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Abstract. *Lactobacillus acidophilus* is a type of probiotic Lactic Acid Bacteria (LAB) which produce more organic acids than other types of LAB. Organic acids produced by probiotics LAB can reduce pH and breaks several molecular bonds. They have the effects of functional foods. *Lactobacillus acidophilus* can be said as a probiotic if the amount in food products reaches a minimum of 107 CFU/mL. Encapsulation is a process of probiotics coating to maintain the viability in final products due to food processing. The aim of this study wasto determine the viability of encapsulated *Lactobacillus acidophilus* through freeze and spray drying methods with the same encapsulation material. The study was conducted using Randomized Block Design (RBD) with three replicates. The research was conducted in Padjadjaran University and Indonesian Education University during August to October 2018. The results showed that solvents with Pasteurized milk can produce better viability and yield than distilled water. Pasteurized milk can further increase the amount of *Lactobacillus acidophilus* because the main substrate of the bacteria is lactose. The viability of freeze dried culture was not significantly different from the viability of spray dried culture but the yield value is significantly different.

1. Introduction

Nowadays, the existence of synbiotic food as part of the functional food is increasing along with increasing public awareness of health. Synbiotic foods have beneficial effects on health because they contain probiotic bacteria and prebiotic components. The prebiotic components were not digested in the small intestine but is digested in the large intestine by probiotic bacteria to enhance the body's immune system through several molecular mechanisms [1]. Prebiotics are carbohydrates in the form of oligosaccharides or dietary fiber which cannot be digested and are not absorbed by the body. The prebiotic components include inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), Lactose, β-glucans, and Xylooligosaccharides (XOS) [2].

Probiotics are living bacteria which have beneficial effects on their hosts if consumed in sufficient quantities[3]. Some probiotics include various species of the genus Lactobacillus and Bifidobacterium such as *B. bifidum, B. breve, B. infantis, B. longum, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus reuteri, and Lactobacillus rhamnosus* [4]. In this study, the probiotics used in the encapsulation experiment were *Lactobacillus acidophilus*. *Lactobacillus*

acidophilus is a natural microflora in human's digestive tract which produce lactic acid as the main product of sugar fermentation. These bacteria can also produce bacteriocins which can stimulate the formation of antibodies [5]. This bacterium can be attached to the gastrointestinal epithelial cells, found in the intestines of adults and originally isolated from the feces of healthy infants aged 1-2 months, and breast milk. The specialty of this bacterium is that it can break down azo bonds from sulfasalazine which produce Azulfidine, which can be used for the treatment of colitis [6].

Lactobacillus acidophilus needs to get the initial encapsulation treatment before added as a food ingredient. The aim of encapsulation was to protect *Lactobacillus acidophilus* from damage caused by the processing, storage, and distribution of food, considering the consumed amount must reach a minimum of 107 Cfu/gr to be classified as probiotic [7]. In addition, encapsulation can maintain the number and viability of the bacteria's cells in the digestive tract [6].

Encapsulation can be done by freeze drying and spray drying methods [8]. In freeze-drying, water in foodstuffs is dried in two stages: freezing of food until the water in the material undergoes freezing[9] (food freezing is done in the freezer), the next stage is removing water that has frozen into water vapor through the sublimation process with a freeze dryer. In spray drying, foodstuffs are passed on through the nozzle to form droplets in the drying chamber, then droplets are dried immediately with hot air and foodstuffs that have become powder are separated by cyclones and accommodated in a reservoir[10]. Frozen drying encapsulation process was carried out for 72 hours; while the spray drying method was carried out within 30 minutes.

The material that has been used as a coating in *Lactobacillus acidophilus* encapsulation were carbohydrate, starch, gum, whey, casein, calcium, and alginate[11]. The combination of two types of ingredients will result in better cell viability than using only one type of coating material[12]. The coating material used in this study is a combination of maltodextrin as a source of carbohydrates and skim milk as a source of calcium. The best composition of coating ingredients of *Lactobacillus acidophilus* according to previous studies was 20% maltodextrin and 10% skim milk from the volume of distilled water as a solvent in inoculum fermentation[13]. In the evaluation of encapsulation with a spray dryer, liquid milk is used as a solvent in inoculum fermentation. Liquid milk is a source of casein, lactose, fat, and whey. These components are materials that have also been used as coating materials. The presence of these components is expected to maintain cell viability in the encapsulation process with a spray dryer.

The combination of carbohydrates and casein and also the components of lactose, fat, whey and casein in fresh liquid milk is expected to maintain the cell viability of *Lactobacillus acidophilus* during the encapsulation process mainly through encapsulation of the spray drying method.

2. Materials and methods

2.1. Materials and tools

The experiment took place at the Laboratory of Food Microbiology, Laboratory of Food Processing engineering and laboratory of food chemistry Faculty of Agroindustrial Technology, Padjadjaran University, and also at the Basic Chemistry and Analytical Laboratory in Faculty of Mathematics and Natural Sciences, Indonesian Education University. The experiment was conducted in August - October 2018. The research material was *Lactobacillus acidophilus* which was purchased from the Microbiology Laboratory of Biology Department, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor. Other materials used were MRS (Man Rogosa Sharpe) Merck Agar, liquid milk obtained from the Lembang milk cooperative, NZWP brand skim milk, distilled water, NaCl and Maltodextrin DE 10-12.

The tools used are test tubes, Petri dishes, colony counters, incubators, measuring cups, scotch bottles, vortex mixer, centrifugation tubes, laminar flow, UV-vis spectroscopy, hotplate magnetic stirrers, freeze dryers, frozen coolers, milk thermometers, hand mixers, spray dryers, microscope, autoclave, electric oven, and refrigerator.

2.2. Research design

The experimental design used in this study was a randomized block design with group drying method and treatment of *Lactobacillus acidophilus* inoculum fermentation media. The experiment was designed as follows:

A = encapsulation control (Freeze dry, distilled water)
B = encapsulation 1 (Spray dry, distilled water)
C = encapsulation 2 (Spray dry, pasteurize milk)
D = encapsulation 3 (dry freeze, pasteurize milk)

The response observed was *Lactobacillus acidophilus* cell viability which data were analyzed with ANOVA and LSD test.

2.3. Response design

The response observed in this study were the effect of drying method to *total Lactobacillus acidophilus*, cell viability, and yield of encapsulated *Lactobacillus acidophilus*.

2.4. Lactobacillus acidophilus cell preparation

The pure culture of *Lactobacillus acidophilus* was rejuvenated and propagated in a test tube with a sloping method using MRS media. *Lactobacillus acidophilus* was then incubated at 38° C for 48 hours. Rejuvenation cultures are then decomposed using 0.5 - 2 mL of physiological NaCl solution for each test tube. Checking turbidity according to McFarland 3 at λ = 600 nm and absorbance ± 0.616 which is equivalent to the number of colonies (3.0 × 10 ^ 8 CFU/ml) on a spectrophotometer. The next culture solution is inoculated as much as 10% part of the solvent in 10% skim milk dissolved in distilled water or pasteurized liquid milk at 72° C for 15 seconds. The inoculum was incubated for 4 hours at 40° C before use [13].

2.5. Encapsulation by freeze-drying method

Maltodextrin (20%) was added into the inoculum solution. The mixture of coating material and inoculum was homogenized with a vortex mixer, then placed in a falcon tube, covered with film and frozen in a freezer at -400C for 24 hours. Then the material was dried with a cold dryer at -500C for 24 hours.

2.6. Encapsulation by spray drying method

Maltodextrin (20%) was added into the inoculum solution. The mixture of coating material and inoculum was homogenized with a vortex mixer, then placed in a sterile glass beaker, covered with plastic wrap. The material is then dried with a spray dryer at 150° C inlet temperature, a flow rate of 15ml / minute, an outlet temperature of 80° C.

2.7. Lactobacillus acidophilus total cell

Sample (1 ml) was diluted with 9 ml of Physiological NaCl solution 0.85% aseptically, take a sample of 1 ml each. At the last three dilutions, MRS agar medium + 0.5% glacial acetic acid (40-45° C) was poured as much as 12-15 ml into a petri dish and shaken to flatten the sample on the media [13]. Wrap the petri dish after it has frozen and incubated it upside down at 37° C for 48 hours, calculate the number of colonies growing on agar,

$$ColoniespermL = \frac{Numberofcoloniespercup}{Dilutionfactor}$$

2.8. Cell viability test

Calculation cell viability in the culture of freeze dried or spray dried culture *Lactobacillus acidophilus* based on the ratio of log number of bacteria per gram after and before the encapsulation process and expressed in percent (%) [13]. The formula for calculating viability is as follows:

$$\text{Viability (\%)} = \frac{\text{Number of colonies after } drying (\text{Log CFU ml}^{-1})}{\text{Number of colonies before } drying (\text{Log CFU ml}^{-1})} \text{x } 100$$

2.9. Encapsulation yield

The yield is a presentation of the ratio between the weight of the product and the weight of the suspension. The yield value is useful to find out how many products can be used. The higher the yield value of a product, the more products can be used [14]. The yield is expressed in percent (%) and can be calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Encapluation final weight (g)}}{\text{Suspension volume (mL)}} \text{x } 100$$

3. Results and discussions

3.1. Total bacteria and viability of Lactobacillus acidophilus

Viability is a resistance parameter of microbial from pure culture throughout coating process by freeze dried or sprays dried process. The Calculation of total bacteria was done using the Total Plate Count (TPC) test. Before calculating cell viability, a total of *Lactobacillus acidophilus* bacteria (**Table 1**) was tested.

Table 1. Total Bacteria of *Lactobacillus acidophilus*

Condition	Total bacteria of *Lactobacillus acidophilus* (Log CFU/g)			
	A	**B**	**C**	**D**
Pure culture	12.09	12.09	12.09	12.09
Inoculum	11.84	11.84	11.69	11.90
Suspension	11.14	11.77	10.83	11.46
Encapsulated	10.05	11.36	7.75	10.10

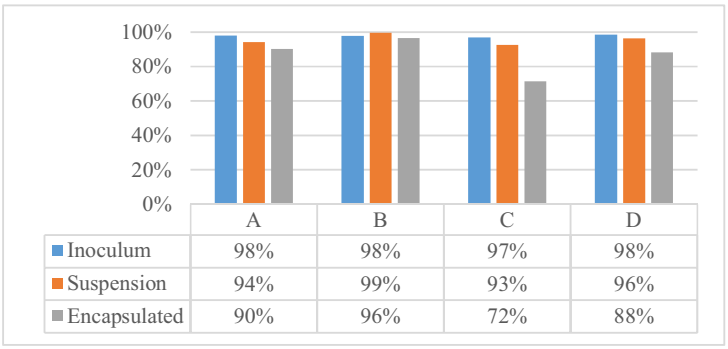
The viability of cells in the inoculum, suspension, and cells after encapsulation showed a decrease which caused by the adaptation process and dilution in each processing stage. The decreasing number of *Lactobacillus acidophilus* in the results of inoculum fermentation and cell culture suspension was because of the addition of pasteurized milk or distilled water as a solvent. The dissolution can cause dilution of a pure culture of *Lactobacillus acidophilus*. A decrease in the number of cells after spray drying can be caused by dehydration and cell inactivation due to heat. The decrease in the number of cells varies depending on lactic acid bacterial strains, treatment, and composition of encapsulation materials.

The total number of bacteria in *Lactobacillus acidophilus* bacterial suspension was influenced by skim milk and pasteurized milk nutrition. *Lactobacillus acidophilus* is a Lactic Acid bacteria and during its growth requires lactose as a carbon and nitrogen source for growth [15]. Lactose in pasteurized milk is able to provide good protection against the effects of freeze and spray drying. The lactose constituent components are in the form of glucose and galactose which have low molecular weight so that lactose can enter bacterial cells and provide protection from two sides of the cell membrane during the freeze-drying process [12]. Lactose is the main component in skim milk and pasteurizes milk. On the other hand, skim milk is used as one of the coating materials because it consists of casein, whey, and calcium which have the effect of protecting the core material.

Milk protein consists of casein and whey. Casein is very stable against high temperatures. Heating at 100° C for 24 hours or heating at 140° C for 20 minutes does not cause coagulation. Unlike whey which is completely denatured at 90° C for 10 minutes. Casein is a phosphoprotein containing 0.85% phosphorus, while whey does not contain phosphorus [15].

In the process of material evaporation, both encapsulations with a spray dryer and freeze dryer, calcium from skim milk will experience cracks on the surface [16]. This crack facilitates the release of heat or cold from inside the particles after drying, which results in reduced heat or cold damage to the cell. Even so, the calcium content that is too high in coating material can cause stiffness of the cell membrane so that cells can be damaged.

From the results of the calculation of total bacteria in each stage of encapsulation, it can be seen the value of viability through the comparison of the number of bacteria after the process and before the next process (**Figure 1**). Although statistically not significantly different, the order of treatment from the start with the highest viability at the encapsulation stage was B (freeze drying, pasteurized milk), A (freeze drying, distilled water), D (spray drying, pasteurized milk) and C (spray drying, distilled water.



	A	B	C	D
Inoculum	98%	98%	97%	98%
Suspension	94%	99%	93%	96%
Encapsulated	90%	96%	72%	88%

Figure 1. The viability of dried cultures *Lactobacillus acidophilus* bacterial suspension

Pasteurized milk is a fat emulsion in water containing sugar, salt, minerals, and protein in the form of colloids. The composition of cow's milk consists of water, fat, and non-fat dry ingredients. Material dry fat marks consist of protein, lactose, minerals, acids, enzymes, and vitamin. Composition of pasteurized milk such are 3.6% fat; 3,2% protein; 4,7%, lactose; 0.8% mineral material. Proteins present in milk are mostly casein (76%) and whey protein (18%)[12]. Casein consists of a mixture of three protein components, namely alpha protein (40-60%), casein beta (20-30%), and gamma casein (3-7%). After casein is separated from milk, the rest which is a solution is called whey. Approximately 0.5-0.7% of soluble protein ingredients and left in whey, namely lactoglobulin and lactalbumin proteins. Lactose is a carbohydrate or milk sugar that is only found in milk and is only formed by mammals. Lactose content in pasteurizing milk is about 5%. Lactose dissolves easily with 1/6-1/2 times the glucose level, where if milk is heated, lactose will form lactulose which dissolves easily and has a rather sweet taste.

The composition of pasteurized milk as described above which causes *Lactobacillus acidophilus* encapsulation using pasteurized milk solvents to have better viability than the use of distilled water. casein can maintain damage to the cytoplasmic membrane, and milk fat can provide space for *Lactobacillus acidophilus* in the encapsulated protein-carbohydrate matrix[12]. this is in line with the results of previous studies which stated that encapsulation using pasteurized milk can produce cells with better viability.

In addition, to skim milk, the coating material used was maltodextrin. Maltodextrin is a derivative of an oligosaccharide which is an energy material for the growth of good bacteria (prebiotics) because the components of maltodextrin are classified as complex carbohydrates. Maltodextrin is widely used as a coating material because it is easy to process, has high solubility, is able to form a matrix, has strong binding strength, low viscosity and is stable in oil and water emulsions[17]. Maltodextrin has a

good ability to inhibit oxidation reactions so that the encapsulated culture produced has a good shelf life compared to using Arabic gum.

3.2. The yield of encapsulated Lactobacillus acidophilus

Figure 2 presented *Lactobacillus acidophilus* encapsulation results were carried out through the freeze-drying method (left) and spray-drying (right). From **Figure 2** it can be seen that the freeze-dried culture has the appearance of coarse hollow powder even some are still in the form of a solid cavity. Whereas the spray dried culture has a very soft powder texture.

Figure 2. Encapsulated *Lactobacillus acidophilus* by freeze-drying (left) and by spray-drying (right).

The freeze-dried culture looks a bit less white compared to the spray dried culture which looks paler. This is due to the freeze-drying time that is longer than the spray drying. Although using high temperatures, spray dried method takes in a very short time, so as to minimize the possibility of cell damage.

Statistically, the yield of the treatment tested was significantly different (**Table 2**). The yield of the pasteurized milk treatment was higher than the treatment with distilled water.

Table 2. The Yield of Encapsulated *Lactobacillus acidophilus*

Treatment	Yield (gr dried culture/mL suspension)
A	23,94% a
B	27,19% b
C	12,67% c
D	18,52% c

Viscosity is one factor that affects the yield of cell encapsulation. The higher the viscosity layers surrounding the core material, *Lactobacillus acidophilus* will form faster, so the essence of the ingredients was protected, thus microencapsulation yield was greater[11]. The use of pasteurized milk can increase the viscosity of bacterial suspensions.

From table 2 it can be seen that the freeze-dried culture yield was higher than the spray dried culture, it is possibly because the volume of air from the frozen dried culture (Figure 2) resulting from its highly porous shape. The spray dried culture particle is smoother than freeze-dried culture. The surface area of the material is inversely proportional to the weight, so the greater the surface area, the lighter the weight [7].

4. Conclusion and suggestion

The results showed that encapsulation with the freeze and spray drying method produced similar *Lactobacillus acidophilus* cell viability values. Pasteurized milk can improve cell viability and increase the yield of encapsulation results, *Lactobacillus acidophilus* encapsulation by spray drying method has a lower moisture content, finer powder, and better color. Based on the conclusions, further

research is needed regarding other types of fillers that can be used to improve cell viability through the spray drying process.

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