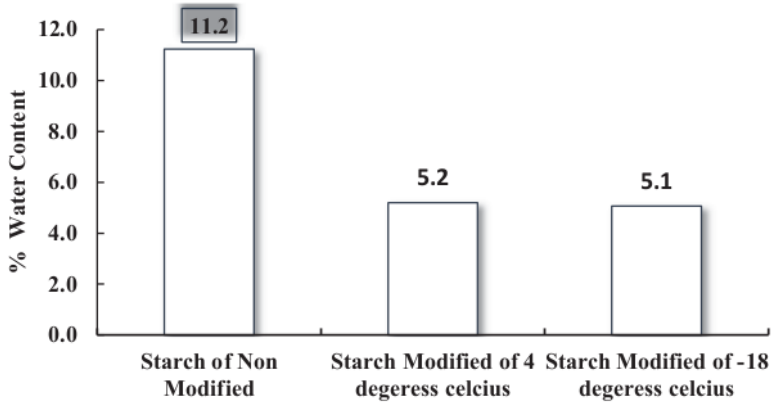

FIGURE 3. Water content of starch variance

According to [17] water content from starch no modified was more content than starch-modified and it shown that was a suit in standard water content of natural and modified starch. The variance content water from variance starch was different because drying process from starch not same and process modified from starch could be the main point to make difference [18]. So that, water content was important factor to be discovered while the starch of no modified and starch of modified was used as a main ingredient.

CONCLUSION

The conclusions of this research are shown that modified starch 4 ^{o}C is the best temperature to apply in autoclaving-cooling method to obtain the best characteristic from starch resistance that is 25% of resistant starch content, 28% of amylose content, and 5% of water content. So, the best modified (resistant starch) was suitable and potential to use for the ingredient of edible film.

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