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Artikel

Antibacterial Edible Coating of *Ipomea batatas* Incorporated with *Lactobacillus acidophilus*

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ABSTRACT: This study was conducted to analyze the effect of variations concentration of *Lactobacillus acidophilus* in edible coatings and their antibacterial activity against *E. coli* using the well diffusion method. This study used an edible coating of prebiotic from purple sweet potato starch and *Lactobacillus acidophilus* with dilution variations (10-6, 10-7, 10-8, 10-9) as probiotics. In this study physical properties were tested for edible coatings such as color tests with visual observations, water content by the gravimetric method and viscosity using Viscometer Ostwald. The content of organic acids in edible coating solutions was measured using the HPLC method. Organoleptic test was conducted with taste and color parameters on the A-E scale of 30 respondents. Research results showed edible coating discoloration before and after incorporation from deep purple to brown. The value of water content and viscosity also changed from 62,8% to 71,4% and 569,97 cp to 486,64 cp respectively. The best antibacterial activity of edible coating incorporation with *Lactobacillus acidophilus* was 263,76 mm2. Organoleptic test on grapes that have been coated with edible coating showed that covered with edible coating contains probiotics (*Lactobacillus acidophilus*) has no effect on respondents' perception. Lactic acid and acetic acid were exist in edible coating which were incorporated with *Lactobacillus acidophilus*.

INTRODUCTION

Healthy food is needed by the community. The food industry is competing to create food products that can improve food functionality that is not only delicious but also beneficial for the health of consumers. One of them is by producing food containing probiotics. Probiotics are defined as live microbial cells which, if consumed in sufficient quantities, will provide health. The addition of probiotics to food will greatly help increase the nutritional value of food. In addition, the presence of probiotics will provide benefits in improving intestinal physiological function, increasing the immune response in the body, and inhibiting the growth of pathogenic bacteria in the digestive system in the intestines [1].

Research on coating food products with edible coatings/films has been widely conducted and has been proven to extend shelf life and improve product quality. The most potential and widely studied polymer material for edible coatings/films is starch polymer. Edible coatings using polysaccharide (carrageenan) are widely used, especially in fruits and vegetables, because they have the ability to act as a permeable membrane that is selective against carbon dioxide and oxygen gas exchange [2], [3].

The development of edible coatings has begun to be directed towards making edible coatings that are good for health, namely by incorporating probiotics in edible coatings. Therefore, many researchers carry out new formulations on edible coatings as a carrier

medium by adding probiotics to increase the benefits of food products. The advantages of LAB (*Lactobacillus acidophilus*) besides being believed to improve health in the human body, the presence of probiotics can prevent decay and contamination from other microorganisms. Lactic acid bacteria (*Lactobacillus acidophilus*) can also produce compounds that are antibacterial [4].

Purple sweet potato starch has a high nutritional content. In addition, purple sweet potato starch also contains antioxidant compounds. The antioxidants contained in purple sweet potato starch are a type of anthocyanin. Various positive benefits of anthocyanin for maintaining human health are to protect the stomach from damage, inhibit tumor cells, and improve vision. In addition, these compounds are also able to prevent obesity and diabetes, improve brain memory abilities and prevent neurological diseases, and ward off free radicals in the body. The sweet taste of purple sweet potato starch is obtained through an enzymatic process, by utilizing the high starch content in purple sweet potato. Starch is converted into simpler carbohydrates with the addition of 0.06% starch α-amylase enzyme and 0.08% starch amyloglucosidase enzyme, so that the level of sweetness increases. The properties of starch are also suitable for edible coatings or films because they can form a film that is strong enough [5].

The most potential and widely researched polymer material for edible coating is starch-based. Purple sweet potato flour has a high starch content. Starch is a type of polysaccharide that is abundant in nature, bio-degradable, easy to obtain, and cheap. Research on the manufacture of starch-based edible coatings has been carried out, as has been done by [6-8].

Lactobacillus acidophilus is one of eight common types of lactic acid bacteria (LAB). *Lactobacillus acidophilus* can grow either with oxygen or without oxygen, these bacteria can live in very acidic environments, such as at pH 4-5 or below and these bacteria are homofermentative bacteria, namely bacteria that produce lactic acid as the only end product. In this study, lactic acid bacteria (*Lactobacillus acidophilus*) will be incorporated in an edible coating with the aim of producing an edible coating that is antibacterial and good for digestion so that it can become functional food. Based on the research results of [9] the edible coating containing lactic acid bacteria (LAB) can extend the shelf life of food and provide health benefits if consumed. To test the ability incorporating of probiotic in edible coatings, it is necessary to test the viability of probiotic bacteria in edible coatings, test the antibacterial properties of edible coatings and measure the organic acids produced by probiotics in edible coatings. In this research we made edible coatings and incorporating lactic acid bacteria (*Lactobacillus acidophilus*) in the edible coating and determining the physical properties of the edible coating. Determine the antibacterial power of the edible coating containing lactic acid bacteria (*Lactobacillus acidophilus*) against *E. coli*. Determine the content of organic acids produced by lactic acid bacteria (*Lactobacillus acidophilus*) in the edible coating of purple sweet potato starch. Organoleptic testing of edible coating applications for coating grape fruit. This research is expected to provide information in producing edible coating from purple sweet potato starch (*Ipomea batatas L*) which is incorporated with lactic acid bacteria (*Lactobacillus acidophilus*) which has antibacterial properties that can inhibit pathogenic bacteria (*Escherichia coli*).

RESULT AND DISCUSSION

Physical Properties of Edible Coating

The process of making edible coating starts with 6 g of purple sweet potato starch added with 150 mL of distilled water then stirred for 15 minutes until the granules contained in the broken starch are then filtered, the purple sweet potato starch solution is added with 3.75 g of glycerol and 3 g of CMC after it is heated on a hot plate with a temperature of 70°C while continuing to stir for 20 minutes, then cool it at room temperature then you will get the desired edible coating solution. The time needed to make the edible coating is around 1-2 hours. After the edible coating dough is cooled, the *Lactobacillus acidophilus* bacteria are incorporated into 50 mL each of the edible coating solution and added as much as 3 mL of *Lactobacillus acidophilus* bacteria with various dilutions (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}).

(a) (b)

Figure 1. (a) Edible coating before incorporation with LAB and (b) Edible coating after incorporation with LAB

Edible Coating Color

The first physical characteristic test that was carried out was using observations of the color of the edible coating before and after incorporation with LAB. There is a fundamental difference from the color of the edible coating before incorporation in the form of dark purple, while after incorporation it is brown. Change in the color affected by MRSB which is incorporated into the edible coating.

Edible Coating Moisture Content

Water content is closely related to the durability of materials during storage. The lower the water content of the material, the safer the material is from damage due to microorganism contaminants. The value of water content in the edible coating before incorporation with LAB was 62.8% and after incorporation with LAB, the water content contained in the edible coating was 71.4%. The amount of water content after incorporation is greater than the amount of water content before incorporation. There is a possibility that it is caused by the addition of MRSB so that the amount of water content in the edible coating after being incorporated is greater than before being incorporated.

Viscosity Edible Coating

Viscosity or viscosity is the resistance to the flow of a liquid or the ratio of shear stress (force applied) to shear rate (speed). The viscosity of a solution is influenced by several factors, namely temperature, solution concentration, molecular weight and solute.

Viscosity is done to determine the size or level of thickness of a liquid. Good viscosity of edible coating ranges from 400-500 cp. The time of the edible coating before incorporation was 2 hours 13 minutes 22 seconds with a viscosity value of 569.97 cp. After incorporation the time obtained was 1 hour 53 minutes 52 seconds with a viscosity value of 486.84 cp. The change in the viscosity value is influenced by the concentration and the addition of MRSB.

Antibacterial Activity of Edible Coating After Incorporation

The growth of a type of microorganism in food can produce metabolites or change conditions in such a way that other microorganism species are stunted or their growth stops. Many microorganisms form metabolites that have antimicrobial power, one of which is lactic acid bacteria. Lactic acid bacteria are useful for improving the quality and safety of foodstuffs through natural inhibition of pathogenic microorganisms. Lactic acid bacteria produce several antimicrobial components, namely organic acids, carbon dioxide, hydrogen peroxide, diacetyl, reuterin, and bacteriocins. The organic acids produced by lactic acid bacteria are lactic acid and acetic acid. Lactic acid is the main metabolite of lactic acid bacteria. The inhibitory effect occurs because organic acid molecules enter the cell membrane and lower the cytoplasmic pH. Lactic acid bacteria antimicrobial test against pathogenic bacteria using the well diffusion method. According to [14] the advantage of the well diffusion method is that all metabolites produced by lactic acid bacteria can be produced during the antimicrobial test. Inhibitory activity tests against pathogenic bacteria were carried out including *Lactobacillus acidophilus* cultures, edible coatings that had been incorporated with *Lactobacillus acidophilus* bacteria, MRSB and edible coatings. The results of research on antibacterial tests against pathogenic bacteria showed that there was a difference in inhibition between *Lactobacillus acidophilus* cultures and edible coatings that had been incorporated with *Lactobacillus acidophilus* bacteria. Lactobacillus Culture acidophilus and edible coating which have been incorporated by *Lactobacillus acidophilus* bacteria which only provide clear zones have antimicrobial power against *E. coli* test bacteria. The zone of inhibition is measured from the edge of the well to the outer circle of the clear zone [15].

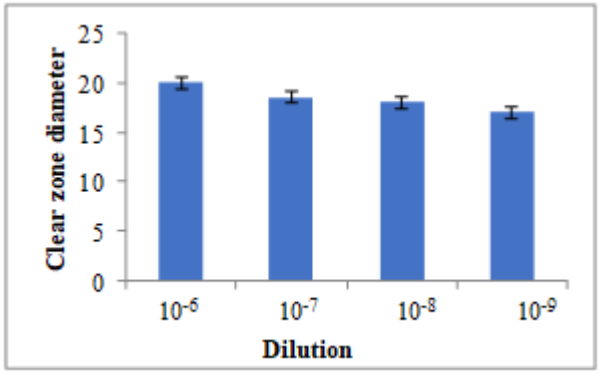

Figure 2. Effect of LAB dilution on the inhibition zone

One of criteria of lactic acid bacteria used as probiotic culture is its ability to inhibit pathogenic bacteria and be able to compete with pathogenic bacteria to maintain the

balance of micro flora in the intestine. Determination of clear zones resulting from edible coatings against *E. coli* can be seen in figure 2.

The size of the area of the inhibition zone is equivalent to the diameter of the clear zone produced by the edible coating which has been incorporated with *L. acidophilus* bacteria. The inhibition zone diameter of *L. acidophilus* at 48 hours incubation time against pathogenic bacteria *E. coli* with different dilutions of *L. acidophilus* bacteria resulted in the inhibition zone diameter that was not significantly different. Based on research by [16-18], the criteria antibacterial inhibition with an inhibitory area of 20 mm or more is included in the very strong criteria, 10-20 mm area of inhibition is the criterion strong, the area of 5-10 mm is moderate and the area of 5 mm or less is weak. Based on the data obtained, it was known that the edible coating dilution 10-6 has a clear zone diameter of 20 mm. Thus, it can be concluded that the inhibitory activity of the 10-6 dilution of the edible coating against *E. coli* is in the very strong category. Then, edible coating with dilutions of 10-7, 10-8, and 10-9 has clear zone diameters of 18.5 mm, 18 mm, and 17 mm, so it can be concluded that the antibacterial activity was in the strong category. The inhibitory power produced by these two substances comes from organic acids contained in metabolites of Lactic acid bacteria which can damage or disrupt cell membrane stability.

This condition causes bacterial cells to leak and ultimately accelerates the death of bacterial cells. The antibacterial effect of organic acids is the result of a decrease in the pH value of acetic acid, propionic acid, butyric acid, and lactic acid produced by lactic acid bacteria through homo fermentative fermentation, interacting with cell membranes and resulting in intracellular acidification and denaturation of pathogenic bacterial proteins that cause bacterial growth to cease. There was a synergistic work between acetic acid and lactic acid in inhibiting the growth of *E. coli* because of the strong acidic effect of lactic acid, the amount of inhibitory acetic acid that is not dissociated from acetic acid increases.

Antibacterial Activity of Edible Coating After Incorporation

This organoleptic test was carried out on 30 respondents consisting of students to assess the color and taste of grapes coated with an edible coating before and after being incorporated with LAB with different bacterial dilutions ranging from 10-6, 10-7, 10-8, and 10-9 CFU/mL uses 5 rating scales, namely very dislike (1), dislike (2), neutral / ordinary (3), like (4) and very like (5). The data were then analyzed using ANOVA.

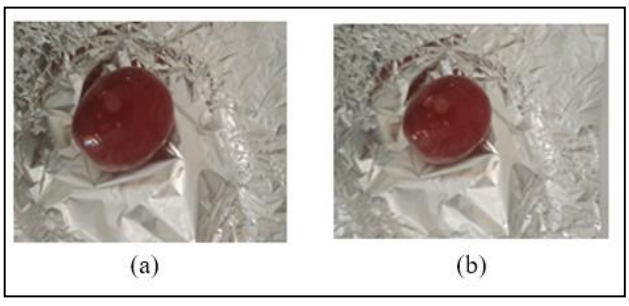

Figure 3. Grape fruit than had been used to coat with edible film gel of Sweet purple potatoes Starch : (a) before incorporation with LAB (b) The grapes after incorporation with LAB

One of the requirements for edible coating is that it is tasteless so it doesn't interfere with the taste of the coated product. Organoleptic test results Fresh grapes for color and taste parameters as shown in appendix 6 show that grapes that have been coated with edible coating before and after incorporation get the value of the P edible coating before and after incorporation greater than 0.05. From the ANOVA and homogeneity table, it was obtained a P value > 0.05, which means that there was no significant difference in terms of color and taste.

Analysis of Organic Acid Determination by HPLC on Edible Coating after Incorporation with LAB

Chemical content analysis was carried out to identify the organic acid metabolites of LAB contained in the edible coating after being incorporated with LAB, as a comparison, the standard pa lactic and acetic acid were used. This analysis was performed using the HPLC (High Performance Liquid Chromatography) method.

Table 1. Results of HPLC analysis of lactic and acetic acids on edible coating after incorporation with LAB

No	Organic acid	Retention Time (Minutes)		Concentration (ppm)		Molecular formula
		Edible Coating	Standard	*Edible Coating*	Standard	
1.	Lactic Acid	3,800	3,792	78,04	100	$C_3H_6O_3$
2	Acetic Acid	3,968	3,965	99,90	100	CH_3COOH

Table 1 shows that the retention time of organic acids contained in the edible coating after being incorporated with LAB is not much different from the standard. From Chromatogram of HPLC8, it is known that the retention time of lactic acid and acetic acid was detected at 3 minutes where lactic acid was detected earlier than acetic acid. Lactic acid is more polar than acetic acid so it has a lower retention time. The mobile phase used is methanol and aqua demineralized which are polar, while the stationary phase used is column C18 which is semi polar. In the column there is a process of separating compounds based on the "like dissolve like" principle. This means that compounds that are the same as the mobile phase will be brought faster to the detector and have a shorter retention time [19]. Table 1 explains that acetic acid has a greater concentration value than lactic acid, this is because acetic acid has a smaller short chain compared to lactic acid. Where the short chain can work as an inhibitor of pathogenic bacteria, the Ka value is better and is easily dissociated.

CONCLUSION

The water content of the edible coating before and after incorporation was 62.8% and 71.4%, and the viscosity values before and after incorporation were 569.97 cp and 486.64 cp. The best antibacterial properties containing probiotics are owned by the edible coating with a dilution of 10-6 of 263.76 mm2 with a diameter of 20 mm, so that it is included in the criteria for strong inhibitory activity. Organic acids detected in accordance with the standards contained in the probiotic edible coating are lactic acid and acetic acid, which respectively contain 78.04 ppm and 99.90 ppm. The number of probiotic concentrations that were incorporated into the edible coating did not affect the respondent's assessment (not significantly different) (P> 0.05) in organoleptic testing.

EXPERIMENTAL SECTION

Materials used

The raw materials used in this study were purple sweet potato starch obtained from the market. Lactic acid bacteria (*Lactobacillus acidophilus*), and pathogenic bacteria *E. coli*. Other ingredients used are distilled water, grapes obtained from the market, carboxyl methyl cellulose (CMC), MRSB (Man-Rogosa-Sharpe Broth) media, MRSA (Man-Rogosa-SharpeAgar), NB (Nutrient Broth), NA (Nutrient Agar), heat resistant plastic bag, 0.85% NaCl solution, cysteine and glycerol. High Performance Liquid Chromatography (HPLC) instrumentation were used to analyze Organic acid.

Microbiological Analysis

All tools are washed and then dried in the oven. Wrapped the cover paper in the petri dish and then put it in a heat-resistant plastic bag. Furthermore, all the tools that have been wrapped are put in an autoclave to be sterilized at a temperature of 1210C, a pressure of 1 atm and left for 15 minutes. This method of sterilization is called wet heat sterilization, which is by boiling it using an autoclave according to a predetermined temperature and time. This method uses water vapor to sterilize tools and materials to be used for research.

Preparation of Test Bacteria Media [10]

Making the media begins with weighing the nutrient media so that as much as 20 grams in 1 liter of distilled water and as much as 8 grams of NB and 1 L of distilled water are put into Erlenmeyer, respectively. Then, stirring while heated using a hot plate and magnetic stirrer until the solution is homogeneous. Then autoclaved for 15 minutes until it reaches a maximum temperature of 121°C. After being sterilized, the media was cooled at room temperature until the temperature reached 40°C.

Preparation of the media begins with weighing 0.6513 grams of MRSA media in 10 mL of distilled water and 0.522 grams of MRSB in 10 mL of distilled water, then each of which is put into Erlenmeyer. Then, stirring while heated using a hot plate and magnetic stirrer until the solution is homogeneous and boiling. Then autoclaved for 15 minutes until it reaches a maximum temperature of 121°C. After being sterilized, the media was cooled at room temperature until the temperature reached 40°C.

Preparation of Probiotic and pathogenic Bacteria

Rejuvenation of lactic acid bacteria was carried out by inoculating one loop of lactic acid bacteria from agar slants into a test tube containing 9 mL of MRSB. Then covered with cotton and then mix homogenously to precipitate the bacteria in the MRSB media. After that, it was incubated for 48 hours at 37°C. One mL of incubated lactic acid bacteria was inserted into sterile MRSA media, then the plates were tightly closed and incubated again for 48 hours at 37°C [11].

The rejuvenation of *E. coli* bacteria is carried out by inoculating one loop of *E. coli* bacteria from agar slants and is inserted into a test tube containing 9 mL of NB. Then covered with cotton and then vortexed to even out the bacteria in the NB medium. After that it was incubated for 24 hours at 37°C. 1 mL of the incubated *E.*

coli bacteria was inserted into sterile NA media, then the plates were tightly closed and incubated again for 24 hours at 37°C.

Edible Coating from Purple Sweet Potato Starch

Coating solution was obtained from sweet potato starch edible coating. Edible coating formula were 6g sweet potato starch, 100 ml distilled water, and 2 ml glycerol. Edible coating solution were prepared by dissolving sweet potato starch on distilled water at 60°C heating temperature on a hotplate and then stirred until the mixtures became clear. Glycerol then added to the mixtures and followed by heating at 60°C during 30. minutes. Lactic acid bacteria was mixed after the last heating of solution. Grape fruit were dipped in each coating solution and dried at drying box. Coated fillets were placed at Styrofoam plates, wrapped by wrapping plastic, and stored at refrigerator (4 ± 1ºC) for 8 days. Samples were analyzed microbiologically and chemically at 0, 2, 4, 6 and 8 days of storage.

Incorporation of Lactic Acid Bacteria in Edible Coating Solution

Incorporation of lactic acid bacteria will be carried out after the Edible coating solution reaches room temperature. Lactic Acid Bacteria (LAB) to be incorporation with edible coating were taken with 3 mL of lactic acid bacteria, 10-6, 10-7, 10-8 and 10-9 dilutions which were put into 50 mL edible coating respectively and then stirred with a spatula homogeneous and incubated for 24 hours at 37°C.

Analysis of Chemical Properties and Physical Properties of Edible Coatings that have been made

Viscosity Test

Viscosity test to determine the level of viscosity of the edible coating making. Testing using an Ostwald viscometer with the work order of the edible coating solution inserted in the tube cylinder until the mark, leave the edible coating liquid until all the edible coating liquid drops to the tar mark until the edible coating runs out then measure and record the time used. The viscosity of the edible coating using the Ostwald viscometer can be calculated using the formula.

Calculation:

$\eta = t \times \rho$

η = viscosities

t = Time

ρ = density of water (0,89 cp)

Water Content Test (SNI 01-3182-1992)

The edible coating solution is weighed as much as 5 g, placed in a porcelain crucible and heated in an electric oven which has a temperature control of 105°C for 5 hours. Cool in a desiccator to room temperature and weigh. Then heat again for 30 minutes and cool inside desiccator. The steps are usually performed repeatedly (usually 3-4 times) until the weight loss between two successive weights is less than 0.001g. Calculation:

Water content, percent weight / weight

$$A = \frac{m_o - m_1}{m_o} \; x \; 100\,\%$$

A = water content
mo = Weight Edible coating at first
m1 = Weight Edible coating once heated.

Preparation of Fruit to be Covered with Edible Coating [12]
Choose grapes that are not defective or physically damaged, then wash the grapes thoroughly using hot water so that all dirt or wax stuck to the fruit is gone. After washing, drain and dry the grapes at room temperature, soak the dried grapes in an edible coating solution containing lactic acid bacteria which has been incubated for 2 hours, then the grapes that have been coated with the Edible coating are left to stand for 4 hours.

Antibacterial Activity of Lactic Acid Bacteria by the Well Method
The well method (agar diffusion) is based on the ability of the tested antibacterial compounds to produce an inhibition zone around the test well against the bacteria used. The tested bacteria (*E. coli*) were inoculated into each NA growth medium by pour plate (0.1 mL of culture into the growth medium) then homogenized and allowed to harden. The NA media that has hardened is then made a well with a sterile drill in the section 4 wells of petri dishes. The first well is filled with MRSB media, the second well is filled with edible coatings, the third well is filled with an edible coating containing lactic acid bacteria, and the fourth well is filled with MRSB and lactic acid bacteria then incubated at 37oC for 48 hour. The diameter of the clear zone formed around the well is measured as the zone of inhibition.

Determination of Organic Acids contained in Edible Coating using the HPLC Method
A sample of 10 mL contains an edible coating containing probiotic bacteria dissolved in 100 mL of MRSB. The mixture is then filtered with filter paper and then filtered again with Millipore. The standard organic acid was put as much as 10 µL into the HPLC, adjust the flow rate of 0.5 mL/minute at a wavelength of 210 nm. Then it will go through the column and the results will be read by the detector in the form of a chromatogram with the retention time, peak, standard area. After that the sample was incubated with the same treatment as the standard organic acids.
The operational conditions of the HPLC appliance are as follows:

Mobile phase	: Methanol : Aquabidest = 70:30
Injection Volume	: 1.0 µL
λ	: 210 nm
Flow Speed	: 1 µL/minutes
Detector	: 0.2 A
Column	: C18

Organoleptic Test
One of the requirements for edible coating is tasteless and clear, for that reason organoleptic testing was carried out on fresh grapes that have been coated with an edible coating containing probiotic bacteria. This is to determine the respondent's level of preference for coated wine. The organoleptic test carried out was the hedonic test of color and taste parameters on the A-E scale. Each of the assessment

criteria is (A) very like, (B) like, (C) neutral / ordinary, (D) dislikes, and (E) very dislikes. The data obtained were processed statistically using ANOVA through the SPSS 15 program. In the acceptance test, there is no comparison or standard sample and respondents are prohibited from remembering or comparing with the samples tested previous. Responses should be quick and spontaneous. Even the responses that have been given should not be withdrawn even though later doubts arise. The acceptance test is more subjective than the differentiation test. Therefore, some respondents who are extremely happy or hate a commodity or material can no longer be used to carry out acceptance tests. The fruit will be coated with an edible coating and tested on at least 30 respondents [13].

Data analysis

The data that will be obtained from this research include, well test area data, data on inhibition of *E. coli* bacteria and retention time produced by HPLC. The data obtained from the HPLC method is the retention time to find the concentration of organic acids. Then the formula is used:

Organic acids = (Sample area / Standard area) x Standard concentration x (PF/BT)
Note:
PF = dilution factor (mL)
BT = Number of samples used (mL)

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