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THE INFLUENCE OF ENCAPSULANT MATERIALS IN *FREEZE DRYING* PROCESS ON *Lactobacillus plantarum* 1 RN9 VIABILITY

Pengaruh Jenis Bahan Enkapsulan Pada Proses Freeze Drying Terhadap Viabilitas Lactobacillus Plantarum 1 Rn9

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ABSTRACT

Lactic acid bacteria are important microorganisms in food fermentation technology. *Lactobacillus plantarum* 1 RN9 is a LAB isolated from bamboo and can be used as a starter culture in making curd. During the processing of LAB culture it can be damaged so that it can eliminate its function as a probiotic. On the other hand, storing culture in a fresh condition cannot be carried out for a long time. Thus we need a preservation method (preservation) of lactic acid bacteria that can maintain its viability and superior of the properties of an isolate. Encapsulation is one way to maintain the viability of probiotics and protect probiotics from damage due to unfavorable environmental conditions such as gastric acid and bile salts (Wu *et al*., 2000). The purpose of this study was to determine the effect of the type of encapsulant on the yield and viability of *L. plantarum* 1 RN9 during the freeze drying (FD) process. The encapsulant material used was skim milk, lactose, maltodextrin with observational parameters including yield, total LAB and viability of LAB. Based on the results of the study showed that the yield of dry cultures of *L. plantarum* 1 RN9 ranged from 26.42% to 41.08%, which statistically showed a significant difference (P> 0.05) between treatments. The highest yield was obtained in skim milk encapsulant by 41.08% then lactose was 39.44% and maltodextrin 26.42%. The viability of *L. plantarum* 1 RN9 culture after freeze drying with lactose encapsulant and maltodextrin decreased by 2.3 to 2.5 log cycles but still had high viability while viability with skim milk encapculation increase. The total LAB with skim milk encapsulants increased by 1 log cycle from 10.3 log CFU/g to 11.3 log CFU/g. Based on the results of the studyit can be conclused the use of skim milk encapsulants on *L. plantarum* 1 RN9 gives the best results compared of lactose and matodextrin with a yield of 41.08% and viability increases 1 log cycles ie 1.6 x 10^{11} CFU/g.

Keywords: *Lactobacillus plantarum 1 RN9, encapsulant, skim milk, lactose, maltodextrin.*

ABSTRAK

Bakteri asam laktat merupakan mikroorganisme yang penting dalam teknologi fermentasi pangan. Lactobacillus plantarum 1 RN9 adalah BAL yang diisolasi dari bambu dan dapat digunakan sebagai kultur starter dalam pembuatan dadih. Selama proses pengolahan, kultur BAL dapat mengalami kerusakan sehingga dapat menghilangkan fungsinya sebagai probiotik. Disisi lain, penyimpanan kultur dalam keadaan segar tidak dapat dilakukan untuk jangka waktu yang lama. Dengan demikian perlu suatu metode pengawetan (preservasi) BAL yang dapat mempertahankan

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viabilitas dan sifat-sifat unggul dari suatu isolat. Enkapsulasi merupakan salah satu cara untuk mempertahankan viabilitas probiotik dan melindungi probiotik dari kerusakan akibat kondisi lingkungan yang tidak menguntungkan seperti asam lambung dan garam empedu (Wu et al. 2000). Tujuan penelitian ini adalah mengetahui pengaruh jenis enkapsulan terhadap rendemen dan viabilitas Lactobacillus plantarum 1 RN9 selama proses freeze drying (FD). Bahan enkapsulan yang digunakan adalah susu skim, laktosa, maltodekstrin dengan parameter pengamatan meliputi rendemen, total BAL dan viabilitas BAL. Berdasarkan hasil penelitian menunjukkan bahwa rendemen kultur kering Lactobacillus plantarum 1 RN9 berkisar antara 26,42% sampai 41,08%, dimana secara statistik menunjukkan perbedaan yang nyata (P>0,05) antar perlakuan. Rendemen tertinggi diperoleh pada bahan enkapsulan susu skim sebesar 41,08% selanjutnya laktosa 39,44% dan maltodekstrin 26,42%. Viabilitas kultur Lactobacillus plantarum 1 RN9 setelah proses freezed drying dengan enkapsulan laktosa dan maltodekstrin mengalami penurunan sebesar 2,3 sampai 2,5 siklus log namun masih tetap mempunyai viabilitas yang tinggi sedangkan viabilitas dengan enkapsulan susu skim mengalami peningkatan. Total BAL dengan enkapsulan susu skim meningkat 1 siklus log dari 10,3 log CFU/g meningkat menjadi 11, 3 log CFU/g. Berdasarkan hasil penelitian dapat disimpulkan penggunaan bahan enkapsulan susu skim pada Lactobacillus plantarum 1 RN9 memberikan hasil terbaik dibandingkan laktosa dan maltodekstrin dengan rendemen 41,08% dan viabilitas meningkat 1 siklus log yaitu 1,6 x 10^{11} CFU/g.

Kata kunci: *Lactobacillus plantarum* 1 RN9*, enkapsulan, susu skim, laktosa, maltodekstrin.*

INTRODUCTION

Food storage and processing can reduce the number of Lactic Acid Bacteria (LAB) so that its role as a probiotic will also decrease. Lactic acid bacterial cell damage caused by processing treatment can cause the product to malfunction as a probiotic. On the other hand, storing culture in a fresh condition cannot be done for a long period of time. Thus we need a preservation method (preservation) of LAB that can maintain its viability.

The preservation process is one of the stages to maintain the properties and advantages of a lactic acid bacterial isolate that has the potential as a probiotic. Preservation of LAB can be done by spray drying, freezing and freeze drying (Fu and Etzel, 1995). Damage to lactic acid bacterial cells due to the freeze drying process can be minimized by the addition of certain encapsulant ingredients before the freezing and drying process is carried out (Tamime, 1981). Encapsulation is one way to maintain the viability of probiotics and protect probiotics from damage due to unfavorable environmental conditions such as stomach acid and bile salts (Wu *et al*., 2000). Decreased cell viability during the freeze drying process is due to freezing itself mainly damaging cell membranes. To reduce damage and maintain cell viability during the freeze drying process, it is necessary to find the type of encapsulant material that can reduce as little as possible the decrease in viability of the freeze dried culture produced. Commonly used encapsulant ingredients are skim milk, lactose, maltodextrin, sucrose with different protective abilities. Based on this, it is necessary to know the effect of the type of encapsulant material on the viability of *L. plantarum* 1 RN9.

METHODS

L. plantarum 1 RN9 isolate was encapsulated using freeze drying method by using 3 types of encapsulant ingredients namely skim milk, lactose and maltodextrin. The initial stage of encapsulation is the multiplication of Lactobacillus plantarum 1 RN9 cells in stages. A total of 3 beads *L. plantarum* 1 RN9 in 20% glycerol stock were put into 5 ml sterile MRSB and incubated at 37ºC for 24 hours. Furthermore, the culture in MRSB was inoculated in 45 ml of sterile MRSB as a multiplication of step 1. Phase 2 propagation was done by inoculating 45 ml of fresh *L. plantarum* 1 RN9 culture in 950 ml of sterile MRSB so that at the end of the 3rd propagation, 1000 ml of fresh *L. plantarum* 1 RN9 inoculation was obtained. *L. plantarum* 1 RN9 culture was further separated between cell mass and growth media by centrifuge use at 4ºC at a speed of 10000 rpm for 10 minutes. Cell washing was done 3 times using 0.85% sterile NaCl. The obtained cell mass is then dissolved in 10% encapsulant and in the shaker for 1 hour. The purpose of the shaking process with the shaker so that the encapsulant material can enter and dispersed envelops all cell masses evenly so as to provide maximum protective effect. Furthermore, the encapsulant solution is rapidly frozen for 24 hours at -80ºC until perfect ice is formed. The frozen material is then dried using the freeze drying tool. The principle of drying using the freeze drying method involves the working principle of fluid in removing water or other solvents from frozen products by the sublimation process. Sublimation occurs when frozen liquid turns directly into gas without passing through the liquid phase. After the dry material is further destroyed and analyzed. The stages of making *L. plantarum* 1 RN9 encapsulated dry culture can be seen as in Figure 1 and the process of making dry culture of LAB by freeze drying can be seen in Figure 2.

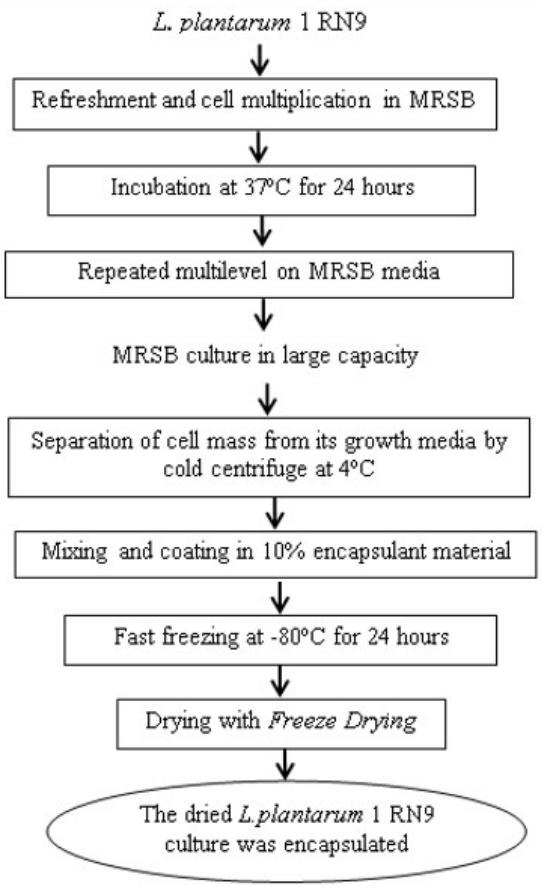

Figure 1. Flowchart of encapsulated dry culture

Figure 2. Freeze drying process of *L. plantarum* 1 RN9 culture.

Yield (Vogel *et al*., 1996)

The yield is the percentage of product by comparing the initial weight of the material with the final weight. The yield of dry culture *L. plantarum* 1 RN9 is the percentage volume/weight of *L. plantarum* 1 RN9 dry culture produced from the amount of encapsulant material and mass of wet cells used in the freeze drying process.

$$\text{Yield (\% v/b)} = \frac{\text{Weight of dry cultured obtained}}{\text{Encapsulant volume and wet cell mass}} \times 100\ \%$$

Viability of *Lactobacillus plantarum* 1 RN9 (Harrigan, 1998)

The number of lactic acid bacteria during freeze drying needs to be calculated to determine the effect of the freeze drying process and protective material (encapsulant) on culture preservation. Determination of total LAB using the plate count method, which is when the culture before freeze drying is taken as much as 1 ml of culture, then diluted until dilution 10^{-8}, as much as 0.1 ml of the dilution results are planted into a solid MRSA media then incubated at 37^{o}C for 48 hours. The same is done in freeze-dried cultures. 0.1 gram of dry culture is taken and diluted to 10^{-8}, then 0.1 ml of dilution is taken and into a solid MRSA medium and then incubated at 37°C for 48 hours. Total LAB before and after the freeze drying process were compared.

LAB resistance to *freeze drying* (Δ log reduction) :

= log (Σ colonies incubated before FD) - log (Σ colonies incubated after FD)

RESULT AND DISCUSSION

The freeze drying process of *L. plantarum* 1 RN9 isolates was carried out using a freeze drier with a process temperature of -50^{o}C; 0.01 Mpa for 2 days. The basis for selecting the Freeze drying method is because this method does not use high temperatures so it affects the LAB viability which is quite high. While the use of encapsulant materials is due to the fact that this material is food grade so it is safe for food applications, has a strong protective power and a relatively cheap price compared to other materials.

Freeze drying, also known as lyophilization, is a drying technique where the product is frozen first then using energy in the form of heat and at low pressure, the water content of the material in the form of ice will be evaporated by sublimation. The principle of drying using the freeze drying method involves the working principle of fluid in removing water or other solvents from frozen products by the sublimation process. Sublimation occurs when frozen liquid turns directly into gas without passing through the liquid phase. Freeze drying is the best drying method to prevent chemical changes and minimize nutrient loss during the drying process. Freeze dried culture has a clear appearance, is dense and has good cell viability. Freeze drying can maintain the rigid form of the dried material so that it can produce a porous, non-wrinkled dry product. Food products that experience freeze drying will lose more than 90% water and because the drying process takes place at low temperatures, the freeze drying method is very safe so that it can produce high-quality dry products compared to other drying methods (Winarno 1993).

Yield

Yield is the ratio between dry cultures after encapsulation compared with the mass weight of wet cells produced after cultivation. The average value of dry culture encapsulated *L. plantarum* 1 RN9 culture can be seen in Figure 3.

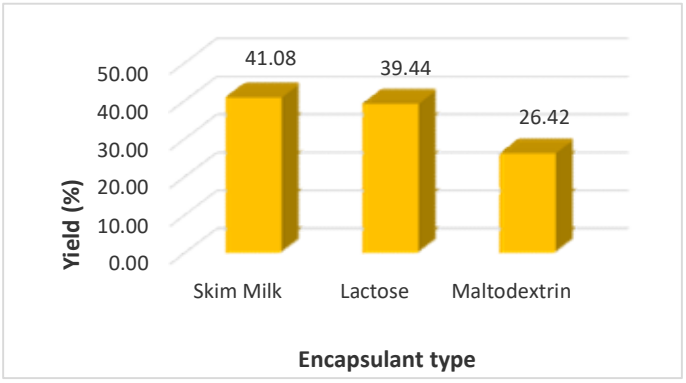

Figure 3. Average yield of dry cultures encapsulated *L. plantarum* 1 RN9 cultures

Based on Figure 3 the average yield of the encapsulated *L. plantarum* 1 RN9 culture produced ranged from 26.42% to 41.08%, which statistically showed a significant difference (P> 0.05) between these treatments. The highest yield was obtained in the skim milk encapsulant material by 41.08% then lactose 39.44% and finally maltodextrin by 26.42%.

Viability of *Lactobacillus plantarum* 1 RN9

Lactic acid bacteria endurance after the freeze drying process is determined by comparing the total LAB before and after freeze drying. Lactic acid bacteria resistance data after the freeze drying process is needed to determine the effect of the type of protective material that is able to maintain the viability of LAB after going through the freeze drying process. The average value of total LAB before and after the freeze drying (FD) process can be seen in Figure 4.

Based on Figure 4. shows that the dry culture of *L. plantarum* 1 RN9 after freezing drying with a protective agent for skim milk, lactose and maltodextrin decreased cell number but still has high viability except with a skim milk protective material. The results of a study conducted by Carvalho *et al*., (2002) showed that there was no significant difference in the use of various encapsulant compounds to cell resistance during freeze drying but still produced a high level of cell resistance. In the use of skim milk encapsulants, the total LAB increased by 1 log cycle ie from 10.3 log CFU/g to 11.3 log CFU/g. While the use of lactose and maltodextrin encapsulant materials on average decreased viability by 2.3 to 2.5 log cycles of CFU/g.

The effect of encapsulant protection from each LAB culture showed a different response. The number of lactic acid bacteria after freeze drying for all types of protective materials ranged from 10^8-10^{11} CFU/g. In research conducted by Harmayani *et al*., (2001) decreased the number of bacteria before and after freeze drying by 2 log cycles, from 10^{13} CFU/ml to 10^{11} CFU/ml. The number of live lactic acid bacterial cells in dry culture is high enough to be able to provide health effects for the body. According to the International Dairy Federation, the minimum number of live probiotic cells in dairy products to play a role in improving digestive health is 10^6 CFU/g cells per gram of product (Sultana *et al*., 2000).

Decreased cell resistance during freeze drying may be due to the effects of the freezing and drying processes. Freezing processes that play a role in reducing cell resistance as occurs in the cell cooling stage

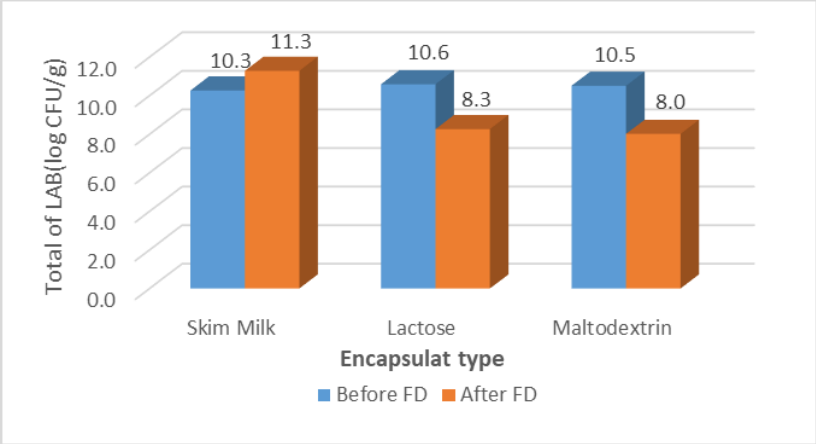

Figure 4. Average total LAB before and after the freeze drying (FD)

and cooling medium to reach the freezing point, intracellular and extracellular ice formation, an increase in solution concentration, storage and thawing (Johnson & Etzel 1995). The freezing process causes the cell to lose its stability so that it becomes easily damaged during drying. The main factors causing damage due to drying of bacterial cells due to osmotic shock with membrane damage and the transfer of hydrogen bonds that affect the properties of hydrophilic macromolecules in the cell (Ray 1993). Besides the decrease in cell viability during the freeze drying process is also due to the reduction in water in the drying process. Drying material can cause loss of water from the material so that the concentration of biomolecules and ions in the cell increases and causes cellular activity to stop, at this time the cell is suffering from stress (Novelina 2005).

CONCLUSION

From the results of the study it can be concluded that:

1. Skim milk, lactose dan maltodextrin as encapsulant material has a very significant effect on the yield and viability of *L. plantarum* 1 RN9 dry culture during the freeze drying process.
2. The 10% skim milk encapsulant material gives the best results compared to lactose and maltodextrin, with a yield reaching 41.08% and the viability of *L. plantarum* 1 RN9 increases by 1 log cycle ie from 2.1 x 10^{10} CFU/g to 2.0 x 10^{11} CFU / g.

Further research needs to be done to test changes in the characteristics of *L. plantarum* 1 RN9 probiotics after undergoing the process of freeze drying.

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