International Journal of Engineering & Technology, 7 (4) (2018) 5242-5244

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET
doi: 10.14419/ijet.v7i4.24255
Research paper

Extrusion encapsulation of *Lactobacillus bulgaricus* coated by carrageenan – alginate with additional tofu waste flour prebiotic

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Abstract

Survival phase of probiotic *Lactobacillus bulgaricus*, depends on its living conditions, which are processing stages, storage condition and acidity level in digestive tract. The severity states of environment and improper treatment may cause a reduction of probiotic viability in food products. Extrusion method was used to encapsulate the bacteria by using combination of encapsulating agents and a certain percentage of prebiotic in order to enhance the viability of probiotic in acid and cold condition. This study used two factors, which were capsule agents (alginate and carrageenan), and percentages of tofu waste flour (1.5%, 2%, 2.5%, and 3%). The results showed that carrageenan was better in protecting probiotics at pH 2 with a total LAB of 4.23 LogCFU/gram, 42.5% viability of LAB and 41% efficiency encapsulation compared to alginate 3.92 LogCFU/gram, 38.2% viability and 37.9% efficiency encapsulation. The addition of tofu waste flour causes an increase in the growth of probiotic bacteria up to 10.5 LogCFU/gram. In the simulation conditions of gastric acid (pH 2), the combination of the use of carrageenan with the optimal percentage of tofu waste flour (3%) results in the most effective encapsulation conditions for probiotic viability and the highest yield of encapsulation.

Keywords: *Alginate, Carrageenan, Tofu waste flour, Extrusion, Encapsulation, Probiotic*.

1. Introduction

Lactic Acid Bacteria (LAB) is a group of probiotic bacteria commonly used in probiotic food products. This type of bacteria has functional properties and plays an important role for human health, especially in the intestine tract. One type of these bacteria is *Lactobacillus bulgaricus* [1]. It requires a certain environmental conditions for optimum growth, and has low tolerance to extreme temperature and acid conditions. Viability or survival of probiotic bacteria is very important to be noted because its functional properties in health will be achieved if these bacteria are alive in the digestive tract.

Survival condition of probiotic bacteria in food products is difficult to achieve due to a series of high or low temperature treatments in production line and a very high acid level in stomach. Therefore, the formation of a protective capsule through extrusion encapsulation method is an effective way to protect bacterial cells against inappropriate conditions [2]. The capsule layer can maintain the life of probiotic bacteria in human body [3, 4].

Extrusion is the simplest and the most common technique of probiotic encapsulation. It forms hydrocolloid capsule with moderately small size (100 µm – 3 mm) that gives unique sensation to the liquid and semi liquid food product [4].

The quality and effectiveness of encapsulation are also determined by type of capsule agents. Several types of encapsulating materials are inulin, starch, chitosan, alginate, and carrageenan [4]. The ability of these ingredients to form gels is a defining factor of capsule's capability in protecting bacterial cells. Alginates and carrageenan are polysaccharides from algae or seaweed which have gel-forming properties, but are composed of different molecular components. Alginate structure is heterogeneous consisting of mannuronic and guluronic acids, while carrageenan is composed of galactose [5]. This difference affects the condition of the capsule formed.

In addition to protection against bacterial cells, the improvement of probiotic viability can also be done by adding nutritional sources for bacterial growth, namely prebiotic. Tofu waste flour is byproduct from tofu process that is considered as prebiotic due to its oligosaccharide content [6]. The use of tofu waste as a prebiotic is also a new innovation in handling domestic industrial waste. The purpose of this study was to study and determine the effect of the type of encapsulating materials and concentration of tofu waste flour on probiotic viability in the capsules made by extrusion method.

2. Materials and Methods

2.1. Tools and Materials

In this study, the ingredients used were *Lactobacillus bulgaricus* strains, carrageenan, alginate, MRS agar and broth (Merck), KCL and $CaCl_2$. The tools used were digital scales, laminar flow cabinets (Bassaire Model 04HB; Astecair 3000L), incubators (Eyela Personal Incubator SLI-170D; Eyela Soft Incubator SLI-450N), centrifuge (Hettich Zentrifugen EBA 20), waterbath (Memmert),

thermometer, and glassware. It also required syringes for extrusion of encapsulation process.

2.2. Research Design

This study used a Completely Randomized Design (CRD) consisting of two factors: the type of encapsulating material (alginate and carrageenan) and the percentage of tofu waste flour (1.5%, 2%, 2.5%, and 3%). The data obtained were processed statistically using ANOVA.

2.3 Production of Tofu Waste Flour

Wet tofu waste was pressed using a hydraulic press. Then the tofu was steamed for ± 15 minutes and dried by sun drying method, which is ± 8 hours per day. Drying process was done up to ± 3 days. Dried waste was crushed and sieved with 140 mesh size to produce flour.

2.4 Preparation of Probiotic Capsule

L. bulgaricus in pellet form was dissolved into distilled water to be used as a culture solution. The encapsulating solution made from carrageenan and alginate were homogenized and sterilized. This solution was mixed with tofu waste flour related to the concentration of treatment. Culture and encapsulating solutions were mixed with a ratio of 1: 4. This mixture was extruded or dripped using syringe into KCL and $CaCl_2$ as hardener solutions. Lastly, probiotic capsule was stored in refrigerator [7, 8].

3. Results and discussion

3.1. Probiotic viability in pH 2

The survival level of *Lactobacillus bulgaricus* probiotic in capsule can be measured by altering its living condition with high acidity, in order to make an intestine simulation at pH 2.

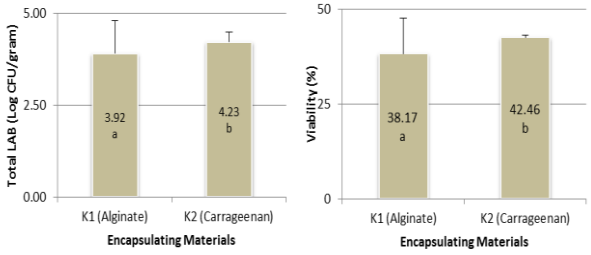

Fig. 1: Intestine simulation (pH 2): (a) Effect of encapsulating materials on total lactic acid bacteria; (b) Viability of probiotic bacteria

Based on Figure 1, it can be seen that at low pH, the number of probiotic in alginate capsule (3.92 LogCFU/gram) is less than the bacteria in carrageenan capsule (4.23 LogCFU/gram). It is approved by the viability data of *Lactobacillus bulgaricus* in carrageenan capsule reaches about 42.46%, which is higher 4.29% than alginate (38.17%) in intestine simulation. This is presumably due to the nature of porosity and the level of alginate resistance in acidic condition. Alginate capsules have a thin and semipermeable membrane layer, which makes it possible for acid solution to decompose the capsule wall or capsule layer. It affects to the reduction of its protection properties for encapsulated bacteria [9]. This condition may cause the release of bacterial cells into high acidic media, while some acid also diffuses into the inner capsule. The exposure to a very acid circumstance causes the death of bacteria, so that total of lactic acid bacteria (LAB) becomes low (3.92 LogCFU/gram).

Better protection capability is owned by carrageenan capsules. This can be seen from the results showing that total LAB in the carrageenan capsule is 4.23 LogCFU/gram. This is consistent with the characteristics of carrageenan capsules which have a higher viscosity and thicker gel in forming capsules. A thick gel layer can protect probiotic bacterial cells from extreme acid conditions.

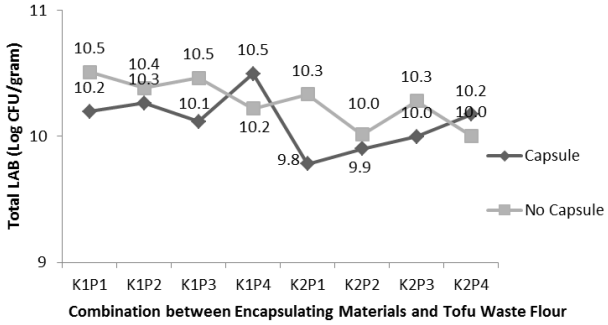

Fig. 2: Comparison Encapsulated LAB and Unencapsulated LAB

Figure 2 shows a comparison between total LAB in encapsulated and unencapsulated samples. All of those samples were added by tofu waste flour as prebiotic. Overall, the data shows that the capsule samples without encapsulation shows that the total LAB is higher than the encapsulated samples. This is presumably because samples without capsule allow bacteria move freely and use optimally the prebiotic as a source of energy to grow, while capsule formation might inhibit the movement of bacteria in the media. In addition, the usage of prebiotic (tofu waste flour) can improve survival and the growth of probiotic since it contains 640.25 µg/g raffinose and 169.87 µg/g stacchiose [10]. From the data, both of encapsulated samples K_1P_4 and K_2P_4, figures out that the highest percentage of prebiotics can encourage survival and viability of bacteria inside alginate and carrageenan capsules (10.5 Log/CFU/gram) with encapsulation technique.

3.2. Effectiveness and yield of encapsulation

Regarding the effectiveness of extrusion encapsulation, it can be examined by comparing number of survival *Lactobacillus bulgaricus* inside capsule with total amount of its initial culture in normal pH. Based on Table 1, the data shows that carrageenan as capsule agent is more effective to protect the probiotic about 41% while alginate has lower efficiency about 38%.

Table 1: Efficiency encapsulation of probiotic capsule using extrusion method in pH 2

Parameters	Encapsulation Efficiency (%)	Encapsulation Yield (%)
Alginate:		
Prebiotic 1.5%	35.2	34.6
Prebiotic 2.0%	36.5	36.4
Prebiotic 2.5%	41.8	41.3
Prebiotic 3.0%	38.0	38.5
Carrageenan:		
Prebiotic 1.5%	39.8	39.8
Prebiotic 2.0%	40.8	42.1
Prebiotic 2.5%	41.3	41.5
Prebiotic 3.0%	41.9	43.3

Yield of encapsulation is the result of numbers of living cells released by capsule divided by numbers of free living cells in its culture [11]. From this calculation, it confirms that carrageenan produces higher percentage of protected living cells compare to alginate which are 41.7% and 37.7% respectively. It may occur due to the thickness of gel formed by carrageenan. It has 700-1000 kDa molecular weight, so that it is more capable in absorbing water and forming gel [12]. This condition relates to the thickness of gel which is more effective in protecting LAB cells inside capsule. Moreover, Table 1 also presents that efficiency and yield of encapsulation increase along with high concentration of prebiotic tofu waste flour used, especially 3%, as it is shown in Figure 2.

3.3. Probiotic viability in low temperature

Another adverse environmental condition of probiotic growth is low temperature. In this study, the effectiveness of carrageenan probiotic capsules produced by extrusion encapsulation was also

evaluated in cold and freeze condition. The results could be used as a consideration in application of probiotic capsule in cold or frozen food product.

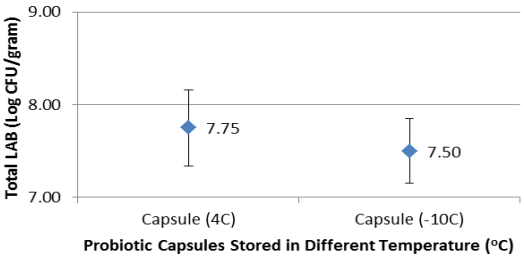

Fig. 3: Effect of low temperature on total lactic acid bacteria

Figure 3 figures out that in both low temperatures, whether cold (4ºC) or freeze (-10ºC) condition, *Lactobacillus bulgaricus* can survive with relatively small differences of numbers, which is 0.25 LogCFU/gram reduction from cold to frozen storage. Comparing with pH 2 (Fig 1), carrageenan probiotic capsule stored in low temperature, both cold and freeze conditions, still could preserve living cells much higher about 3.27 – 3.52 LogCFU/gram than in low pH.

4. Conclusion

The type of encapsulating material used has an effect on the viability of probiotic bacteria, namely carrageenan is able to provide a better protection for *Lactobacillus bulgaricus* at pH 2 compared to alginate, confirmed by its higher total LAB, viability, effectiveness and yield of encapsulation. A proper use of encapsulating material and addition of tofu waste flour can improve the efficiency of probiotic encapsulation and the quality of capsules. Combination between carrageenan and 3% of tofu waste flour offer the optimum survival condition for probiotic and preserve high number of living cells.

Acknowledgement

This research is supported financially by KEMENRISTEKDIKTI, Indonesia, from 2017 to 2018.

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