

Quality evaluation of probiotic capsule prepared from alginate, carrageenan and tofu waste flour based on bacterial activity and organoleptic test

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Abstract. Probiotic capsule is an innovation in functional food sector. It is used to preserve the living cells of probiotic bacteria during processing and storage. In this research, the improvement of probiotic viability is studied by using two kinds of encapsulating biomaterials and different concentration of tofu waste flour. Extrusion is selected method for encapsulation process. The purpose of this study is to examine the quality of probiotic capsule by evaluating the lactic acid bacteria performance and its physical characteristic. The article provides the data of probiotic bacteria activity related to their living cells present in capsule, activity in fermentation media compare to uncapsulated bacteria, and panelists' preferences of capsule's physical properties. The data is analyzed statistically by using ANOVA. The result shows that variables in this study affect the number of bacteria, their metabolic activity in producing acid during fermentation, and physical appearance of the capsule. Combination of alginate and tofu waste flour allows the multiplication of bacteria to a high number, and forms elastic, yellow and cloudy capsule, while with carrageenan, it causes the growth of a few numbers of bacteria which affects to a moderate pH and produces elastic, creamy and transparent capsule.

1. Introduction

Probiotic is essential live bacteria that deliver beneficial effect to human's health if they present in sufficient number [1]. The positive effect of this bacterial group, especially lactic acid bacteria, confirms it as one of functional food. A living condition of probiotic needs to preserve during processing and storage time, until the bacteria are released into gastrointestinal tract [2]. One of effective protection methods for probiotic is encapsulation. It is a technical procedure to protect probiotic bacteria from harmful condition for their growth by immobilizing the cells inside capsule and entrapping them within the gel matrix [3]. This physicochemical process produces tiny particles, ranging from nanometers to millimeters. The formation of these capsules can sustain the life of probiotic bacteria in the product and improve the effectiveness of its life in reaching the digestive tract [4, 5].

In encapsulation, the quality and physicochemical characteristic of capsule are determined by organic macromolecules used as encapsulating biomaterials. The common biomaterials derived from polysaccharide group, such as alginate and carrageenan. Alginate is a polymer of carbohydrate group extracted from seaweed. Alginate has a good capability as a capsule, but its gel is unstable under



acidic conditions [6, 7]. The ratio of its chemical composition between guluronic and mannuronic acid defines the gel strength of its capsule [8, 9]. Carrageenan is also a class of polysaccharides obtained from red algae that contains sulphate and hydrophilic molecules [10]. Chemical binding of D-galactose, its molecular weight, and sulphate along the polymer chain determines hydrophilic characteristic and gel properties [11, 12]. Both of these biomaterials have different characteristics leading to different physicochemical properties of probiotic capsule.

In addition, probiotic bacteria also require source of energy (prebiotic) in their living media for the growth and metabolism activity. Natural prebiotic can be derived from plants, such as soybean. Soybean is a kind of bean commonly used as main ingredient in making tofu. This bean contains of oligosaccharide (raffinose and stachyose) considered as one of prebiotic sources [13]. Oligosaccharide still can be found in its byproduct, which is tofu waste. This waste also contains other nutrition, especially protein and fiber. The high nutritional value of tofu waste brings it as a prospective reusable material to be applied in several innovations, namely as prebiotic in probiotic encapsulation. The tofu waste performs as an important ingredient in encouraging living capability and multiplication of probiotic bacteria. Probiotic capsule made from alginate, carrageenan and tofu waste flour combination shows specific quality. Therefore, this study aims to examine through laboratorial tests regarding bacterial activity and panelists' preferences and description in terms of its physical characteristic prior to the capsule apply into food product.

2. Materials and Methods

Materials used in this research were probiotic bacteria; *Lactobacillus bulgaricus*, encapsulating materials; carrageenan and alginate, and prebiotic source; tofu waste flour. Regarding research analysis, bacteria enumeration [17] used MRS agar and broth, and pH calculation used milk as fermentation media following yogurt production method [18, 19]. Organoleptic test followed the procedure [20]. The tools used were syringe 2.5 cc, digital scales, laminar flow cabinet, incubator, water bath, thermometer, centrifuge, hot plate, and glassware.

This study used two factors, which were the variety of encapsulating biomaterials (alginate and carrageenan) and the percentage of tofu waste flour (1.5%, 2%, 2.5%, and 3%). The data comparison was statistically calculated by using ANOVA, in order to examine the significant level of variables to the tested product.

This research used extrusion method for encapsulation technique as it is easy and commonly used in laboratorial scale. *L. bulgaricus* in pellet form was dissolved into sterile distilled water for a culture solution. The encapsulating solution, either carrageenan or alginate, was homogenized and sterilized. This solution was mixed with tofu waste flour. The *Lactobacillus bulgaricus* culture solution and encapsulating solution were mixed with a ratio of 1: 4. This mixture was dripped by using syringe into the hardener solution [14, 15]. The tofu waste flour used in the encapsulation process had been prepared before making capsule. Tofu waste was taken from one of tofu industries in Banda Aceh, Indonesia. The tofu waste was pressed and steamed for \pm 15 minutes before drying outdoor for 8 hours per day. Drying process took about 3 days. The dried waste was crushed and sieved with 140 mesh size [16].

Analysis performed in this study involved of laboratorial test; bacteria enumeration and pH analysis of fermentation media, and panelists' perception. According to bacteria enumeration, probiotic capsule was added with phosphate buffer and stirred for 2 hours for 1.5%, 2% and 5 hours for 2.5%, 3 % of additional tofu waste flour. After dilution, 100 μ l injected to MRS agar and incubated in 37°C for 72 hours [17]. Fermentation followed the procedure of yogurt fermentation. Skim milk and sugar were added to milk then homogenized and pasteurized at 80°C for 15-30 minutes. The mixture was cooling down until 40-45°C. *Lactobacillus bulgaricus* as starter culture and probiotic capsule were added into the milk. It was incubated at 37°C for 24 hours [18, 19]. In terms of organoleptic test, it used 15 panelists that assessed all probiotic capsules and presented their observation in score [20].

3. Results and Discussion

3.1. Bacterial Activity (Enumeration of total LAB and pH of fermentation)

The enumeration of total lactic acid bacteria (LAB) was conducted by calculating the number of living cells inside 1 gram of probiotic capsule. Based on Table 1, a high number of bacteria are discovered in alginate capsule rather than carrageenan capsule. It correlates to the quantity of capsule in 1 gram. Due to low weight of alginate capsule, so it requires more capsules to reach 1 gram compare to carrageenan capsule which has more weight and only requires a few capsules to obtain 1 gram as a total. In each capsule of alginate, there might be some living bacteria, therefore the more number of capsules, and the more bacteria are examined (19.61×10^9 CFU/gram).

Table 1. Total of Lactic Acid Bacteria (LAB)

Variables	Total of Lactic Acid Bacteria (1×10^9 CFU/gram)
Encapsulating Biomaterials :	
Alginate (K_1)	19.61 ^b
Carrageenan (K_2)	11.32 ^a
Concentration of Tofu Waste Flour :	
1.5% (P_1)	10.85 ^a
2.0% (P_2)	12.40 ^b
2.5% (P_3)	15.07 ^c
3.0% (P_4)	23.53 ^d

Moreover, the characteristic of biomaterials also determine the growth of bacteria. Alginate capsule has a liquid center bordered by semipermeable membrane [21, 22]. Semipermeable with thin layer and small diameter facilitates the movement of cells and nutrition, so that the bacteria could grow well [23]. The high viscosity and thick gel of carrageenan capsule could interrupt and restrict the diffusion and dispersion of nutrition for bacteria. Also, cell of bacteria could not move and pass through the membrane to absorb the nutrition due to its thickness. It may cause mortality to the bacteria and affect to the low number of LAB (11.32×10^9 CFU/gram). Additional prebiotic in capsule could increase the viability, endurance capability and growth of probiotic bacteria [24]. Moreover, prebiotic also could improve the quality and robustness of capsule by repairing the gel's porosity. Table 1 shows clearly the improvement of LAB along with the increase of tofu waste flour concentration. The highest amount of LAB is represented by P_4 or 3% (23.53×10^9 CFU/gram). This number fulfills the requirement of probiotics dose in a day, at least $10^6 - 10^7$ CFU/gram [25].

Bacterial activity inside capsule also could be evaluated from pH reached during fermentation. In this test, the capability of encapsulated bacteria in metabolic system was compared with starter culture *Lactobacillus bulgaricus* without capsule. *L. bulgaricus* without capsule produces the lowest pH, 4.0, due to its direct interaction with the media, while alginate and carrageenan capsule have a higher pH; 4.68 and 4.88 respectively (Figure 1a). Encapsulation could inhibit proliferation cells of probiotic in food product; therefore the sensory characteristic of product might not deteriorate and could preserve its quality [26]. In making capsule, alginate has porous layer properties that causes cells moved unrestrictedly through membrane [6], then release acid to media and lowering pH (4.68). Moreover, its higher number of total LAB (Table 1) compare to carrageenan is responsible to the lower pH in fermentation.

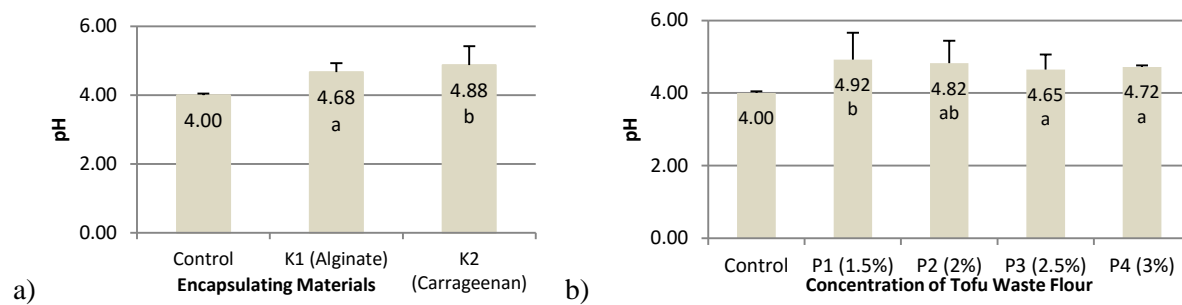


Figure 1. pH during fermentation; a) Encapsulating biomaterials; b) Tofu waste flour.

All capsules with tofu waste flour have a higher pH rather than sample without the flour (control). It confirms the function of prebiotic is not only as energy source for bacteria (Table 1), but also as supporting materials to build up good capsule by repairing porosity of biomaterials [27, 28]. Figure 1b shows that pH around 4.65 – 4.92 occurs due to the bacteria could not across easily from capsule to media. Moreover, a complex chemical structure of this prebiotic makes it is not able to be degraded optimally by metabolic reaction during fermentation time (24 hours), so that it produces low acid level. Therefore, pH reached is moderately high.

3.2. Organoleptic Test

Table 2. Preference Level of Capsule Physical Characteristics

Encapsulating Biomaterials	Preference Level of Organoleptic Parameters		
	Texture	Color	Odor
Alginate (K ₁)	2.83 ^a	2.55 ^a	2.78 ^a
Carrageenan (K ₂)	3.81 ^b	3.58 ^b	3.12 ^b

Scale: 1=very dislike; 2=dislike; 3=neutral; 4=like; 5=very like

Organoleptic test used in this research were hedonic and description tests. Hedonic test is aimed to assess the preference level of panelists in term of specific parameter. Regarding Table 1, it shows that panelists prefer capsule made from carrageenan, according to texture (3.81 or like) and color (3.58 or like) than alginate (2.83 or neutral) and (2.55 or neutral) respectively. The texture of carrageenan capsule is tenderer due to its capability in trapping and absorbing more water [12, 29]. Carrageenan flour has creamy to yellowish color, while alginate has yellow to brown color, which relates to the color of their raw material [30]. Moreover, hydrophilic properties of carrageenan makes it binds with more water, and produces capsule with more shiny and white. Panelists assume that both of encapsulating biomaterials produce almost none odor (neutral) (Table 2). The preference level of both capsules either alginate or carrageenan occupies approximately 2.78 – 3.12 (neutral).

Description test is intended to evaluate the specific physical characteristic of capsule based on panelists' perspective. From Figure 2a, it illustrates that, overall, the alginate and carrageenan capsules have similar elastic texture (3.8 – 4.4 or elastic). Based on color description, panelists express different perspective between alginate capsule and carrageenan capsule, in which alginate capsule (K₁P₁ – K₁P₄) has yellow color (3.9 – 4.5), while carrageenan capsule (K₂P₁ – K₂P₄) reflects creamy color (1.8 – 2.3). As overall appearance, panelists think that alginate (K₁P₁ – K₁P₄) produces cloudy or turbid capsule, while carrageenan (K₂P₁ – K₂P₄) is responsible to semitransparent appearance of the capsule. It is also related to the amount of water binding and its natural characteristics [30].

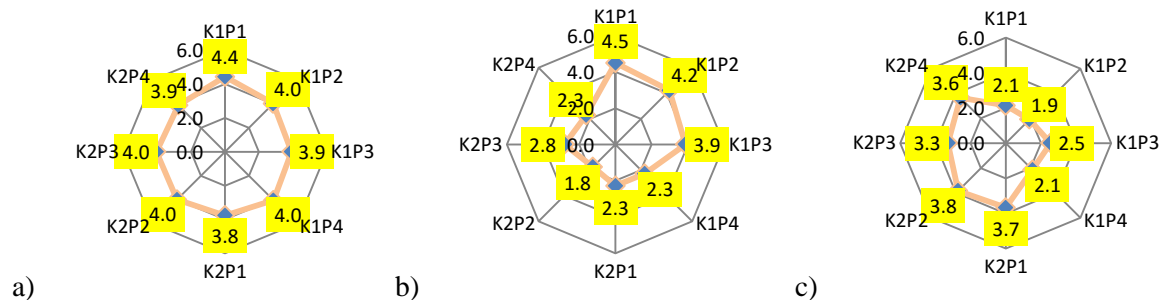


Figure 2. The Description of Capsule's Quality; a) Elasticity; b) Color; c) Appearance.

Elasticity scale: 1=very soft; 2=soft; 3=slightly elastic; 4=elastic; 5=very elastic; 6=rigid. Color scale: 1=broken white; 2=cream; 3=light yellow; 4=yellow; 5=dark yellow; 6=brown yellow. Appearance scale: 1=opaque; 2=cloudy; 3=slightly cloudy; 4=semitransparent; 5=transparent; 6=clear

4. Conclusions

Encapsulating biomaterials and prebiotic addition could affect the probiotic living cells and capsule physical characteristic related to panelists' perspective. Alginate could provide a better growth condition for bacteria in capsule with higher total LAB (19.6161×10^9 CFU/gram) than carrageenan (11.32×10^9 CFU/gram). The porosity of alginate makes it reaches lower pH in 24 hours than carrageenan. Additional tofu waste flour prebiotic in encapsulation process can improve the growth of bacteria and their survival condition in capsule; 3% has 23.53×10^9 CFU/gram. Its chemical compounds affect to the longer fermentation time in reaching proper pH; (pH 4.72 of 3% prebiotic). Panelists prefer carrageenan capsule than alginate (texture and color). Alginate capsule illustrates similar elasticity to carrageenan, yellow and cloudy appearance, while carrageenan capsule has creamy and transparent performance. Overall, even though carrageenan confirms lower total LAB, its combination with high percentage of tofu waste flour (3%) could produce a good quality of probiotic capsule.

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