Viability of encapsulated *Lactobacillus casei* using glucomannan iles-iles and skim milk to low pH and bile salts

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**Abstract.** The aim of this study was to determine the effect of glucomannan iles-iles concentration as an encapsulating agent and skim milk concentration as the protectant agent on the viability of *L. casei* in the encapsulation process using spray drying, and to determine its viability in low pH and bile salts. The experiment used a randomized complete block design with two factors. The first factor was the concentration of glucomannan in three levels, A1 (5%), A2 (10%), A3 (15%). The second factor was the concentration of skim milk as a protectant agent with three levels, B1 (9%), B2 (12%) and B3 (15%). The concentration of glucomannan iles-iles and skim milk had no significant effect on the cell number of *L. casei*. Based on the number of *L. casei* after the spray drying process. Decrease in viability *L. casei* after spray drying and stored for 3 months, about 2 log cycles. The highest viability of encapsulated *L. casei* cells against low pH and bile salts was obtained at 5% glucomannan concentration and 9% skim milk.

**Keywords:** glucomannan iles-iles, skim milk, *L. casei*, viability, spray drying

1. **Introduction**
Nowadays, people with degenerative diseases are increasing, therefore aware of the importance of good health and lifestyle are getting attention. The people are selective in choosing food products that are consumed, so as to avoid diseases due to diet and the wrong lifestyle. Nowadays many functional food products are developed, namely products that benefit human health if consumed. The concept of functional food now leads to the concept of synbiotics between prebiotics and probiotics. Food containing probiotics in order to function in the body must be alive, and able to pass through the human digestive system. While in the stomach there are inhibitors of stomach acid which results in low pH. This situation has very strong destructive for the viability of probiotic bacteria. Acid inhibition of bacterial cell growth occurs through the effect of denaturing enzymes on the cell surface, damage to lipopolysaccharide and outer membrane and decreasing cytoplasmic pH through increased membrane permeability. In addition, bacterial cells have experienced considerable stress due to the effects of drying and freezing. In the small intestine, there is also a bile salt. The function of bile salts in the small intestine is as an emulsifier in the process of digestion of fat (fat emulsification). For microorganisms in the small intestine bile salts are "biological detergents" which are liquids that have the ability to dissolve phospholipids, cholesterol, and protein.
Both of these factors are inhibitors for lactic acid bacteria and can cause death. Therefore, it needs to protect cells, so they stay alive. One way is by encapsulation. One of the ingredients that can be used as encapsulating material is glucomannan from iles-iles. Glucomannan extracted from iles-iles has the ability to form a gel so that it can be used as a capsule material. The ability as an encapsulating agent of glucomannan must be studied and examined again specifically for the concentration of encapsulating material because it will affect the diameter of microcapsule particles and their ability to protect bacterial cells.

One of the encapsulation methods that can be used is spray drying. Spray drying is able to produce smaller microcapsules (less than 1 mm) so that microcapsules can be used for further processing into synbiotic food products that are more practical for consumption. The disadvantage of the spray drying method is the decrease in cell viability significantly due to high temperatures. Therefore, a protective material is needed that is able to maintain the viability of bacteria. One of the protective agents that is often used in the spray drying method is skim milk. Synbiotic encapsulation added with encapsulant ingredients such as skim milk can produce high viability of probiotics [1].

Resistance to stomach acid is an important requirement for an isolate to be a probiotic. This is because if the isolate enters the human digestive tract, it must be able to withstand gastric acid pH of around 2.5 [2]. Selection of LAB from Indonesian fermented foods and the results showed almost all isolates had good resistance to grow at low pH with a decrease in the number of colonies at low pH compared to controls not to 1 log/ml, except lactobacillus Plantarum FNCC 107 decreased 1.1 log/ml unit [3].

The resistant of lactic acid bacteria isolates to bile salts is also an important requirement for probiotics, as is resistance to stomach acid. Farida (2005) states that lactobacillus is a normal microflora found in the human digestive tract and has varying resistance to bile salts. Jacobsen, et al. 1999), all microbes that have succeeded in living after being grown in MRSA plus 0.3% bile salt, are stated to be resistant to bile salts. The concentration of bile salt of 0.3% is a critical concentration, a value high enough to select isolates that are resistant to bile salts. Lactobacillus which is resistant to bile salts are found in the upper part of the small intestine (jejunum). The amount of BAL found in the jejunum was lower than that of the ileum, cecum, and colon. This is due to the highest concentration of bile salts in the jejunum part of the ileum because the location is closest when bile salts enter the intestinal tract [4]. Some lactobacillus has enzymes with activities to hydrolyze bile salts (bile salt hydrolase, BSH). This enzyme is able to change the physical-chemical abilities possessed by bile salts, so it is not toxic to BAL [5]. The higher the concentration of bile salts, the higher the number of dead lactobacillus cells [3,6].

Based on the description above, it is necessary to do research on the viability of synbiotic microcapsules encapsulated with iles-iles glucomannan by the addition of skim milk after spray drying and its viability to the conditions of low pH and bile salts. The aim of this study was to determine the effect of glucomannan iles-iles concentration as an encapsulating agent and skim milk concentration as the protectant agent on the viability of L. casei in the encapsulation process using spray drying, and to determine its viability against low pH and bile salts.

2. Material and method
The material used in this study was using MRS media (Merck), agar, iles-iles flour, bile salt (Merck), HCL, L. casei, NaOH and aquadest,

2.1. Preparation of glucomannan
Preparation of glucomannan was carried out by weighing the iles iles flour that had been processed before, then iles iles flour plus aquadest with a ratio of 1:30 (10g:300ml aquadest). Then it was heated and stirred constantly at 750C for 1 hour with a stirring speed of 560 rpm. After that, cooling is carried out at room temperature for about 15-20 minutes. Then extracted with 96% ethanol with a ratio of 1:2 and stirred constantly at 560 rpm for 1 hour. Then filtering is done using gauze cloth. Dregs or solid
parts that are not filtered are dried using an oven at a temperature of 70-80°C until they dry out. After drying, milled and sieved with a 60 mesh sieve.

2.2. Preparation of MRS media and subculture of L. casei
Prepared MRS Broth media as much as 5 grams and added with 100 ml of distilled water, then heated to boil. Then put into the test tube 10 ml. Bacterial culture was carried out by inoculating one isolate L. casei into 10 ml liquid MRS, then incubated for 18-24 hours at 30 °C.

2.3. Cell biomass preparation
Pure culture of L. casei bacteria from MRS Broth media which was 24 hours old was centrifuged at 3000 rpm for 25 minutes. The results of sediments were taken to obtain pellets or L. casei cell biomass while the supernatant was removed. Next, 1ml of sterile aquades are given into biomass and then the biomass is ready to be mixed into the encapsulating material solution.

2.4. Preparation of encapsulating L. casei
Glucomannan prepared according to concentration 5%, 10% and 15% w/v. The skim milk prepared according to concentration 9%, 12% and 15% w/v. The mixtures of glucomannan and skim milk then stirred and heated on a hotplate until it dissolves completely and after that sterilized for 3 minutes at 100 °C. Lactobacillus casei biomass was mixed aseptically and then homogenized and put into the spray dryer machine. Set the inlet temperature to 120 °C, 15% pump, 90% aspirator, and cleaner nozzle 4. The inlet temperature is the spray drying temperature. The pump is the speed of the material delivery pump to the spray drying machine. After finishing spray drying, the results of the spray are weighed to determine the yield of the yield and analysis. During the analysis, the spray drying samples were stored at cold temperatures to prevent capsule damage and add silica gel to maintain the moisture content of the ingredients.

2.5. The resistance of encapsulated L. casei to low pH
The MRS media was prepared as much as 9 ml, then the pH was adjusted to 2 then each one gram of synbiotic microcapsules was added and left for 3 hours. Then the number of bacteria is calculated using the plate count method.

2.6. The resistance of encapsulated L. casei to bile salt
Prepared MRS media as much as 9 ml, then added bile salt with a concentration of 1%, then each of them added 1 g of encapsulated L. casei and left for 3 hours. Then the number of bacteria is calculated using the plate count method.

3. Results and discussion
Drying conditions are very dependent on the coating material used and the core material. Incompatibility between the coating material and drying conditions can result in leakage or the occurrence of "ballooning" and swelling "puffing" effects and can reduce retention. In this study, the Scanning Electron Microscopy (SEM) technique was used to determine the structure of synbiotic microcapsules with glucomannan encapsulation and skim milk as a protective material. SEM images of synbiotic microcapsules can be seen in figure 1.
Figure 1. Encapsulated L. casei with glucomannan and skim milk as an encapsulating agent.

The Scanning Electron Microscopy (SEM) picture of encapsulated L. casei has around and irregular appearance of microcapsules. From the picture, it can also be seen that there are cracks that occur from encapsulants and protective agents that are thought to facilitate heat from the core when the temperature inside the core is higher than the temperature in the drying chamber. Cores in the form of probiotic bacteria will be protected from adverse environmental effects so that cell death can be suppressed.

3.1. Viability of encapsulated L. casei after spray drying

The number of bacteria after spray drying can be seen in Table 1.

| Glucomannan concentration | Skim milk concentration | Average |
|---------------------------|-------------------------|---------|
|                           | B1 (9%) | B2 (12%) | B3 (15%) |
| A1 (5%)                   | 2.06    | 1.77     | 1.37     | 1.73     |
| A2 (10%)                  | 1.50    | 2.74     | 1.46     | 1.90     |
| A3 (15%)                  | 1.52    | 1.75     | 1.83     | 2.01     |
| Average                   | 1.69    | 2.08     | 1.55     |

Based on Table 1, it can be seen that there was no significant difference in each treatment of the number of bacteria after spray drying. This is because biomass added to each sample also has no significant difference and the performance of spray drying runs in the same conditions for all treatments. But the higher the concentration of glucomannan, the higher the number of bacteria even though the difference is not significant with other treatments. This is because the higher the concentration of glucomannan, the more cells to be absorbed. Glucomannan is also a source of nutrition for L. casei bacteria by breaking down glucomannan bonds into glucose and mannose groups which are then used as sources of carbon or nutrients for bacterial growth. During cell growth, carbon sources are used as food to grow and multiply cells and metabolize cells which produce lactic acid [7]. The variation in the concentration of skim milk did not give a significant difference to the number of bacteria after spray drying. However, the concentration of 12% protective agent provides the highest average caused by its ability to protect L. casei cells thoroughly but also does not provide a coating that is too thick. The concentration of 10% protective agent is able to provide better protection because it is more optimal in protecting cells from excessive heat received by cells during the encapsulation process in the spray dryer inlet and outlet [8].
3.2. Decreasing of cell viability

The decrease in the viability of bacterial cells can be seen in Table 2.

Table 2. The decrease of the viability of bacterial cells after spray drying (log CFU).

| Glucomannan concentration | Skim milk concentration | Average |
|---------------------------|-------------------------|---------|
|                           | B1 (9%) | B2 (12%) | B3 (15%) |       |
| A1 (5%)                  | 2.13    | 2.07     | 2.19     | 2.13  |
| A2 (10%)                 | 2.06    | 1.84     | 2.09     | 1.99  |
| A3 (15%)                 | 2.17    | 2.07     | 1.98     | 2.01  |
| Average                   | 2.12    | 1.99     | 2.08     |       |

Based on Table 2, it can be seen that microcapsules with a 10% glucomannan encapsulating material have the smallest decrease in the number of bacteria. The smaller the decrease in the number of bacteria, the better the treatment to maintain the viability of lactic acid bacteria. The 10% glucomannan concentration is considered to be able to overly cover probiotic bacteria so that it can suppress bacterial death while providing nutrients for its growth. The low concentration of glucomannan causes the bacteria not to be completely covered, while too much glucomannan concentration causes the capsule diameter to be too large so that the heat trapped in the core cannot be channelled out [9].

Associated with the concentration of skim milk, all treatments also did not show a significant difference because the adjacent concentration ranges were 9%, 12%, and 15%. The concentration of 10% skim milk is able to provide better protection because it is more optimal in protecting cells from excessive heat received by cells during the encapsulation process in the spray dryer inlet and outlet [8]. At a concentration of 10% skim milk, encapsulated cells also have the ability to release heat better than the core, when the temperature in the core is hotter than the temperature in the drying chamber. The advantage is that the heat inside the core can be reduced so that cell death due to heat decreases. This has resulted in all treatments not showing a significant difference in the number of bacteria because the variation in concentration only ranges from 9% to 15%. The concentration that is too thick will cause the performance of the spray dryer not to work properly.

After the surface structure of microcapsules with skim milk as the encapsulating material was observed using the Scanning Electron Microscopy (SEM) technique, it was seen that on the capsule surface there were very fine cracks. These cracks are then thought to facilitate so that heat can escape from the core, so that cell viability during the encapsulation process can be maintained [9].

3.3. Viability of encapsulated L. casei after incubation in broth with pH 2

Viability of encapsulated L. casei after incubation in broth with pH 2 can be seen in Table 3.

Table 3. The number of L. casei after incubation in broth with pH 2 (x 10⁶ CFU/g).

| Glucomannan concentration | Skim milk concentration | Average |
|---------------------------|-------------------------|---------|
|                           | B1 (9%) | B2 (12%) | B3 (15%) |       |
| A1 (5%)                  | 2.07    | 1.79     | 1.11     | 1.66  |
| A2 (10%)                 | 1.63    | 1.64     | 1.66     | 1.64  |
| A3 (15%)                 | 2.03    | 1.43     | 1.57     | 1.86  |
| Average                   | 1.91    | 1.62     | 1.45     |       |

Based on Table 3 it can be seen that the number of L. casei bacteria against low pH with 15% glucomannan concentration produces the highest number of cells which is 1.86 (x10⁶ CFU/ml) because glucomannan is able to absorb cells more strongly and the structure of microcapsules is more tightly so that outside influences can be minimized. Glucomannan has water-soluble properties, gels, adhesives, expands, transparent (forming film), melts, and settles. The advantage of glucomannan is
the unique character as a thickening agent, among others, is having a water absorption capacity of more than 100 times its own weight [10]. Based on table 3 it can be seen that the number of *L. casei* bacteria encapsulated with 9% skim milk produces the highest number of cells which is 1.91 (x10^6 CFU/ml). This happens because the higher the glucomannan or casein absorbed so that the amount of *L. casei* growth decreases (12% skim milk) with the number of bacteria 1.6257 (x10^6 CFU/ml) and (15% skim milk) with the amount of 1.4541 (x10^6 CFU/ml). Even though casein is an amphoteric protein that has acidic or basic properties, it usually has alkaline properties. The provision of skim milk is used to achieve solid non-fat content because skim milk is a medium for the growth of lactic acid bacteria. Decreasing pH in MRSB as an *L. casei* growth medium is used hydrochloric acid (HCl), it is used to approach acidic conditions in the stomach which also contain HCl. The normal gastric pH is around 3-4. Based on the results obtained in testing the resistance of LAB at low pH, lactic acid bacteria found in glucomannan are thought to pass through the acidic gastric tract. These results are consistent with the results of several studies that show that LAB, especially lactobacillus, is one of the bacteria that is most resistant to acidic conditions.

3.4. Viability of encapsulated *L. casei* after incubation in broth with bile salt 1%

Viability of encapsulated *L. casei* after incubation in broth with bile salt 1% can be seen in table 4.

| Glucomannan concentration | Skim milk concentration | Average |
|---------------------------|-------------------------|---------|
| B1 (9%)                   | B2 (12%)                | B3 (15%)|         |
| A1 (5 %)                  | 1.89                    | 1.71    | 1.62    | 1.74    |
| A2 (10%)                  | 1.51                    | 1.61    | 1.68    | 1.60    |
| A3 (15%)                  | 1.76                    | 1.79    | 1.49    | 1.66    |
| Average                   | 1.72                    | 1.70    | 1.60    |

Based on table 4 it can be seen that the calculation of the number of *L. casei* bacteria against 1% bile salt with 5% glucomannan concentration produces the highest number of cells which is 1.74 (x10^6 CFU/ml). This happens because glucomannan is able to absorb cells more strongly and the structure of microcapsules is more tightly so that outside influences can be minimized. Based on table 7 it can be seen that the number of *L. casei* bacteria encapsulated with 9% skim milk produces the highest number of cells which is 1.72 (x10^6 CFU/ml). This happens because the higher the glucomannan or casein absorbed so that the amount of *L. casei* growth decreases (12% skim milk) with the number of bacteria 1.70 (x10^6 CFU/ml) and (skim milk 15%) with the amount of 1.60 (x10^6 CFU/ml). Even though casein is an amphoteric protein that has acidic or basic properties, it usually has alkaline properties. The provision of skim milk is used to achieve solid non-fat content because skim milk is a medium for the growth of lactic acid bacteria. The decrease in the number of bacteria due to the presence of 1% bile salt is due to a leak in the cells incubated by bile salts but not cause the cells to experience lysis. Tolerance of bile salts is caused by the role of polysaccharides as one of the constituents of gram-positive bacterial cell walls but the mechanism involved in them is not yet clear [11]. Inhibition of bile salts to the growth of bacteria caused by bile salts amphipathic structure so that it dissolves or breaks down all cell substances containing lipids. Bacterial cell walls and bacterial cell membranes contain lipids so that the entry of bile salts into cell walls and cell membranes will cause cell walls and cell membranes to become damaged and lose their function as protective bacteria and filters. If the bacteria are damaged or lose function on the cell wall, it will cause bacteria to be unable to withstand the osmotic pressure, causing cell lysis or evisceration which results in cell death [12].
4. Conclusion
The concentration of glucomannan iles-iles and skim milk had no significant effect on the cell number of L. casei. Based on the number of L. casei after the spray drying process. Decrease in viability L. casei after spray drying and stored for 3 months, about 2 log cycles. The highest viability of encapsulated L. casei cells against low pH and bile salts was obtained at 5% glucomannan concentration and 9% skim.

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Acknowledgment
The author would like to thank Dr. Meidi Syaflan, MP and Ferdianto Erwin Kaka who have helped during the research and DP2M DIKTI who have provided research funding through competitive scheme in 2016/2017 fiscal year.