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PREBIOTIC TEST OF HYDROLYSIS PRODUCT OF CELLULOSE EXTRACT FROM BANANA LEAVES KEPOK (Musa Paradisiaca Linn) BY β-AMYLASE

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ABSTRACT--*Banana leaves have one agricultural waste containing high enough cellulose Enzymatically hydrolysis cellulose will produce oligosaccharides and other compounds that have chains shorter. That compounds from hydrolysis processes, in general, can be as a prebiotic compound that can decrease the cell number of Escherichia coli and increase the cell number of Lactobacillus bacteria in the human digestive tract. Based on the information provided, the study was aimed at hydrolysis crude cellulose extract from banana leaves of using enzyme β - amylase and to determine the prebiotic character from hydrolysis banana leaves of banana. The study was conducted by a completely randomized design. Crude cellulose extraction was obtained by process delignification cellulose using NaOH 9%. Crude cellulose extract was hydrolysis by β - amylase enzyme with different hydrolysis temperatures (35, 40, 45, 50 and 55°C) and different hydrolysis time (3, 4, 6 and 7 hours). Test Prebiotic was conducted by method dilution and spread plate, in this case, Escherichia coli was added with solution hydrolysis and was grown on agar nutrient medium. The results showed that at 55°C the cell number of Escherichia coli decreased from 1,2 x 10^7 to 4 x 10^5 CFU/ml and the best hydrolysis time was 7 hours where the cell number of Escherichia coli decreased to 3 x 10^5 CFU/ml.*

Keywords*--banana leaves, cellulose, β-amilase, prebiotic, Escherichia coli.*

I. INTRODUCTION

Banana is one type of fruit that is preferred because of its delicious taste. In addition, banana plants are almost evenly grown throughout Indonesia. Almost all regions have banana plants with a variety of different species. One type of bananas widely consumed is banana *kepok* (*Musa paradisiaca* L). Banana *kepok* is grouped in *monocot* plants. The stem of banana is called banana leaves arranged by leaf midrib compiled tightly and regularly. Banana leaves of banana contain 63-64% cellulose, 20% *hemicellulose* and 5% *lignin* (Lokantara & Suardana, 2007). Various region communities in Indonesia use banana leaves of banana *kepok* as a medication used for preventing bleeding after childbirth, diabetes, kidney inflammation, nourish hair and new wound. The communities in Nusa Tenggara Timur and Sulawesi *Tengah* use banana leaves of *kepok* to cool the body and to heal the wounds (Wardani, 2014).

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Cellulose compound is grouped in polymer organic arranged by straight glucose chain. The monomer glucose and other monomer glucose are connected by β-1,4 glycosidic bonds. Cellulose is in crystalline form and is not soluble in water or organic solvent. In a plant, cellulose bonds another polysaccharide such as hemicelluloses or lignin to form plant cell walls (Caballero, 2003).

The oligosaccharide, disaccharide, and monosaccharide will be produced if cellulose is hydrolyzed using acid or enzyme Escherichia Oligosaccharide can be used in food processing such as increasing food quality and modifying food taste and in health such as increasing the bacteria population in the digestive tract. This bacterium has a role as a prebiotic. In addition, prebiotic bacteria can decrease wicked bacteria such as *Escherichia coli* and *Salmonella* (Aganis, 2008).

Prebiotic is an undigested compound by an enzyme in the digestion system however prebiotic affects gut microbiota (Rycroft et al., 2001). In general, the prebiotic type is commonly used in the Oligosaccharide form. Oligosaccharide can be obtained from a tuber, another food source contains high fiber such as coconut pulp that contain mannan or from a source of cellulose on banana leaves of bananas.

Polysaccharide extract has a potency to be prebiotic because this extract can increase the growth of beneficial bacteria such as lactic acid bacteria (Gibson & Manning, 2004). Oligosaccharide from hydrolyzed sago palm fruit powder 0,4% in the feed had a significant effect on feed consumption, increasing body weight, final body weight, mortality and had no negative effect on broiler performancEscherichia (Daud et al., 2009).

The study conducted by *Mayana* et al. (2016) using red onion (*Allium cepa)* extract was a source of *prebiotic* in *Osteochilus vitattus* fish feed. Their results showed that there was an increase in body weight between 2,32-3,21 g, specific growth rate between 1,24-1,41%/day, length 2,45-2,57 cm, feed conversion ratio 2,57-3,03, feed efficiency 33,40%-39,25% and life sustainability 100% (Mayana et al., 2016). Meanwhile in vitro test by Susanti et al. (2013) about sweet potato tuber extract as source of prebiotic showed that sweet potato bett-1 variety tuber extract and sukuh could suppress the growth of Escherichia coli and stimulate the growth of Lactobacillus bacteria so that it extracts has a potency to be as a prebiotic(Susanti et al., 2013) (Husna et al., 2018).

Hydrolysis of polysaccharide compound from plant source can be done with chemical (acid or base) and enzymatic process. Many factors such as pH, time, temperature, pressure, used acid and enzyme concentration affect the hydrolysis process (Susanti et al., 2013). A study of chitosan depolymerization by enzymatic hydrolysis using the α-amylase enzyme in which the α-amylase enzyme was able to reduce chitosan molecular weight from 1680-1750 kDa to 144.18 kDa (Handayani et al., 2013). Rokhati et al. (2013) compared the hydrolysis process using α-amylase and β-amylase They found that the optimum temperature for the hydrolysis process using α-amylase is 90 °C and β-amylase is 50 °C (Rokhati et al., 2013).

II. EXPERIMENTAL

Equipment's and Material

The materials used in this study were banana leaves of kepok, b-amylase, *Escherichia coli* bacteria, NaOH, NA (Nutrient Agar) media, Luria Bertani Broth (LB) media, aquadest, alcohol, phosphorus buffer pH 5, NaCl 0,85%, spiritus, tissue, aluminium foil, plastic wrapping, cotton and filter paper. The tools used in this study were blender, Petridis, ose, incubator, autoclave, drop pipette, micropipette, stirring rod, compartment, knife, cutting

board, test tube rack, test tube, water bath, thermometer, colony counter, haemocytometer, matches, stopwatch, LAF (Laminar Air Flow) and glass tools commonly used.

III. RESEARCH PROCEDURE

Cellulose extraction of banana leaves of kepok

The banana leaves of kepok were cut and dried and then it was refined to be powder. Powdered banana leaves could be as a sample The sample was delignification using NaOH 9% solution with a ratio between NaOH 9% solution and sample was 1:9. The sample was soaked for 90 minutes and filtered. The residue obtained was washed by aquadest until the neutral pH than the residue was dried. The dried residue was used as a sample in hydrolysis processes using ß-amylase enzyme

IV. ENZYMATIC HYDROLYSIS PROCESS

Temperature variations

Cellulose extraction was hydrolysis by mixing 1 g sample with 25 ml of 1% β-Amylase enzyme and 1 ml of phosphate buffer pH 5, the mixture was shaken. The mixture was heated with temperature variations of 35, 40, 45, 50 and 55°C for 3 hours. The solution was filtered. The prebiotic activity from the filtered solution was tested by *Escherichia coli* using the method spread plate

Time variations

Hydrolysis of cellulose extract was carried out by mixing 1 g of cellulose extract powder, 25 ml of 1% β-Amylase enzyme and 1 ml of phosphate buffer pH 5, this mixture was shaken. The mixture was heated on the best temperature obtained with time variation of 3, 4, 5, 6 and 7 hours. The solution was filtered. The prebiotic activity from the filtered solution was tested by *ESCHERICHIA coli* using the spread plate method

Prebiotic activity test of hydrolysis product

The prebiotic activity test of hydrolysis product was carried out by using *Escherichia coli* suspension. The 4 ml of *Escherichia coli* suspension was added with 1 ml of filtrated hydrolysis product and this mixture was shaken with a vortex. After shaking it was cooled for 5 minutes. Furthermore, 0,1 ml of *Escherichia coli* suspension added by filtrated hydrolysis product which was collected from the surface of that mixture and was spread out on solid NA medium and then it was incubated for 24 hours. Furthermore, the number of *ESCHERICHIA coli* colonies was observed.

V. RESULT AND DISCUSSION

Cellulose extract

Extraction is the separation process of certain components from either plant or animal tissue using an appropriate solution following decided procedure (Rokhati et al., 2013). The aim of extraction is the separation of certain material from others. The Extraction of cellulose from the banana leaves of kepok banana was done

by process delignification. Delignification process using NaOH was chosen because the procedure was easily conducted and the material could be easily obtained. The aim of delignification process was to dissolve the lignin compound contained in the sample

NaOH was used to impair or to get rid of lignin and hemicellulose which protect cellulose from cellulosEscherichia NaOH can also break hydrogen bond particularly on intermolecular cellulose bond furthermore cellulose is not bound (Kurniaty, 2017). Delignication process can be seen in Figure 1.

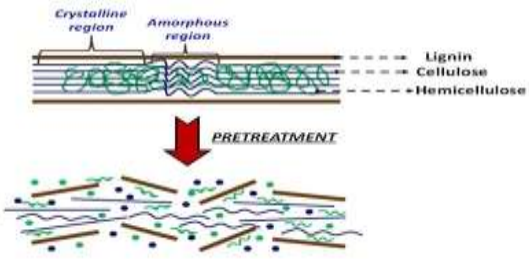

Figure 1: Degradation process of lignin and hemicelulose by NaOH (Tiwari et al., 2011).

Delignification process to obtain crude cellulose extract can be seen in Figure 2. Refined banana leaves a sample of kepok in the fibre form was brown (a). After delignification process, the colour of refined banana leaves was changing to be white (b), the colour of extracted lignin compound by NaOH 9% was brownish-black (c)

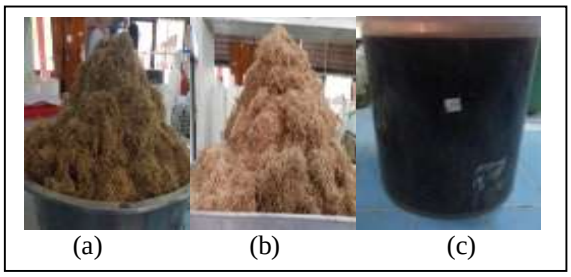

Figure 2: Delignification process of banana leaves with NaOH 9%.

In delignification process using NaOH 9% obtained residue pH of 13. The residue was washed by water to be neutral pH. Meanwhile in delignification process of cellulose extract yield obtained 487,37 g or equal with 48,74%. In the delignification process above, lignin was dissolved by NaOH, it can be shown in the colour alteration of NaOH solution from black to brown. OH- ion from NaOH will break up lignin band, meanwhile Na+ ion will bind lignin to form phenolic salt, on the other hand, phenolic salt was easily dissolved. Hemicellulose compound at the same time was dissolved in NaOH. The existence of NaOH in the solution system will cause damage to hydrogen, ester and ether bond between hemicellulose and cellulose or between hemicellulose and lignin. Its structure is amorphous furthermore it was easily degraded and it was dissolved in NaOH. Breaking of bonds is due to exposure to sunlight in the subsequent drying process. As a result of this delignification treatment, lignin and hemicellulose compounds are free from cellulose compounds, because cellulose is not soluble in NaOH solution.

Dissolved lignin is characterized by the presence of brownish-black solution in the filtered solution (Feki Desfran Zely, 2014). In this case, a decrease in sample weight was obtained from the lignification process (Barrett, 2009). Hemicellulose can be removed from lignocellulose compounds using alkaline solvents such as NaOH,

NH4OH, and KOH. Among these three solvents, NaOH is the best solvent (Idiawati, 2013). Hemicellulose compounds in the presence of NaOH molecules will dissolve together with lignin. The bonds that are broken/damaged in this process are hydrogen, esters, and ether. Damage to these bonds occurs in the lignocellulose compounds

Prebiotic test of hydrolisis products with temperature variations

Amylase enzyme is one of the enzymes that can degrade long-chain carbohydrate compounds to produce short-chain carbohydrates. In this study, the β-amylase enzyme was used to hydrolysis crude extracts cellulose of kepok banana leaves, where the hydrolysis process was carried out with temperature variations. The results of prebiotic test can be seen in Figure 3. The prebiotic properties of the hydrolysis of crude cellulose extract of kepok banana leaves showed that the number of colonies produced was positively correlated with the increase in hydrolysis temperature

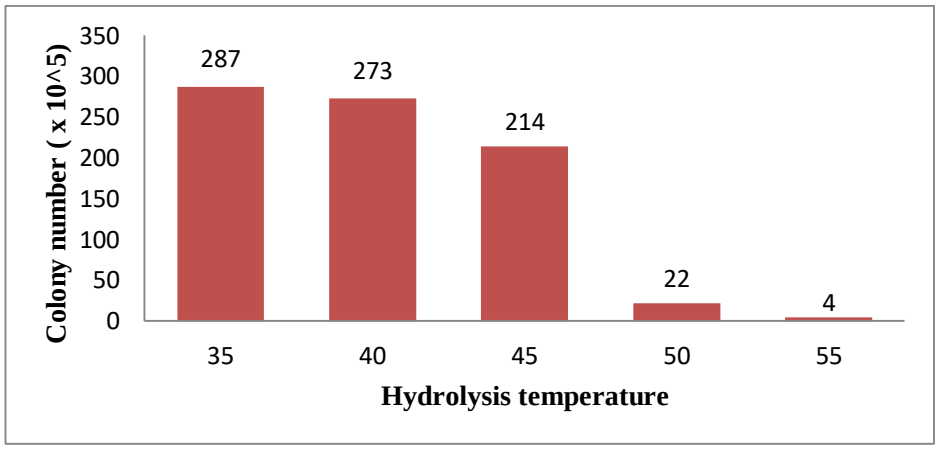

Figure 3: Effect of hydrolysis temperature to the crude cellulose extract of kepok banana stem on the number of Escherichia coli bacteria colonies on the tube surfacEscherichia

By increasing hydrolysis temperature, the hydrolysis product produced better prebiotic properties. It can be seen that the number of *Escherichia coli* found on the tube surface was decreasing. At the hydrolysis temperature of 35 ºC, the number of colonies decreased from 3.2 x 10^7 to 2,87 x 10^5. The most decrease in the colony number was obtained at a hydrolysis temperature of 55 °C, where the number of colonies was 4 x 10^5.

In this case, *Escherichia coli* bound to the hydrolysis product so that these bacteria will be carried out to the bottom of the tube due to the ballasting process. This result is consistent with the addition of prebiotic compounds in the animal feed where *Escherichia coli* in the digestive tract is reduced while in animal feces the number of *Escherichia* coli is increased because these bacteria bind prebiotic compounds so bacteria were carried out together with the animal's feces.

The potential prebiotic activity of growol (traditional fermented food from cassava) against digestive bacteria *Lactobacillus sp* and *Escherichia coli* (Puspita & Desty, 2019). The prebiotic activity of growol was more increasing the growth of probiotic bacteria compared to enteric bacteria. In addition, the result obtained indicated that the higher used hydrolysis temperature increased the prebiotic of hydrolysis product to decrease the number of *Escherichia coli*. It was suspected that the number of oligosaccharide compounds formed in the cutting of β-1,4 glycoside bonds process and the amount of hydrolysis product resulted would be proportional to the number of

Escherichia coli released with feces. This was because the used β-amylase enzyme had a slow activity to cut β-1,4 glycoside bonds in cellulose compounds so that β-amylase requires high temperature to accelerate cutting of cellulose bond. The best hydrolysis temperature in this experiment was obtained at 55ºC. This temperature resulted in a good prebiotic activity which was able to suppress and bind *Escherichia coli*. Theoretically, the β-amylase enzyme can cut amylase into disaccharide molecules. The β-amylase enzyme has a maximum and stable activity at 55º C. β-amylase has maximum activity at 60°C (Ni'maturohmah & Yunianta, 2015).

Hydrolysis temperature had a statistically significant effect on the number of *Escherichia coli* colonies at the tube surface There was a significantly different between the number of *E coli* at 35 °C and at 45°C. However, there was not significantly different between the number of *E coli* in 35 °C and at 40 °C and between the number of *Escherichia coli* in 50 °C and at 55°C. Furthermore recomended temperature to be applied to cellullose hydrolysis process was 55°C.

Prebiotic test of hydrolysis product by time variation

In this study, the β-amylase enzyme was used to hydrolyze crude cellulose extract from banana leaves of kapok banana with time variations of 3, 4, 5, 6 and 7 hours. Hydrolyzed potato starch with an amylase enzyme The best time for hydrolysis was 4 hours. Meanwhile, the best hydrolysis temperature was 55 °C (Rohmayanti, 2013).

The results showed that the highest colony number was 390 x 105, this colony number was achieved in 3 hours of hydrolysis time, meanwhile, the lowest colony number was 3 x 10^5 in 7 hours of hydrolysis time It can be seen in figure 4. Increasing hydrolysis time yield in decreasing the number of bacteria colonies. Hydrolysis time of 3, 4 and 5 hours obtained colony number of 390 x 10^5, 270 x 10^5 and 160 x 10^5 CFU/mL, respectively. Meanwhile, the number of bacteria cell after the addition of the hydrolysis product at 6 and 7 hours of hydrolysis time was reduced at 5 x 10^5 and 3 x 10^5 CFU/mL

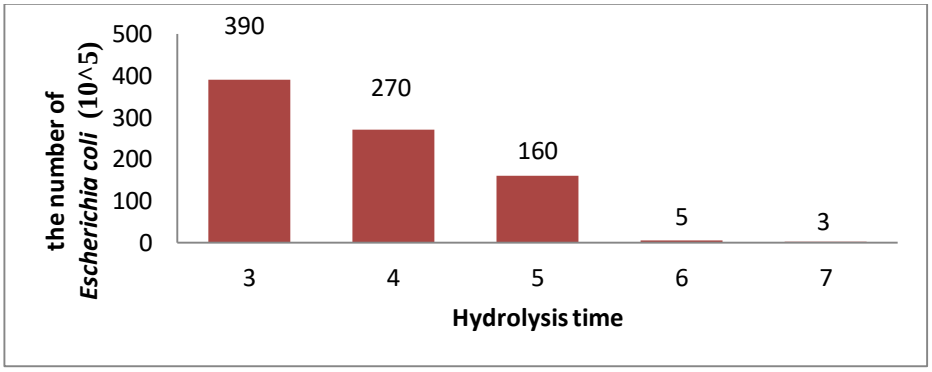

Figure 4: Effect of hydrolysis time of crude cellulose extract of kapok banana pseodo stem to the number of Escherichia coli left at tube surface

Figure 4 showed that there was a negative correlation between a number of colonies and hydrolysis time The number of colony bacterial was decreased when hydrolysis time was increased from 3 to 7 hours. It caused by increasing prebiotic activity decreased the number of *Escherichia coli* at the tube surface It was suspected that prebiotic compound formed an increased in the breaking process of the β-1,4 glycoside bond. This is also caused

by the used old β-amylase enzyme, increasing interaction between enzyme and substrate furthermore hydrolysis process is more reacting which causes high prebiotic activity.

Based on the above result, the number of hydrolysis product was equal to the number of precipitated *Escherichia coli*. Increasing hydrolysis time obtained in the number of short-chain of polysaccharide, oligosaccharide, disaccharide, and monosaccharide (Hajar et al., 2016). Those compounds in the digestive system had a characteristic as a prebiotic. Based on the statistical analysis, the number of *Escherichia coli* colonies at the tube surface was not significantly affected by hydrolysis time.

VI. CONCLUSION

The highest prebiotic activity of hydrolysis product of crude cellulose extract from kepok banana leaves was obtained at 55 0C which the number of *Escherichia coli* at tube surface decreased from 3,2 x 10^7 to 4 x 10^5 CFU/mL, while the best hydrolysis time was 7 hours which resulted the number of bacteria at tube surface was 3x10^5 CFU/mL

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