Characteristics based of edible film made from whey with isolated lactic acid bacteria from tempoyak as probiotics packaging

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Abstract. Edible film made from whey, a waste obtained from milk and used as primary packaging. This research aimed at isolated and identification of lactic acid bacteria derived from tempoyak, subsequently applied in the manufacture of edible film and have the properties of probiotics. Physical properties measured from the edible film are moisture content, pH, solubility and the thickness of the time. This research uses material from waste milk whey and tempoyak. The method used is an experiment using a random design group that consists of four isolates tempoyak addition, treatment A (0%), B (4%), C (8%), into a solution of whey and six replicates. Treatment results show to a thickness ranging from 0.20-0.25 mm and already meet the standards JIS 1975.
Keywords – edible film, whey, isolated lactic acid bacteria, tempoyak, probiotics packanging.

1. Introduction

Tempoyak is a type of traditional Malaysian food made by Durian that is undergoing the fermentation process. The fermentation process is spontaneous tempoyak involving microorganisms that exist in nature. The production of tempoyak is quite simply where the meat Durian fruit that comes from old, anaerobic conditions are left in during the 3-7 days at room temperature. Sometimes someone makes you add salt 1-3% [1].

In the process of fermentation of lactic acid bacteria producing tempoyak, this is due to the synthesis of Durian, which consists of about 15-20% [2], which is the source of energy in the development of lactic acid bacteria. Species *Lactobacillus* It is the predominant lactic acid bacteria found in isolated tempoyak from Indonesia and Malaysia. However, lactic acid bacteria in tempoyak also depend on the location where such products exist. *Lb. plantarum*, *Lb. brevis*, *Lb. mali*, *Lb. fermentum* found in tempoyak, that comes from the Malaysia [3], While in Indonesia, according to a study [4] and [5] There Isolate *Lb. plantarum, Lb. casei*, *Lb. corynebacterium* and *Lb. fersantum*, the latest research [6], found *Fructobacillus durionis* and *Lb. plantarum* as dominant lactic acid bacteria in tempoyak from Malaysia with total lactic acid bacteria 8.88-10, 42 log CFU/g.

One way of keeping food was put (*packaging*) and the right materials. According to [7], also known as packaging, and is one way of maintaining agricultural materials, because the packaging can extend the life of the material. Together with human consciousness will this problem than the type of packaging of biological materials and derived from renewable materials (*renewable*) and financially. One of the

types of packaging that is environmentally friendly packaging is edible (*edible packaging*). The advantages of *edible packaging* It is able to protect the original appearance of food products, the product can be maintained and can be eaten as well as safe for the environment and is the packaging of the future [8].

Edible film is a thin layer made of edible materials, formed over the food ingredient which serves as an obstacle to mass transport (e.g., moisture, oxygen, dissolved substances and fat) and or as *carrier* Food ingredients or additives and or to improve food handling [9].

In this study the author will use the basic ingredients whey. Whey generally a waste of casein or cheese. Possible food and energy whey will be lost if not used, given the whey contains about 55% of the total diet from milk [10]. In addition to hydrocolloid, other construction materials, edible It is a polysaccharide of cellulose and its derivatives. While the lipid used can be derived from natural wax (*beeswax, Carnauba wax, paraffin wax*), glycerin, fatty acids, and emulsifier.

Based on research [11] whey by using the carboxymethylcellulose (CMC) with as much as 1% and 3% glycerol significantly impacts against the thickness of the film but had no effect on moisture content, pH and solubility. In review research on the edible Film and *coating* [8], stating the edible film is very active food packaging is required as the future, some biopolymer It shows excellent performance as a carrier for the active compounds that are exported so taken food packaging is worth consuming for now and the future. Further research by [6], found *Fructobacillus durionis* and *Lb. plantarum* as dominant lactic acid bacteria in tempoyak from Malaysia with total lactic acid bacteria 8.88-10, 42 Log CFU/g, which can be used as active compounds antimicrobial in the production edible film.

1.1. The purpose of the research

The research purpose is to determine lactic acid bacteria from tempoyak on edible film from waste of cheese as probiotic packaging.

2. Material and methods

2.1. Material of research

This research material is Durian Tempoyak derived from the region of Pariaman, whey from the manufacture cheese "Lasi" in District Agam, West Sumatera, acid acetate, CMC, glycerine, acetic acid, ethanol, *Man Rogossa Sharpe Agar* (MRSA) (Merck), MRSB (Merck), *Muller Hilton Agar* (MHA) (Biolife), *Nutrient Agar (NA)* (Merck), *buffer peptone water*, aquadest, alcohol, spirits, HCl 0,1N, NaOH 0,1N.

The equipment used is *cool box, t*hermometer, pH meters, bowls, petri dish, incubators, needles, counting cup, analytical scales, Erlenmeyer, Bunsen, Test tubes, *beaker glass*, the dropper drops, hockey stick, *autoclave, centrifuge, vortex,* Spectrophotometer and small pipette.

2.2. Isolate lactic acid bacteria conventional

2.2.1. Sampling

Tempoyak samples taken from the area of Pariaman.

2.2.2. The measurement of physical and chemical properties and total lactic acid bacteria Tempoyak

- Physical and chemical properties of tempoyak i.e.: pH, Moisture content [12]
- Total Lactic acid bacteria [13]

2.2.3. The isolation of lactic acid bacteria [13]

2.2.4. Characteristics of lactic acid bacteria [13]

- Morphology
- Biochemistry
    - Gram pigmentation
    - Catalase Test

2.3. Procedure Edible Film [7], [14]

The realization of edible film whey with the addition of treatment isolate lactic acid bacteria A (0%), B (4%), C (8%), with 6 replications as a group, then carried out measurements of physical properties edible film whey

2.3.1. Moisture content [12]

2.3.2. pH [12]

2.3.3. Thickness of the film [15]

2.3.4. Solubility [16]

Data Analyses Statistics with variance analysis and if treatment shows different results for Real ($P < 0.05$) then conducted further tests using *Duncan's Multiple Range Test (DMRT).*

3. Result and discussion

3.1. Isolate lactic acid bacteria from tempoyak

The value of chemistry tempoyak the value of pH, moisture, protein, fat content can be treated in Table 1.

Table 1. The chemical composition of tempoyak.

The value of chemistry	Quantity
pH	3.89
Moisture content (%)	70.21
Protein (%)	5.04
Fat content (%)	6.11

Tempoyak is composed of proteins, fats and carbohydrates, and has a sour taste because of pH low. pH this low moisture content producing 3.89, moisture content 70.21%, protein 5.04% 6.11% fat content, tempoyak, associated with the age of tempoyak is greater. This is due to the accumulation of lactic acid produced during the development of lactic acid bacteria. [17] In traditional fermentation occurs spontaneously, germs that are naturally present in food will increase and lead to a succession of the dominant microbe or substitution in the next process. Lactic acid bacteria were changed by producing more acid, so that pH down about 3.89 even lower.

3.1.1. Isolation and cleaning of balancing

The calculation of the total tempoyak by Padang Pariaman District as many 16 x 10^8 CFU/g. Some bacteria are taken randomly by the moisture content in order. Further marked for the nature of the classification. After the isolated LAB found with a characteristic rounded, creamy-white and colored convex. After that, proceed with the determination of the morphology of the LAB is selected. The identification made in this investigation is microscopic and macroscopic.

Compared to some previous reports on the types of processed milk food [17]. This may indicate that the viability of the tempoyak, which in the product is a basic requirement for the development of functional food more. It is therefore necessary to the characterization microbial diversity and synthesis of conventional dairy products.

3.1.2. Characterization of isolate elected

Macroscopic (Shape, size and color) LAB Colony found white-beige, round, and Convex for MRS Agar Merck. Macroscopic observation, after cleansing is done up to two times smoother than the census of bacteria to be used for further testing, i.e. the identification of microscopic. Microscopic determination in this research is to adhere to physiological properties Isolate LAB. Observations made from test grams. Gram test conducted to know the gram positive or gram-negative bacteria is characterized by the absorption of color reagents by the bacteria. If the bacteria absorb the color reagent violet colored crystal violet, gram-positive bacteria (+), while Gram-negative bacteria (-) is a reagent

color safranin which is red. The results of the laboratory isolated gram pigmentation of curd from Padang Pariaman recovered formed the morphology of the bacteria, i.e. rods (Bacillus).

Gram pigmentation results in each Isolate LAB obtained in this study is Gram positive (+) bacteria. Characterized by bacteria that absorb the color purple violet and has the form Bacillus round rods. This is in line with the declaration by [18] stating that Gram-positive bacteria will get violet purple color. Even if it has been washed with alcohol and when given safranin red, the bacteria will still be purple, while the red shows gram-negative bacteria.

Biochemical test conducted with catalase. Catalase test is done in an Isolate LAB to learn the potential of bacteria produce catalase enzymes and isolate tolerance to oxygen. Biochemical test made with hydrogen peroxide (H_2O_2) on the review of bacteria for glass material. Observations made were the occurrence or absence of gas bubbles in the study of bacteria. In the event of a gas bubble showed that the bacteria is catalase positive, while the absence of gas is catalase negative. Because of the characteristics of the parcels are usually Gram-positive pigmentation, reacts negatively to catalase and not to the spore. The results of the experiments carried out in the Isolate They have a negative catalase which is characterized by the absence of gas bubbles in the bacteria exhausted in H_2O_2. The results of this study are like [19] who gets a negative catalase isolate laboratory of the intestines of shrimps. And also received a negative catalase isolate Mango [20].

In addition to biochemical testing, catalase test is a test of the type of fermentation. The purpose of this test is to classify the BAL belonged to the groups homofermentative or heterofermentative. Observation is done by examining the formation of air bubbles in the tube Durham. Research results isolate tempoyak Padang Pariaman are bacteria homofermentative in the treatment of tempoyak fermentation without the addition.

There are two types of lactic acid fermentation bacteria, namely homofermentative and heterofermentative [21]. Homofermentative lactic acid bacteria produce lactic acid as the main fermentation products, while lactic acid bacteria lactic acid additionally heterofermentative also produce ethanol, other acids such as acetic acid and Gas Co.$_2$, so when lactic acid bacteria tested to produce accommodation gas. In the Tube Durham lactic acid bacteria, said heterofermentative, while isolate which does not produce or produces a gas called homofermentative. Results of research [22], antibacterial activity research of lactic acid bacteria *Lactobacillus delbreukii* Also obtained BAL formula homofermentative of 6 isolates isolated from yogurt on the commercial market.

3.1.3. The Activity of Antimicrobial

To select and get isolate the most likely LAB became a candidate probiotic. In addition, the ability to know candidates' probiotics suppress the growth of pathogenic bacteria *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli O157*. The screening process is conducted using qualitative diffusion methods well. In this study do not use positive controls such as antibiotics, because tests are done to learn the potential of Isolate LAB was found to block the growth of pathogenic bacteria. The diameter of the clear belt is formed in the Isolate as shown in Table 2 below.

Table 2. Diameter clear band test antimicrobial (mm).

Isolate	Clear Zone (mm)		
	E. coli O_{157}	*S. aureus*	*L. monocytogenes*
Tempoyak	12,3	19,3	17,3

Larger obstruction zones were taken from isolated tempoyak (19.3 mm) in *Staphylococcus aureus*, (17.3 mm) in *Listeria monocytogene* and suspension of low *Escheria Coli* (12.3 mm). This is the third-test bacteria that have a different character. And the opinion of [23], each test bacteria has resilience separately against many kinds of organic acids. *L. monocytogenes* They have a greater susceptibility to lactic acid than acetic acid. *E. coli* high sensitivity against lactic acid and acetic acid, while *S. aureus*. They have the highest resistance to acids compared to other bacteria. As seen by [24] that the indicator

showing the ability of packages to inhibit the growth of pathogenic bacteria can be seen from the clear zone produced during the test Antimicrobial and related to the ability of packages to produce lactic and secondary metabolites bacteriocin.

The resulting results show that the Isolate Padang Pariaman's LAB were able to block the growth of pathogenic bacteria that can harm humans, especially bacteria *Staphylococcus aureus*. Probiotics can be used as a treatment at the sight of inhibitory zones tested for pathogenic bacteria.

3.2. Characteristics of Physic Edible Film Whey

Track Moisture content, pH, the thickness, solubility and total lactic acid bacteria colony edible film whey with the addition of lactic acid bacteria from tempoyak can be seen in Table 3.

Table 3. Characteristics of physic and total LAB edible film whey.

Treatments	Moisture content (%)	pH	Thickness (mm)	Time Solubility (seconds)	Total Colony LAB (x 10^6CFU/ml)
A	19,5[a]	7,07[a]	0,21	32,89[a]	0[a]
B	15,1[b]	7,23[b]	0,23	33,48[a]	7[b]
C	15,9[b]	7,31[b]	0,24	34,68[b]	15[c]

On the table, be considered that the average value of the moisture content, pH actual different shows, with the addition of lactic acid bacteria than without treatment, is A (0%). The moisture content has decreased with the addition of lactic acid bacteria this is because isolate LAB free water binds to edible film in addition to the use of other ingredients like glycerin that is hydrophilic [11]. While increasing the pH, caused by alkali from glycerol that cannot be lowered by adding lactic acid bacteria. Based on international standards (Japan JIS Z 1707:1997) Maximum moisture content is 13%, the average moisture content of the edible film whey this exceeds the standard, this is due to the fact that the long drying in the oven for 18 hours, with temperatures 50^{o}C was not able to reduce moisture content.

Treatment with the addition of lactic acid bacteria has no effect on the thickness due to the volume of each edible film that is poured into petri dish the same 50 ml, based on JIS Z 1707:1997, the standard thickness is 0.25 mm. Thus, in this study, the thickness of the edible film whey already meets the standards. Lactic acid bacteria, including safe microorganisms, when added to food: for nature is not toxic and does not produce the toxin, it is then called food grade microorganism Otherwise known as microorganisms *Generally Recognized as Safe* (GRAS) that is not at risk in health, even various types of basil the nutritious health [25].

Time is the actual effect of C solubility treatment (8%) from A and B, this is due to an increasing number of additions of lactic acid bacteria can increase your connective strength of lactic acid bacteria with water connected that is edible film whey time, thus adding solubility. It is also in line with previous research [14], that certain ingredients like glycerin do not dissolve in water because they are non-polar.

Concerning the parameters of all lactic acid bacteria colonies, the treatment effect is really against the treatment, in which the number of lactic acid bacteria also increased, the average temperature in drying does not cause bacteria to die. Optimum growth temperature is ± 37-45 ^{o}C, in general Distract, are anaerobic, catalase negative and oxidase positive, the fermentation produces lactic acid. Lactic acid bacteria can grow in sugars, alcohol, and high levels of salt, able to ferment the monosaccharides and monosaccharides. Most of the parcels can be well developed in environments that have or do not have O_2 (Unconscious in O_2), including *Anaerobic aerotolerant*.

4. Conclucion

Based the results of research concluded the more many additions of LAB affected the in moisture content, pH, time soluble, as well as the total number of LAB from edible film whey. However, no effect of thickness of edible film.

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