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Microbiological and physics testing encapsulation of *Lactobacillus plantarum* with kepok banana flour

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ABSTRACT

Kepok banana flour (KBF) contains fructo-oligosaccharides (FOS) which can stimulate the growth of *Lactobacillus plantarum* bacteria in the digestive tract. The encapsulation technique is done to maintain the viability of probiotic bacteria from environmental conditions by packaging using coating materials like skim milk and maltodextrin. This research aims to determine the effect of adding KBF with different concentrations on *Lactobacillus plantarum* encapsulates. The research design used was a completely randomized design (CRD) with 5 treatments and 4 replications. The treatment given was a concentration of KBF of 0, 2, 4, 6, and 8%. The results obtained were a lactic acid bacteria (LAB) viability value of 8.72 Log CFU/g-9.16 Log CFU/g, a pH value of 6.55-6.43, yield value of 23.39%-23.43%, color of 3.36-3.12, aroma of 2.80-2.88, texture of 3.08-2.92, and overall, 3.12-3.36. The addition of 8% KBF can increase LAB viability, color, and texture, but reduces the pH value, yield, and aroma of *Lactobacillus plantarum* encapsulates and produces a well-encapsulated microcapsule morphology.

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1. INTRODUCTION

Bananas are easy to grow in countries located on the equator such as Indonesia, which has an elevated temperature of 28-35 °C each year [1]. Kepok banana is one type variant of banana cultivar which are widely consumed in Indonesia [2]. Kepok banana is a climatic fruit that is easily damaged or has a short shelf life. Processing kepok banana into flour is one way to prevent banana shelf life [3]. Banana flour can be used as an alternative to wheat flour which is high-nutrition and gluten-free [4].

Kepok banana is a type of plantain banana that contains high starch content. The starch contained in plantain bananas includes resistant starch [5]. The resistant starch content of kepok banana is 27.7%, so it can be classified as one of the fruits that is high in fiber [6]. Within 100 g of plantain banana, there are 60.7 g of carbohydrates, 536 kcal of energy, 3.57 g of protein, and 3.6 g of fiber [7]. In addition, plantain bananas are also high in fructo-oligosaccharide (FOS), which is beneficial for the digestive system [8]. Processing kepok banana into flour can be utilized as a source of prebiotics for probiotic bacteria because it has a high content of resistant starch and FOS [9].

Lactobacillus plantarum is a probiotic bacteria that can grow optimally at a temperature of 30-37 °C and has the potential as a probiotic that is more resistant to acid than other types of bacteria [10]. Probiotics in food products must be protected from various things that can affect processing, namely by microencapsulation. This encapsulation is done by wrapping probiotic bacteria and using certain encapsulant materials that maintain the viability of probiotic bacteria from environmental conditions [11].

One method that can be used in the encapsulation process is freeze drying. The damage to probiotic bacteria cells by freeze drying can be minimized by the addition of coating materials such as skim milk and maltodextrin [12]. The coating material can produce a fairly high viability of *Lactobacillus* bacteria, besides the freeze-drying method that is maintained at the appropriate temperature and humidity can maintain the viability of *Lactobacillus* bacteria [13]. The growth of *Lactobacillus* bacteria is influenced by low pH (6.2 to 5.0). Low pH can cause *Lactobacillus* bacteria growth to be more optimal [14]. The process of making banana flour utilizes citric acid to produce banana flour that tends to be acidic [15]. Citric acid soaking in flour making can result in a low flour pH [16]. Therefore, this study was conducted to make kepok banana flour (KBF) functional by adding probiotic bacteria which is *Lactobacillus plantarum*.

2. RESEARCH METHOD

2.1. Research design

Samples were prepared in a completely randomized design which was carried out in stages including: i) making KBF, ii) making encapsulation of *Lactobacillus plantarum*, and iii) comparative analysis of microbiology, resistant starch, and physics parameters encapsulation of *Lactobacillus plantarum*. The treatment given to *Lactobacillus plantarum* encapsulates is the addition of KBF concentrations of 0, 2, 4, 6, and 8%.

The research was carried out at the Food Chemistry and Nutrition Laboratory and the Food Engineering and Agricultural Products, Faculty of Animal and Agriculture, Diponegoro University in August-October 2023. The main ingredients used in the encapsulation of *Lactobacillus plantarum* are KBF which comes from raw kepok bananas obtained homogeneously from one of the banana plantations in Semarang, pure isolate of *Lactobacillus plantarum* strain American type culture collection (ATCC) 8014, skim milk, maltodextrin, enzyme, de man rogosa sharpe broth (MRSB) and de man rogosa sharpe agar (MRSA) media, pH 4 and 7 buffers, 0.85% physiological NaCl, and citric acid. Meanwhile, the tools used in this research were a freezer dryer, autoclave, freezer, oven, cabinet dryer, spectrophotometry, pH meter, search engine marketing (SEM) tool, incubator, laminar air flow, colony counter, micropipette tip, micropipette, and petri dishes.

2.2. Research procedure

2.2.1. Making kepok banana flour

The production of KBF has been adopted [15] with modifications. The steps for making KBF start with sorting and washing of kepok banana. The kepok banana is then peeled and sliced to a of ±2 mm. The kepok banana was soaked with 1500 ml water and added citric acid according to the treatment (w/v) 0.8% for 3 minutes. The banana pieces are dried using a cabinet dryer at 60 °C for 6 hours. The dried banana is crushed and sived thorough 80 mesh.

2.2.2. Making encapsulation of *Lactobacillus plantarum*

Preparation of *Lactobacillus plantarum* encapsulate has been adopted from [17] which was carried out by incubation of one ose of *Lactobacillus plantarum* bacteria in MRSB, then incubation for 48 hours at a temperature of 37 °C. Next, 5 ml of the harvested *Lactobacillus plantarum* bacterial culture was added to 0.85% physiological NaCl. Then 4 ml of *Lactobacillus plantarum* culture was mixed in 36 ml of 10% skim milk medium and KBF was added with concentrations of 0, 2, 4, 6, and 8% and incubated for 2 hours at 37 °C. The *Lactobacillus plantarum* suspension that has been formed is added with 20% maltodextrin and frozen in a freezer at -24 °C for 24 hours, then the frozen material is placed in a freeze dryer at a temperature of -50 °C and a pressure of 0.040 mbar for 48 hours until a dry culture is formed *Lactobacillus plantarum* bacteria (microcapsules).

2.2.3. Parameters testing

Parameter assessment was carried out on *Lactobacillus plantarum* encapsulation with the addition of 0% KBF as a control, KBF 2%, KBF 4%, KBF 4%, KBF 6%, and KBF 8%. Lactic acid bacteria (LAB) viability testing was carried out on [17] using the pour plate method with 10-7 dilution. Testing of pH values was carried out at [18] using a pH meter. Yield testing was carried out in [19] with weight comparisons. Resistant starch testing is carried out on [20] using enzymes. Microcapsule morphology testing with a modification of the method [21] with 2000x magnification. Organoleptic testing using the scoring method.

2.3. Data analysis

The data were analyzed using an application named SPSS for Windows 26.0. The data obtained from testing LAB viability, resistant starch, pH, and yield were analyzed using the analysis of variance (ANOVA) parametric test with a significance level ($p<0.05$) and when there is a significant difference in treatment will be continued by Duncan's multiple range test (DMRT). The organoleptic testing using Kruskal Wallis analysis. If the significant level has a real effect ($p<0.05$), then proceed with the Mann-Whitney test. While resistant starch was statistically analyzed using the Excel application and microcapsule morphology was descriptive.

3. RESULTS AND DISCUSSION

3.1. Encapsulation of *Lactobacillus plantarum* with kepok banana flour microbiology characteristics

The microbiology characteristic of *Lactobacillus plantarum* with KBF is the LAB viability as described in Figures 1 and 2. The viability of LAB in *Lactobacillus plantarum* before encapsulation can be seen in Figure 1. Meanwhile, the viability of LAB in *Lactobacillus plantarum*, after encapsulation can be seen in Figure 2.

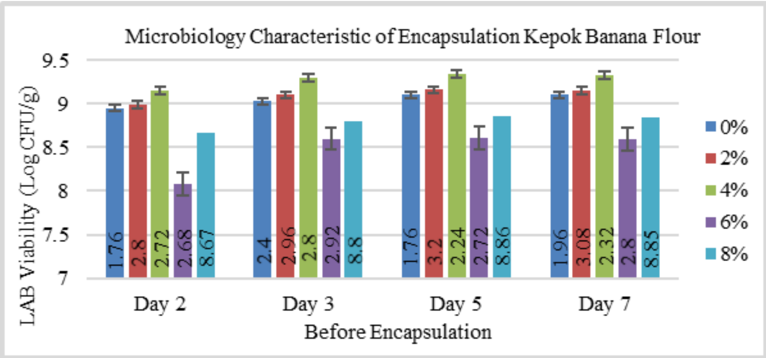


Figure 1. Microbiology characteristic before encapsulation of KBF

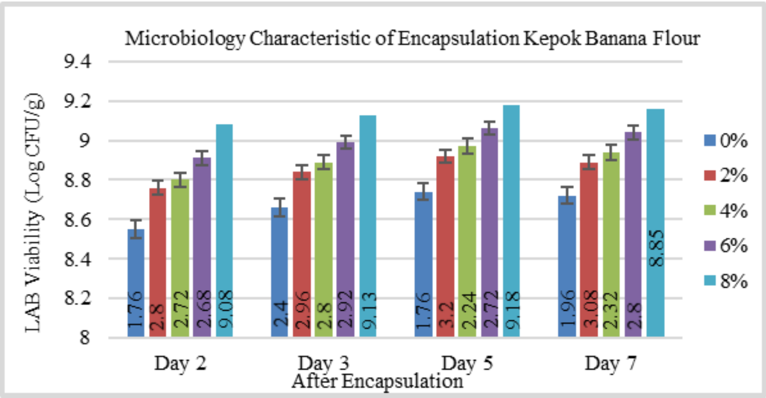


Figure 2. Microbiology characteristic after encapsulation of KBF

LAB viability after encapsulation of *Lactobacillus plantarum* at a concentration of 0-4% decreased in the number of colonies, whereas at concentrations of 6 and 8% KBF *Lactobacillus plantarum* experienced an increase in the number of colonies. The higher addition of KBF indicates that the amount of fiber content contained in it is also high, so when KBF is mixed with the coating material it can create a gel in the sample solution and can be more optimally utilized as a growth medium by bacteria probiotics [22].

The viability of probiotic bacteria can be measured in Log CFU/g, where a high Log CFU/g value indicates better viability. The minimum number of probiotic bacteria used for functional food is no less than 6 Log CFU/g [23]. Based on the data obtained, the viability of the probiotic before and after encapsulation shows that the number of cells is above 6 Log CFU/g, which means it meets the requirements of a probiotic.

The exact number of live cells that trigger probiotic effects ranges from 6 to 9 Log CFU/g [24]. Thus, KBF can encapsulate *Lactobacillus plantarum* optimally.

The longer the fermentation time, the total number of LAB increases. LAB can reproduce by utilizing the nutrients in the *Lactobacillus plantarum* encapsulate medium during the fermentation process to hydrolyze sugar into simpler components, namely CO2, H2O, and lactic acid, so that the number of bacterial cells increases [25]. KBF contains resistant starch which has the potential to act as a prebiotic which can protect probiotics. The probiotic bacteria *Lactobacillus plantarum* can survive in the large intestine when contained in resistant starch granules because resistant starch as a potential prebiotic cannot be digested in the small intestine [26].

3.2. Encapsulation of *Lactobacillus plantarum* with kepok banana flour resistant starch and physics characteristics

Resistant starch and physics characteristics of *Lactobacillus plantarum* with KBF were pH value and yield as described in Figure 3. Figure 3 explains the results of the resistant starch, pH value, and yield tests. Resistant starch only uses 0 and 8% samples to determine the effectiveness of the highest KBF addition of 8%. Meanwhile, the pH value and yield tests use all samples.

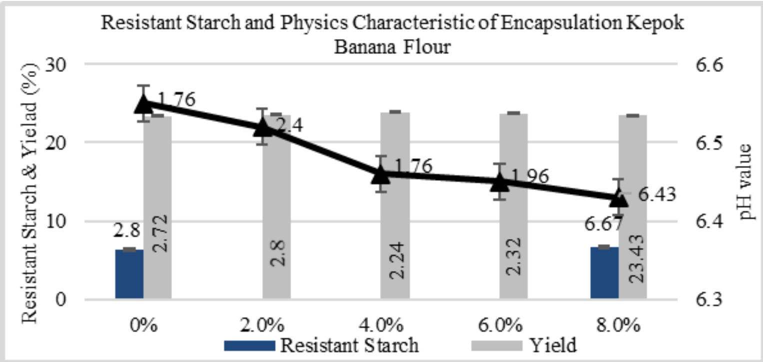


Figure 3. Resistant starch and physics characteristic of encapsulation KBF

Based on the data in Figure 3, the addition of KBF at concentrations of 0 and 8% affects the yield of resistant starch. The freeze-drying method is able to increase the percentage of resistant starch content in encapsulated KBF [27]. The treatment with the addition of 8% KBF produced higher resistant starch than without the addition of KBF. This is influenced by the presence of *Lactobacillus plantarum* which plays a role in increasing the levels of resistant starch in banana flour. The fermentation of LAB by *Lactobacillus plantarum* is able to produce amylase and pululanase enzymes which can convert some of the starch in KBF into resistant starch [28].

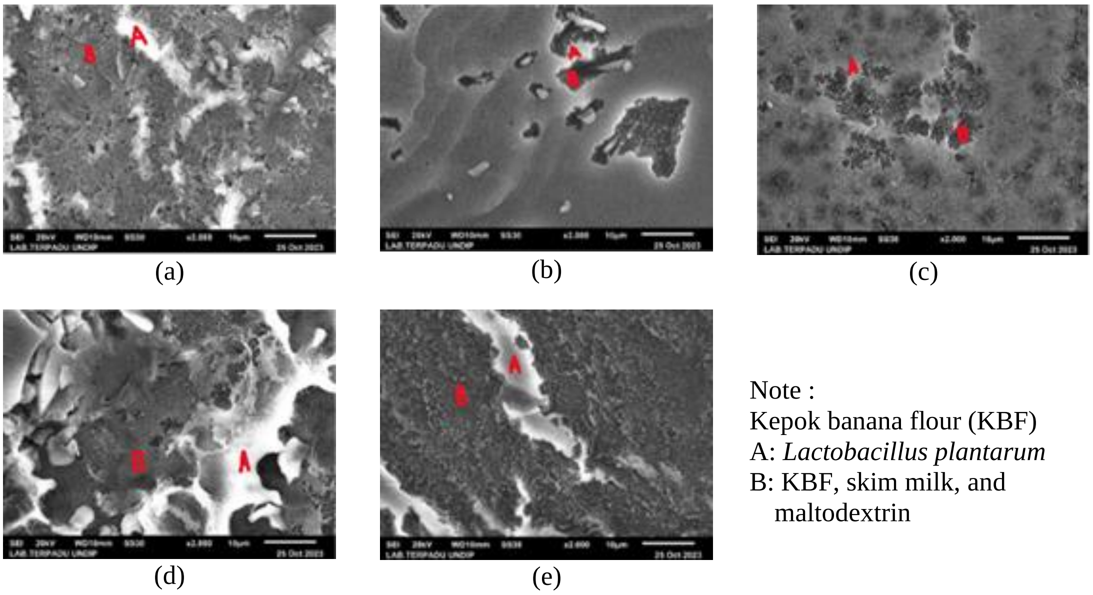
The maltodextrin coating material has an influence on the yield value because maltodextrin has natural characteristics that produce sponge microcapsules with many remaining water molecules [29]. The freeze-drying method used resulted in very few encapsulate samples coming out of the container and many remaining in the container and the addition of maltodextrin which could increase the total solids of the material and increase the volume, so that the encapsulate yield value was high [30]. The coating material maltodextrin and skim milk with the same concentration in each treatment resulted in yield values that were not much different.

Lactobacillus plantarum can thrive in the pH range of 4 to 6 [31]. The data obtained was that the pH of the *Lactobacillus plantarum* encapsulate was in the range of pH 6. The 8% KBF treatment showed pH results that tended to be lower compared to other treatments, this influenced increased bacterial growth. Encapsulation with a freeze dryer can increase the tolerance of *Lactobacillus plantarum* to pH in acidic conditions [32]. The greater the number of probiotic bacterial cells, the more acidic the resulting encapsulate acidity value will be increased [33].

The pH value of *Lactobacillus plantarum* encapsulates which becomes more acidic or decreases with the higher concentration of KBF indicates that the nutritional needs of probiotic bacteria have been met by the addition of KBF so that the number of bacterial cells increases [34]. The pH value which tends to be acidic can help prevent the development of unwanted microorganisms so that it can increase the shelf life of *Lactobacillus plantarum* encapsulates [35].

3.3. Microcapsule morphology

Figure 4 is presented as follows: Figure 4(a) the treatment with the addition of 0% KBF shows quite a lot of *Lactobacillus plantarum*. Figure 4(b) the treatment with the addition of 2% KBF shows *Lactobacillus plantarum* growing slightly. Figure 4(c) treatment with the addition of 4% KBF shows that *Lactobacillus plantarum* is seen growing. Figure 4(d) treatment with the addition of 6% KBF shows *Lactobacillus plantarum* growing a lot. Figure 4(e) treatment with the addition of 8% KBF shows that *Lactobacillus plantarum* grows more optimally than the previous treatment, which is indicated by a white image from the results of SEM observations.



Note :
Kepok banana flour (KBF)
A: *Lactobacillus plantarum*
B: KBF, skim milk, and maltodextrin

Figure 4. SEM of encapsulated *Lactobacillus plantarum* KBF concentration with the same magnification of 2000x where (a) 0%, (b) 2%, (c) 4%, (d) 6%, and (e) 8%

The morphology of *Lactobacillus plantarum* microcapsules shows that the increasing use of KBF is able to function as a carrier medium for *Lactobacillus plantarum*. It can be seen at the same magnification (2000x) that there are more *Lactobacillus plantarum* attached to the surface of the KBF in the 8% KBF formulation. *Lactobacillus plantarum* is shown in the SEM results to be bright in color, which shows that *Lactobacillus plantarum* is under the KBF. KBF and other components in the form of skim, milk, and maltodextrin are darker in color, which means they are on the surface of *Lactobacillus plantarum*. According to Liu *et al.* [36], observing the morphology of *Lactobacillus plantarum* using SEM can be shown by the results of *Lactobacillus plantarum* being rod-shaped and looking shiny white.

The SEM image of *Lactobacillus plantarum* encapsulates (Figure 4) shows that the morphology of the microcapsules is similar to the surface shrinking due to the uniform drying process by freeze drying [37]. A smoother microcapsule surface and uniform structure were demonstrated with a concentration of 8% KBF, 20% maltodextrin, and 10% skim milk. The probiotic bacteria in the encapsulation results are located randomly on the surface of the microcapsule, this is due to the type of matrix with scattered cells and may be located on the surface of the coating material [29].

The aim of using a scanning electron microscope on *Lactobacillus plantarum* encapsulates was to observe the probiotics coated with the coating material. Figure 4 shows that a thin layer of protective matrix is formed covering the cells with regular intercellular spacing and a dense structure [38]. From the SEM image, it can be that its morphology is irregular and heterogeneous. Coating ingredients for skim milk and maltodextrin with the same concentration for each treatment caused the size of the encapsulated *Lactobacillus plantarum* microcapsules to tend to be uniform between treatments [39].

The shape of the microcapsules looks smooth and slightly wrinkled which can be caused by the high ratio of maltodextrin encapsulant compared to skim milk. Skim milk is a protein-based coating material that has a double emulsion system that can produce microcapsules with a shape that does not look wrinkled and smooth, but due to the presence of another encapsulant material, namely maltodextrin with a high concentration, the texture looks wrinkled [40]. Maltodextrin coating does not have good emulsion ability, so the microcapsule shape has wrinkles and size non-uniformity due to the high viscosity of the emulsion [41].

3.4. Organoleptic properties

The organoleptic testing attributes of *Lactobacillus plantarum* encapsulates assessed include color, aroma, texture, and overall organoleptic encapsulation of *Lactobacillus plantarum* with KBF as described in Figure 5. The organoleptic testing was carried out with the help of 25 semi-trained panelists. *Lactobacillus plantarum* encapsulates have a white color, the color of each *Lactobacillus plantarum* encapsulate treatment tends to fluctuate. The addition of high levels of maltodextrin resulted in the encapsulate color of *Lactobacillus plantarum* increasing or being white and tending to be bright, this was due to the increasing number of total solids and white maltodextrin [42]. The color of *Lactobacillus plantarum* encapsulates is also influenced by the color of the skim milk used. The research results of [43] the encapsulation results of red peppers have a red color which comes from the red pepper raw materials used. This can also be interpreted as the encapsulation results of *Lactobacillus plantarum* are white, influenced by the color of the material used during encapsulation.

Lactobacillus plantarum encapsulates tend not to have a typical banana aroma, this is because the concentration of the addition of KBF is not too high. Adding a low concentration of coating material can result in the aroma of the encapsulation still having a distinctive banana aroma because it cannot cover the strong characteristic aroma of kepok banana [39]. However, the addition of KBF also affects the resulting aroma. The small concentration of KBF in the *Lactobacillus plantarum* encapsulation means that the aroma produced cannot be seen significantly between treatments [44]. Overall, the encapsulate produced was as expected, namely, it did not have a typical banana aroma.

Encapsulation of *Lactobacillus plantarum* with the addition of KBF using freeze drying can make the texture of the encapsulate less rough which is caused during the drying process carried out by freezing and low temperatures [45]. *Lactobacillus plantarum* encapsulates have a rough to non-coarse texture which can be caused by the uneven grinding of the encapsulates between treatments. *Lactobacillus plantarum* encapsulates produce a texture that is not rough due to the addition of maltodextrin which can interact with water so that it is able to maintain the texture of the *Lactobacillus plantarum* encapsulates [46].

Lactobacillus plantarum encapsulates have overall criteria that tend to fluctuate. However, with the addition of 8% the KBF had a high score and was liked by the panelists. The treatment with the highest score is the best treatment that can be received by the panelists [47]. Overall acceptance is an assessment of *Lactobacillus plantarum* encapsulates on color, aroma, and texture parameters. These results indicate that the ratio of KBF, coating material, and *Lactobacillus plantarum* at 8% KBF according to the expectations and criteria preferred by the panelists.

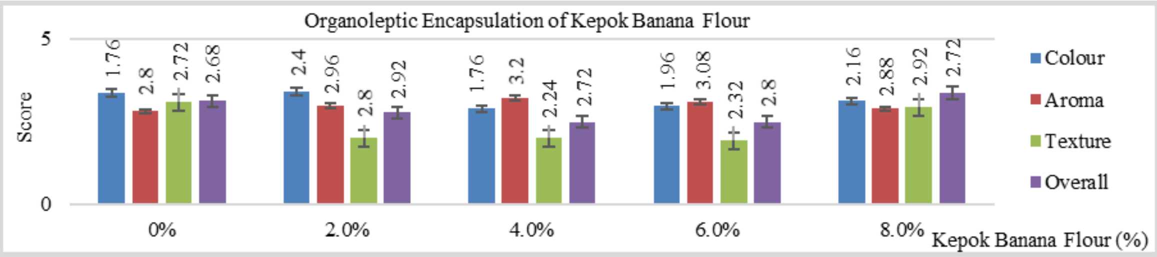


Figure 5. Organoleptic of encapsulation KBF

4. CONCLUSION

This research shows the effect of adding KBF on *Lactobacillus plantarum* encapsulation. Our findings offer definitive evidence that this phenomenon at a KBF concentration of 8% is the best treatment with results in high LAB and resistant starch viability, low pH and yield values, non-grainy texture, white color, non-banana aroma, and overall very favorable levels. This change was caused by the addition of higher KBF concentrations. From the best treatment encapsulated *Lactobacillus plantarum*, research on synbiotic food can be continued because the addition of KBF can act as a prebiotic for *Lactobacillus plantarum*.

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