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Microencapsulation of Lactobacillus acidophilus with Freeze Drying Method and Application to Synbiotic Beverage of Banana Corm Stone

Sumanti Debby Moody [#1], In In Hanidah[#], Indira Lanti Kayaputri[#], Tita Rialita[#], Een Sukarminah[#]
Raisha Audina Prameswari Zakaria[#]

[#] *Department of Food Industrial Technology, Faculty of Agricultural Industrial Technology, Universitas Padjadjaran, West Java, Indonesia*
Email : debby@unpad.ac.id

***Abstract*— Functional foods preferred currently should contain both prebiotics like inulin and frukto-0ligosaccharide and probiotics as *Lactobacillus acidophilus* required for good health. Functional food in the form of the synbiotic beverage can be made using banana corm stone which acts as prebiotic and has phenolic, tannin, and antioxidant with the addition of *Lactobacillus acidophilus* microcapsules roling as probiotic. Probiotics are bacteria can contribute to improving health, especially in the digestive system, one of the probiotic bacteria are beneficial to health is the *Lactobacillus acidophilus* . The number of probiotic bacteria that must be consumed to get the benefits that ≥10^7 CFU/g, so that the viability needs to be maintained. This study aimed to determine the cell viability of L.acidophilus microcapsules and define the effect of *Lactobacillus. acidophilus* microcapsules concentration on microbiological quality and sensory of synbiotic beverage from banana corm stone. The study was conducted using randomized block design (RBD) with five concentrations treatment namely 2%, 4%, 6%, 8%, and 10% (w/v) repeated 3 times. The results showed that the *Lactobacillus. acidophilus* microcapsules bacteria produced had a white color and fine crystalline powder form with a size of 2.2 µm, the yield of 18.62% and cell viability of probiotic bacteria L. acidophilus 93.47%. The addition of *Lactobacillus. acidophilus* microcapsules as much as 8% produced the best synbiotic beverage of banana corm stone with following characteristics: total probiotic bacteria 10.4 Log CFU/ml, pH 7.33, panellist preference values of color, aroma, taste and viscosity of synbiotic beverage of banana corm stone were like slightly to like.**

***Keywords*— banana corm stone; functional food*; Lactobacillus acidophilus* microencapsules; prebiotic; synbiotic beverage.**

I. Introduction

Functional food is fresh or processed food that provides health benefits and can prevent an illness, besides its basic function as a provider of functional food must have three basic functions, namely (1) providing sensory (attractive colors and appearance, good taste), (2) nutritional (high nutritional value), and (3) physiological (providing beneficial physiological influences for the body) [1]. One functional food product is a probiotic food product that is good for increasing gastrointestinal endurance. Probiotics are defined as living microbial cells and if consumed in sufficient quantities will provide health benefits [2]. Functional food that is growing and in demand by the public today is food and beverages containing probitic and prebiotic as the symbiotic beverage of Banana corm stone.

Banana corm flour contains carbohydrates as much as 66.2% which comprises oligosaccharides such as inulin, unused FOS and GOS [3].That oligosaccharide compound is a prebiotic and produces a synbiotic product such as synbiotic drink if added with probiotic bacteria.

Probiotic bacteria are live bacteria which are consumed in sufficient quantities will provide a beneficial effect on the body to create a balance of intestinal microflora [2]. Probiotic bacteria are generally from the class of lactic acid bacteria (LAB), particularly the genus Lactobacillus and Bifidobacterium. One of the probiotic bacteria of the genus Lactobacillus which has been used extensively in the food industry is *Lactobacillus acidophilus*. The probiotic bacteria must also be able to survive during processing and storage. Storage culture in fresh condition can not be done for a long period. Thus a preservation method that can maintain the viability of probiotic bacteria is required. Cell viability of bacteria in probiotic products should range 6-8 Log CFU/ml [4]. The viability of probiotic bacteria will decrease during storage and while in the digestive tract, it is caused by unfavourable environmental factors to their survival, including the existence of a low pH and the presence of bile salts in the digestive system [5] [. One way to improve the resilience and viability of probiotic bacteria is by encapsulation.

Encapsulation is a coating process of a core material, in this case is probiotic bacteria, using particular encapsulation material which is useful for maintaining the viability and protect the probiotic bacteria from damage caused by unfavourable environmental conditions, such as heat, chemical substances, gastric acid, and bile salts [6].
Encapsulation methods used in this research is freeze drying method. Encapsulation of *L. acidophilus* bacteria done is freeze drying method which is made in suspension form and added coating material of 10% skim milk and 20% maltodextrin until it shapes freeze dried culture [7]. The result of the microencapsulation process is called microcapsules. The microcapsules have a size of more than one µm, typically between 1-2000 µm with spherical shape (ball shape) or irregular [8].

The microcapsules of probiotic bacteria will be symbiotic with prebiotic material to produce a product called synbiotic. One foodstuff that can be used as a prebiotic material in the synbiotic drink is banana corm stone. Synbiotic beverage characteristics are determined by the number of probiotic bacteria, and organoleptic properties of the product, such as taste, aroma, color, and texture.

The purposes of this study were [1] to determine cell viability of *L. acidophilus* microcapsules and [2] to define the effect of concentration of bacteria *L. acidophilus* microcapsules on microbiological and sensory quality of synbiotic drink of banana corm stone.

II. Material and Method

A. Materials

Materials used in this study were banana corm stone with brownish-white color, rough and hard texture, obtained from a banana stone grower in Cikuda-Sumedang, microcapsules of L. acidophilus bacteria, skim milk, distilled water, MRS agar (Oxoid) medium, physiological NaCl 0.85 %, alcohol 70% and glacial acetic acid.

B. Research Methods

1). Making of Probiotic Bacteria Lactobacillus acidophilus Microcapsules [11]*:*

The making of probiotic bacteria *L. acidophilus* microcapsules consisted of two phases, namely the manufacture of suspension, as follows: Inoculation of one ose bacteria *L. acidophilus* on aslant MRS agar, then incubated for 24 hours at 37°C. Furthermore shed *L. acidophilus* bacteria culture with sterile distilled water. Turbidity was checked according to Mc Farland 3 at λ 600 nm and absorbance ± 0.616 which is equivalent to the number of bacteria colony 9.0 x 10^8 CFU/ml using a spectrophotometer. Then 4 ml culture of *L. acidophilus* bacteria was mixed in 36 ml of 10% skim milk media, further incubation for 2 hours at 37°C and the resulting *L. acidophilus* bacteria suspension. *L. acidophilus* bacterial suspension that had formed was then added as much as 20% of maltodextrin, then frozen in a freezer with a temperature of -50^{o}C for 24 hours. Encapsulation suspension using Alpha 1-4 LDplus freeze dryer at a temperature of -50°C and a pressure of 0.040 mbar for 24 hours until form a dried culture of *L. acidophilus* bacteria (microcapsules).

2). Testing of Cell Viability of Probiotic Bacteria L. acidophilus [12]*:*

Cell viability of *L. acidophilus* bacteria was determined based on the ratio of the number of bacteria per gram after and before the encapsulation process and stated in percent. As much 1 g of *L. acidophilus* bacterial cell suspension was taken before encapsulated and 1 g of the dried culture of *L. acidophilus* bacteria (microcapsules). Each was put into a test tube containing 9 ml of sterile physiological NaCl (dilution 10^{-1}), then made serial dilutions up to 10^{-10}. Then took 1 ml at three final dilutions (dilution 10^{-8}, 10^{-9}, 10^{-10}) and inserted into a sterile Petri dish and added sterile MRS agar medium, then homogenized using a vortex for 5 minutes. Incubation was done at 37°C for 48 hours. Then calculated colonies grown using TPC method. The formula of viability calculation is as follows

$$\text{Viability}(\%) = \frac{\text{Total probiotic after encapsulation}}{\text{Total probiotic before encapsulation}} \text{x100} \quad (1)$$

Making of Synbiotic Drink of Banana Corm Stone:

The making of a synbiotic drink of banana corm stone consisted of two stages: making banana corm stone flour extract and synbiotic beverage. Banana corm stone flour was dissolved into water at a ratio of flour and water 1:20, then performed heating at a temperature of ± 60°C for 30 minutes. Then it was filtered to obtain Pisang Batu corm flour extract. As much as 100 grams of flour extract mixed with liquid sugar as much as 10% (V/V) and shaken until homogeneous. Then added freeze dried culture appropriate with concentration treatment (2%, 4%, 6%, 8% and 10%) into banana corm stone flour extract which had been added by sugar, then stirred, after that stored at a temperature 4°C for 24 hours. A total number of probiotic bacteria and sensory characteristics of synbiotic beverage was then analyzed.

3). Calculation of total Probiotic Bacteria Using Total Plate Count Method [9]:

As much as 1 ml of synbiotic beverage was added into a test tube containing 9 ml physiological NaCl solution 0.85% (dilution 10^{-1}). Dilution was done up to 10-8, then each of 1 ml at three final dilution was taken (10-6, 10-7 and 10-8), and put into a sterile Petri dish. Furthermore, pouring MRS agar medium + 0.5% glacial acetic acid (40-45°C) of 12-15 ml into the Petri dish and shaken to form a figure 8 to flatten the sample on the media. Incubation at 37°C for 48 hours was done. Calculating the number of colonies grown on agar as follows:

$$\text{Colony/ml} = \text{colony number x} \frac{1}{\text{dilution factor}} \quad (2)$$

4). Measurement of Acidity (pH) of Synbiotic Beverage of Banana Corm Stone:

pH meter was calibrated by dipping the electrode into buffer pH seven so that the figures showed the number of 7.0 (pH neutral). Then the electrode pH meter was dipped into the solution of synbiotic drink for some moment and the tool started for reading. Synbiotic beverage pH value appeared on the screen.

5). Organoleptic testing using hedonic test [8]*:*

Organoleptic testing of the synbiotic beverage of banana corm stone was done with 15 trained panelists. Each panelist was asked to give an assessment of color, aroma, taste and viscosity of synbiotic beverage for every sample code with a number that suitable to the following statements: (1) Dislike, (2) Dislike Slightly, (3) Like Slightly, (4) Like and (5) Like Very Much.

III. Results and Discussion

A. Microcapsules of Probiotic Bacteria Lactobacillus acidophilus

The microcapsules of *L. acidophilus* bacteria produced had a white color and fine crystalline powder form, as shown below:

Fig. 1 *L.acidophilus* Microcapsules

The microcapsule size of *L. acidophilus* bacteria which was done with a fluorescence microscope at 1000 times magnification was 2.2 µm. The yield of *L. acidophilus* bacteria microcapsules was 20.16%. Based on research [18], *L. acidophilus* bacteria microcapsules had a moisture content 3:06%, 18.62% yield, resistance to low pH (pH 2.0) where the number of cells decreased by 0.24 log CFU/g and resilience on bile salts (0.5%) in which the number of cells decreased 1.05 Log CFU/g.

B. Cell Viability of Probiotic Bacteria Lactobacillus acidophilus

Calculation shows that the freeze drying method could maintain the viability of probiotic bacteria *L. acidophilus* up to 93.47%. This means the use of coating material of skim milk 10% and 20% maltodextrin could keep bacterial cell viability. The use of a combination coating of protein and carbohydrate-based can produce optimum efficiency as a microcapsule wall [13].Selection of the coating material is very influential in maintaining the viability of bacteria during the microencapsulation process. According to [14], as a coating material skim milk roles in preventing cell damage by stabilizing the membrane and provide a protective layer on the cells because the lactose presence in skim milk reduces bacterial cell damage during the freeze drying process. This is due to lactose has a low molecular weight so as lactose can enter into bacterial cells and provides protection from two sides of the cell membrane during the freeze drying process [12]. Maltodextrin is included in prebiotic because it is classified as complex carbohydrates. Maltodextrin is often used because of its properties such higher solubility, capable of forming a film, has low hygroscopicity, capable as supporting dispersant, capable of inhibiting crystallization and has a strong bonding capacity [8].

C. Total Probiotic Bacteria Lactobacicillus acidophilus in Synbiotic Beverage

Data of various concentrations addition of *L. acidophilus* microcapsules into a synbiotic beverage of banana corm stone as shown in the chart below:

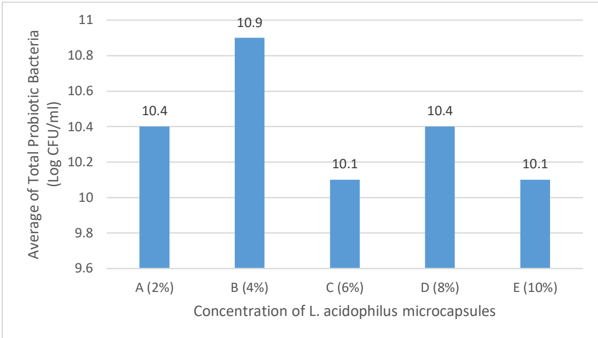

Fig. 2 Effect of concentration *L. acidophilus* microcapsules on total probiotic bacteria in the symbiotic beverage of banana corm stone

Fig. 2 showed the average of total probiotic bacteria *L. acidophilus* barely different between treatments. This was caused by the metabolic activity of bacteria cell was stopped for a while (dormant) due to the storage of product at low temperature and microencapsulation which was performed to *L. acidophilus* bacteria. The optimum temperature for *Lactobacillus* namely 37^{o}C – 42^o C.

According to [15], at low temperature, the enzyme activity of bacteria will reduce or even stop, so that it can lead to the slower growth of bacteria and the bacteria will not perform its metabolic activity. The manufacture of the synbiotic beverage of banana corm stone that was stored at 4°C generated an inhibition to *L. acidophilus* bacteria growth.

Besides, the content of prebiotic substrates contained in various treatments was the same, so there was no difference in nutrition received by *L. acidophilus*. This became the factor why different *L. acidophilus* concentration added into a synbiotic beverage of banana corm stone was not influential to total probiotic bacteria contained therein.

Meanwhile, the microencapsulation performed to *L. acidophilus* can be useful to protect cells from damage and enhance the resilience or viability of probiotic cells during the manufacturing process and storage products [16]. Freezing in bacteria can cause Aw to decrease because the water turns into ice crystals that bacteria cannot use. The condition of dehydration due to the formation of ice experienced by cells causes an increase in the concentration of intracellular solutes which means there is also a difference in osmosis pressure. The high concentration of intracellular solutes can change the intracellular enzyme system, so that bacterial metabolic activity is temporarily stopped. Total bacteria probiotic in the synbiotic beverage of banana corm stone produced ranged between 10.1 Log - 10.4 Log CFU/ml. Those values had met the standards that refer to SNI No. 2891-2009 in which the number of probiotic bacteria at least 7 Log CFU / ml indicates that the product has microbiological characteristics appropriate with applicable standards.

D. pH value

Statistical analysis showed that the difference in the concentration of *L. acidophilus* microcapsules provided a non significantly different effect on the pH value of synbiotic beverage of banana corm stone, as shown in the chart below:

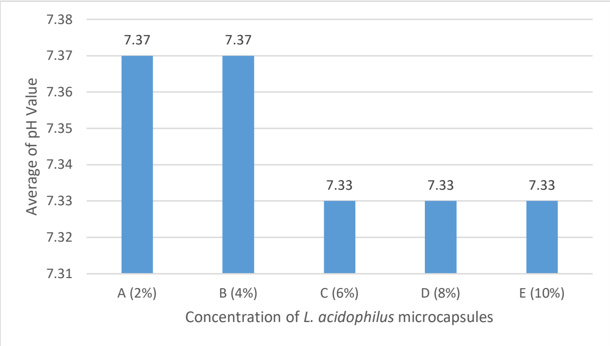

Fig.3. Effect of concentration microcapsules *L. acidophilus* on acidity degree (pH) value of beverage of banana corm stone

Fig. three showed there is no difference in the effect of pH value on various treatments, this result could be caused by *L. acidophilus* bacteria added did not accomplish fermentation process due to storage process of the product was carried out at low temperature (4^oC) and the bacteria added was in encapsulated condition. Synbiotic beverage produced was processed without doing the fermentation process previously. A synbiotic beverage that had been added with *L. acidophilus* bacteria was directly achieved cold storage at 4°C for 24 hours. This made the absence of activity of *L. acidophilus* that lower the pH value of the synbiotic beverage. This is proven by the pH value of the product after added with freeze dried *L. acidophilus* culture that was unchanged from pH of previous banana corm stone, namely 7.3. Sinbiotik drinks with the best formula are the percentage of Lactobacillus casei 2 % and the ratio of sweet potato juice: skim milk (3 : 1) Have the results of the pH value of 5,6, total dissolved solids 26 o Brix, a total of probiotic 1,56 x 10^9 CFU [1]. According to [17] a low temperature environment may slow the metabolism rate of the bacteria, so the bacteria will slowly produce metabolites, in this case is related to breaking of prebiotic dietary fibre into organic acids that can lower the pH.

Apart from the storage temperature, the addition of bacteria in the form of freeze dried culture was one of the factors in the absence of changes in pH in the product. The microencapsulation of probiotic bacteria added will make the product have a stable quality during storage for quite a long time, because bacterial cells are protected from unfavorable environments and cell metabolic activity can be slowed, so that the reaction changes in the product can be slowed and cell viability can be maintained [5].

E. Organoleptic Test

1). Colour and Aroma of Synbiotic Drink of Pisang Batu Corm Flour Extract:

Organoleptic test of color and aroma of the synbiotic beverage of banana corm stone flour extract gave no difference among treatments. Color is a characteristic that defines a product acceptance by consumers.

The addition of microcapsules of *L. acidophilus* slightly changed from the previous color of banana corm stone The flour has a brownish color because it contains tannins and phenols. These phenolic compounds are converted into brown melanin (melanoidin) compounds. Components that can cause enzymatic browning are oxygen, enzymes and substrate. The damaged tissue becomes darker in color after contact with air. The mechanism of enzymatic browning is caused by the breakdown of agricultural materials due to mechanical damage, causing the phenol compounds in the vacuole to come out and meet the polyphenol oxidase enzyme in the cytoplasm in the presence of oxygen and metal catalysts quinone compounds will form. Subsequent reactions occur spontaneously and are no longer dependent on enzymes or oxygen. The form of quinon undergoes hydrolysis into a hydroxy form and then hydroxy quinone polymerizes and becomes a brown polymer which eventually becomes brown melanin[15]. The color of the synbiotic beverage of banana corm stone was influenced by a mixture of white color of and brown color of banana corm stone producing a light brown color preferred by the panelists, with an average values of 3,5-3.6 meaning panelists like slightly to like on color of synbiotic beverage of banana corm stone produced.

The addition of microcapsules *L. acidophilus* had no effect on aroma of synbiotic d of banana corm stone. This beverage had a slightly astringent aroma arising from the content of tannin on banana corm stone. Tannins can cause astringent aroma and taste [18]. The average values of the aroma were from 3.2-3.4. The values showed that panelists like slightly the aroma of synbiotic beverage of banana corm stone.

2). Taste of Synbiotic beverage of Banana Corm Stone:

Statistical analysis showed that various concentration of *L. acidophilus* microcapsules provided a significantly different effect on the preference value of panelists on taste of synbiotic beverage of banana corm stone. The average values of preference for the taste of synbiotic beverage of banana corm stone c as shown in the chart below :

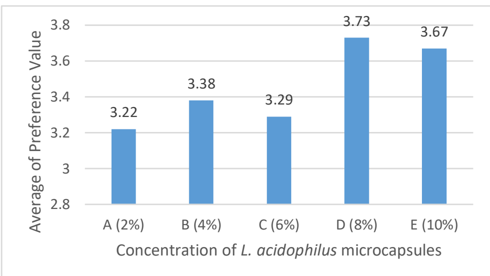

Fig. 4 Effect of concentration microcapsules *L. acidophilus* on the taste of beverage of banana corm stone

Fig. 4 showed preference value of panelists on taste for treatment A was not significantly different with treatment B and C, but significantly different in lower value to treatment D and E, whereas both treatment D and E were not significantly different. It showed that panellists preferred synbiotic beverage of banana corm stone with more of *L. acidophilus* microcapsules.

The addition of more microcapsules can cover the astringent taste caused by tannin content in the banana corm stone. Banana corm stone flour gained the highest phenols content (9.09 g/ 100 g) and tannin content 11.59 g/100 g) and if tannin content in foodstuffs may cause a rough and fairly bitter taste [17]. It's proven on hedonic test on the taste of synbiotic beverage of banana corm stone, panellists preferred the banana corm extract added by 8% and 10% of *L. acidophilus* microcapsules for skim milk taste more obvious. Average values from a panelist of the synbiotic beverage of banana corm stone ranged from 3.2-3.7. These values indicated that the average panelist like slightly to like the taste of synbiotic beverage of banana corm stone product.

3). Viscocity of Synbiotic Beverage of Banana Corm Stone:

Statistical analysis showed that the difference in the concentration of *L. acidophilus* microcapsules provided a significantly different effect on the value of panelist preference on the viscosity of synbiotic beverage of Banana corm stone, as shown in the chart below :

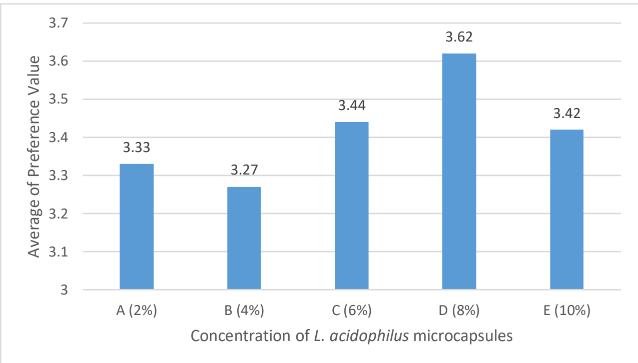

Fig. 5. Effect of concentration microcapsules L. acidophilus on viscoseity of the synbiotic beverage of banana corm stone

Fig. 5 showed the value of panellists preference on the viscosity of treatment B was not significantly different with treatment A, C, and E, but significantly differed in lower value compared to treatment D. Synbiotic beverage of banana corm stone had a viscosity of slightly viscous and increased along with more addition of *L. acidophilus* microcapsules. Synbiotic beverage in treatment A, B, and C were not too thick, treatment E had a very thick viscocity while treatment D had sufficient viscoseity, so it was more preferred by the panellists.

Synbiotic beverage of banana corm stone had a slightly thick viscosity due to the carbohydrate content of banana corm flour itself, which made the viscosity of synbiotic beverage of banana corm stone increased, but remained dissolved in the beverage. This was caused by the use of coating material on freeze dried culture. Skim milk has properties of free flowing and free of clumps, so that it will easily unite when mixed with food ingredients . Meanwhile maltodextrin is a thickening agent (increase the viscosity) and easily dissolves in cold water [11].

Synbiotic beverage of banana corm stone with various concentrations of *L. acidophilus* microcapsules was moderately preferred by the panelists. Fifteen panelists gave the average value of preference 3.27- 3.62. These values indicated that the average panellists like slightly to like on the viscosity of symbiotic beverage of banana corm stone product.

IV. Conclusions

Microcapsules of *L. acidophilus* bacteria resulted had a white color and fine crystalline powder form with a size of 2.2 μm, the yield of 18.62% and cell viability of probiotic bacteria *L. acidophilus* of 93.47%. Concentration addition treatment of *L. Acidophilus* microcapsules as much as 8% produced the best synbiotic beverage of banana corm stone with the following characteristics: total probiotic bacteria 10.4 Log CFU/ml, pH 7.33, value of panelists preference on taste 3.8 (like slightly-like), viscosity 3.6 (like slightly-like), color of 3.5 (like slightly-like), and aroma 3.3 (like slightly-like).

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