



Resistance of *Lactobacillus fermentum* InaCC B1295 Encapsulated in Microcrystalline Cellulose from Palm Leaf Waste to Acidic Conditions Across Various Temperatures and Storage Durations"

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ABSTRACT

Lactobacillus fermentum InaCC B1295 is a probiotic bacterium with limited tolerance to acidic environments, bile fluids, and high temperatures, necessitating physical protection via microcrystalline cellulose (MCC) encapsulation derived from palm leaf waste. This study aimed to evaluate the resistance of encapsulated probiotic bacteria under acidic conditions at various temperatures and storage durations to identify the optimal storage temperature. A factorial complete randomized design (CRD) with two factors was employed: storage temperature (room temperature, 4°C, and -18°C) and storage time (0, 7, 14, 21, 28, 35, and 42 days). The results indicated that storage time and the interaction between storage time and temperature significantly affected the total lactic acid bacteria (LAB) count. However, temperature alone and its interaction with storage time did not significantly impact the percentage reduction in the number of encapsulated *Lactobacillus fermentum* InaCC B1295 at pH 2. Overall, encapsulated bacteria stored at various temperatures demonstrated comparable bacterial resistance.

1.1. INTRODUCTION

1.2. Background

Probiotics can provide health benefits if the microorganisms can survive in the human digestive tract, meaning that the bacteria consumed must survive to pass through the small intestine and stomach acid, so the bacteria must survive at a very low pH. [1]. Research [2], states that some environmental factors that do not support bacteria to live, Long storage and low pH in the digestive tract, can cause the viability of probiotic bacteria to decrease. The requirement for a product to be said to be probiotic is that the product contains probiotic bacteria that are still alive in the digestive tract, more than 10⁶ CFU/g or 10⁶ CFU/ml. [3].

The most widely used microorganisms as probiotic agents are *Lactobacillus* and *Bifidobacteria* strains. *Lactobacillus fermentum* InaCC B1295 bacteria obtained from the collection of Prof. Ir. Usman Pato, M.Sc., Ph.D, a professor of microbiology at Riau University. *Lactobacillus fermentum* is one of a class of heterofermentative lactic acid bacteria because, in addition to producing lactic acid, it can also produce acetic acid, succinic

acid, CO₂, bacteriocin, and H₂O₂, which can be antimicrobial. [4]. *Lactobacillus fermentum* is weak in maintaining itself in a very acidic environment, bile fluids, and high temperatures. The optimum pH value that *Lactobacillus* can tolerate is in the pH 3-5 [5]. The availability of probiotics with physical protection is necessary to maintain their life and fight against adverse environmental conditions. Therefore, encapsulation is one technique to preserve the survival of probiotics during processing until they reach the digestive system.

Encapsulation is a coating technique of a material that can maintain the physical, chemical, and biological properties of an active compound or core material by coating it in a coating material [6]. Encapsulant raw materials can be selected from various natural and synthetic polymers, carbohydrate groups such as starch, dextrin, pectin, sucrose, cellulose, chitosan, alginate, and carrageenan, while lipid groups such as wax, paraffin, monoglycerides and diglycerides, and proteins such as milk, gluten, casein, gelatin, and albumin. One of the carbohydrate encapsulants that has the opportunity to be developed is Microcrystalline Cellulose (MCC) [7].



MCC is a material composed of cellulose fibrils >100 nm in size that can be isolated from wood-based fibers. One of the agricultural products that contain cellulose is palm leaf waste. Research results [8] showed that palm leaves contain 27.95% cellulose, 21.1% hemicellulose, 16.9% lignin, 4.48% ash, 5.3% crude protein, and 0.6% silica. The high cellulose content in palm leaves can be used as MCC as an encapsulant material for *Lactobacillus fermentum* bacteria.

1.3. Literature Study

Lactobacillus fermentum is included in the heterofermentative lactic acid bacteria group because, in addition to producing lactic acid, it also produces acetic acid, succinic acid, CO₂, bacteriocin, and H₂O₂, which can be antimicrobial [4]. *Lactobacillus fermentum* is weak in maintaining itself in a very acidic environment, in bile fluids, and at high temperatures. Encapsulation can provide probiotics with physical protection.

Encapsulation is a technique of coating a material that can maintain the physical, chemical, and biological properties of an active compound or core material by coating it in a coating material [6]. An encapsulant consists of a core (core substance) and a coating material (encapsulant). Factors that influence the success of encapsulation include the physicochemical properties of the core material or active substance, the coating material used, the stage of the encapsulation process, the nature and walls of the microcapsules, and the manufacturing conditions (wet or dry) [6]. Several parameters can be used to assess the success of probiotic encapsulation, including probiotic cell resistance, cell release ability and encapsulant solubility, granule shape, encapsulant density, number of cells in the granule, encapsulant hardening settings, and encapsulant dispersion in the product [9].

Microcrystalline Cellulose (MCC) is cellulose that undergoes fiber separation treatment into microfibrils that are >100 µm in size (Winuprasith and Suphantharika, 2013). MCC is currently a material of interest because it has a specific surface area, high strength and stiffness, low weight, and is biodegradable and renewable [10]. These characteristics give MCC good mechanical properties, so it has the potential to be used in the composite industry, automotive, pulp and paper, electronics, paints, coatings, and so on. MCC can be produced through several methods, especially mechanical methods such as homogenization, microfluidization, microgrinding, refining, ultrasonication, cryocrushing, and electrospinning [11].

1.4. Research Objectives

This study aimed to determine the effect of the resistance of probiotic bacteria encapsulated with microcrystalline collagen from palm leaf waste to acid conditions at various temperatures and storage times to get the most appropriate temperature.

2. Materials And Methods

2.1. Preparation of Tools and Materials

2.1.1 Equipment Sterilization

The equipment was washed thoroughly and sterilized using an oven and autoclave at 121°C pressure 1 atm for 15 minutes [12].

2.1.2 Preparation of MRS Broth Media

Preparation of MRS Broth media refers to [12]. MRS broth as much as 13.78 g was dissolved with distilled water to a volume of 250 ml. The solution was distributed into 18 test tubes of 5 ml

and closed using a setup. Separate 9 test tubes for control and 9 to add 37% HCL and set to pH = 2. Sterilize in an autoclave at 121°C pressure 1 atm for 15 minutes.

2.1.3 MRS agar Media Preparation

Preparation of MRS Agar media refers to [13]. As much as 68.2 g of MRS agar was dissolved in distilled water to a volume of 1,000 ml and stirred. The media was heated on a hot plate and stirred using a magnetic stirrer until homogeneous. Sterilize with an autoclave at 121°C pressure 1 atm for 15 minutes. Pour into a Petri dish sterilized as much as ± 15 ml, then cover and leave until solid.

2.1.4 Preparation of 0.85% Physiological Salt

The preparation of physiological saline refers to [13]. NaCl as much as 8.5 g was dissolved with distilled water to a volume of 1,000 ml and put into test tubes of 5 ml each and then closed using a setup. Furthermore, sterilization was done with an autoclave at 121 ° C pressure 1 atm for 15 minutes.

2.1.5 Bacterial Rejuvenation

Bacterial rejuvenation refers to [13]. Culture isolates of *Lactobacillus fermentum* InaCC B1295 were inoculated with as much as one needle ose into a test tube containing 5 ml MRS broth media and then stirred with a vortex. Then, incubation was carried out at 37°C for 24 hours in an incubator so that the active culture stock could be obtained, which was marked by a change in the color of the media to cloudy.

2.2. Preparation of Encapsulant from MCC of Palm Leaf Waste

2.2.1 Preparation of Microcrystalline Cellulose

The manufacture of Microcrystalline Cellulose refers to [14]. Palm leaves are cut into small pieces with a length of ±0.5-1 cm, then washed with water, then boiled in boiling water (100°C) for 1 hour, after which it is filtered and washed with water until clean, then dried in an oven 60°C for 4 hours. The dried fibers were put into a glass goblet, and then 6% KOH was added to as much as 1,000 ml and soaked at room temperature for 12 hours. After that, the fibers were washed with water for three rinses. Furthermore, the fibers were soaked using a hypochlorite solution for 5 hours, and then the leaf fibers were filtered and washed with water until the pH was neutral (pH = 7).

Furthermore, the palm leaf fibers were dried using an oven at 60°C for 4 hours until the moisture content was ± 5%, and then mashed using a blender until smooth. After that, they were sieved using an 80 mesh sieve to obtain leaf fiber powder. Furthermore, they were sent to Nano Center Indonesia Tangerang Banten to be processed into Microcrystalline Cellulose (MCC).

MCC processing was done by milling the sample using a Planetary Ball Mill machine at 8,000 rpm for 60 minutes with a 15-second flame time and a 2-minute rest period to avoid sample damage caused by heat during milling. The milling results were then sieved using a 100 mesh sieve, and the sieve results that passed were MCC.

2.2.2 Preparation of Microcrystalline Cellulose

Preparation of Microcrystalline Cellulose refers to [15], which was modified. Separation of cells and supernatant is done utilizing an active culture of *L. fermentum* InaCC B1295 that has been stored in the refrigerator for 1-hour centrifuge for 15 minutes at a speed of 2,300 rpm, then washed twice using sterile distilled water as much as 5 ml for 5 minutes until clean cells are obtained from the medium. Furthermore, the clean cells were

removed by adding phosphate buffer 1: 1 with the cells obtained, then the cells obtained were put into a clean container and stored at 4°C in the refrigerator.

2.2.3 Preparation of Polyvinyl Alcohol Solution 8%

The preparation of the 8% polyvinyl alcohol solution refers to [15], which was modified. The preparation of polyvinyl alcohol (PVA) solution was carried out by weighing 96 g of PVA, then adding 1,104 ml of distilled water and heated at 100°C with a magnetic stirrer until dissolved so that an 8% PVA solution was obtained.

2.2.4 Preparation of Sterile Microcrystalline Cellulose Hydrogel

The preparation of this solution refers to [15]. Preparation of sterile MCC hydrogel was done by mixing 8% PVA and MCC by putting 250 g of MCC powder in 250 ml of PVA, then heated with a hot plate and magnetic stirrer and then sterilized with an autoclave at 121 ° C with a pressure of 1 atm for 15 minutes.

2.2.5 Lactic Acid Bacteria Encapsulant Preparation

Preparation of LAB encapsulant refers to [15], which was modified. 40 ml of cell biomass was added to 40 ml of sterile MCC hydrogel, then stirred using a stir bar until well mixed, and the encapsulated LAB was ready for use.

2.2.6 Storage

Storage was carried out by putting each 2 ml of encapsulated LAB into a 5 ml cryovial, then storing at room temperature, 4°C and freezer temperature, then observing the activity test of encapsulated LAB against acid (pH 2) on storage days 0, 7, 14, 21, 28, 35 and 42.

2.3. Observation

Observations made on the encapsulant included MCC particle size (length, width, height, and diameter) and MCC hydrogel viscosity. Total bacteria of *Lactobacillus fermentum* InaCC B1295 and resistance test of *Lactobacillus fermentum* to low pH.

2.3.1. Observation Implementation

a. MCC Particle Size

Characterization techniques are used to determine the size or distribution of MCC particles and bacterial encapsulant characteristics using a particle size analyzer (PSA). MCC powder samples were prepared from palm leaves by dispersing MCC powder from palm leaves into Deionized Water. Vortexed until the MCC powder was dispersed, a 1.0-1.5 ml sample was placed in a disposable cuvette and tested using a Malvern Zetasizer ZS PSA with a 633 nm laser and a 173° detector angle. While the preparation of MCC encapsulant samples from palm leaves was carried out utilizing palm leaf MCC encapsulant gel dispersed into Deionized Water, then vortexed until homogeneous, a sample of 1.0-1.5 ml was placed in a disposable cuvette and tested using PSA Malvern Zetasizer ZS with a 633 nm laser and 173 ° detector angle.

b. Encapsulant Viscosity

Calculation of encapsulant viscosity using an Ostwald viscometer. The determination was made by calculating the time required to flow the encapsulant hydrogel of palm leaf waste MCC in the capillary pipe from a to b. The encapsulant hydrogel was inserted into the viscometer placed on the thermostat. The MCC encapsulant hydrogel was then sucked with a pump until it was above mark a. The liquid was allowed to flow down, and the time required to move from A to B was recorded using a stopwatch. The formula can calculate the calculation of viscosity as in the following equation.

$$\frac{\eta^{\circ}}{\eta} = \frac{P^{\circ} \cdot t^{\circ}}{P \cdot t}$$

Description:

η° = Viscosity of comparison liquid (poise)

η = Viscosity of sample liquid (poise)

P° = Pressure in the comparison liquid (dyne/cm²)

P = Pressure in sample liquid (dyne/cm²)

t° = Comparison liquid flow time (second)

t = Sample liquid flow time (second)

c. Total Bacteria *Lactobacillus Fermentum* InaCC B1295

The total LAB counts were determined using the spread surface plate method. The number of bacteria was analyzed after the medium was incubated for 24 hours at 37°C. Calculating the number of LAB was done by taking 0.1 ml of encapsulated LAB sample using a pump pipette, then put into 1 ml of 0.85% physiological saline for dilution 10-1 and continued until dilution 10-7. Furthermore, 0.1 ml of LAB samples were taken from dilutions 10-5 to 10-7 to be inoculated on MRS agar media by dripping the sample on a cup containing MRS agar and then leveled on the entire surface of the medium with a hockey stick that had been sterilized by burning over a bunsen flame [16].

This inoculation process is carried out in a sterile room, namely laminar airflow. Petri dishes that have been inoculated are then incubated in an incubator for 48 hours at 37°C upside down to avoid dripping water that may adhere to the inner wall of the cup lid. LAB colonies that grow will be counted using a colony counter. The formula calculates the calculation of total LAB as in the following equation.

$$\begin{aligned} & \text{LAB count per ml} \\ & = \text{Number of colonies} \times \frac{1}{\text{Dilution Factor}} \times 10 \end{aligned}$$

Description:

Total LAB expressed in log cfu/ml

d. Resistance test of *Lactobacillus fermentum* to low pH Viability of *Lactobacillus fermentum* InaCCB1295 bacteria

The microbiological test used the spread surface plate method. The number of bacteria was analyzed after the medium was incubated for 24 hours at 37°C. The calculation of the number of LAB was carried out by taking 0.1 ml of encapsulated LAB samples in the control solution and the solution that had been included in the acidic liquid (pH = 2) using a pump pipette, then put into 1 ml of 0.85% physiological saline for dilution 10-1 continued until dilution 10-7. Furthermore, 0.1 ml of encapsulated LAB cells were taken from dilutions 10-5 to 10-7 to be inoculated on MRS agar media by dripping the sample on a cup containing MRS agar and then leveled on the entire surface of the medium with a hockey stick that had been sterilized by burning over a bunsen flame.

The inoculation process is carried out in a sterile room, namely laminar airflow. Petri dishes that have been inoculated are then incubated in an incubator for 48 hours at 37°C upside down to avoid dripping water that may adhere to the inner wall of the cup lid. A colony counter will count LAB colonies that grow [16]. The formula calculates the calculation of LAB viability as in the following equation.

$$\text{LAB viability (\%)} = \frac{\text{LAB count at acidic pH}}{\text{Number of LAB in the Control}} \times 100$$

e. Decrease in *Lactobacillus fermentum* InaCC B1295 bacteria

A total of 1 ml of culture that had been refreshed in 5 ml MRS broth for 24 hours each was inoculated into MRS broth that had been adjusted to pH = 2 using 37% HCl, then incubated for 5 hours at 37°C. At the beginning and end of incubation (0 and 5 hours), the total number of LAB was calculated using the cup count method on MRS agar media [17]. The formula for calculating the decrease in the number of LAB is as in the following equation.

$$\text{LAB Reduction} = \text{number of LAB at control pH} - \text{number of LAB at pH}$$

Description:

LAB count reduction expressed in log CFU/g.

2.4. Data Analysis

The data obtained were then analyzed statistically using analysis of variance (ANOVA). The results of the analysis of the data were obtained if $F_{\text{count}} \geq F_{\text{table}}$, then further tests were carried out with Duncan's New Multiple Range Test (DNMRT) at the 5% level.

3. RESULT AND DISCUSSION

3.1. Microcrystalline Cellulose (MCC) Particle Size

Particle size is a geometric characteristic typically assigned to material objects ranging in size from nanometers to millimeters. The larger the mesh screen size number, the finer the material size. The test results of MCC from palm leaves using PSA can be seen in Table 1, Figures 1 and 2.

Table 1. MCC measurement results using PSA

Sample	Size d.nm	Intensity %	St Deviation d.nm	Average Size d.nm	Pdl
MCC Powder	Peak 1 1765	34.4	205.0	1.949	0,527
	Peak 2 972,5	32.8	78.20		
	Peak 3 3112	32.8	440.5		
MCC Encapsulant	Peak 1 2035	32.2	110.4	1.916	0,515
	Peak 2 3687	28.2	371.2		
	Peak 3 26,07	23.1	2673		

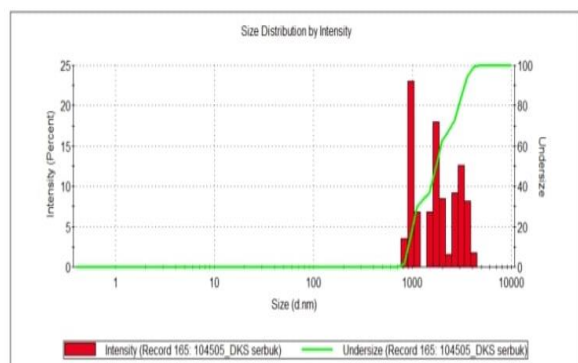


Figure 1. Testing results of MCC powder from oil palm leaves using PSA

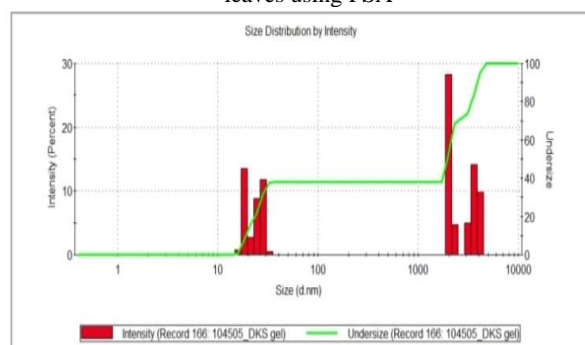


Figure 2. Test results of MCC encapsulant from palm leaves using PSA

From PSA testing, it is known that the size of MCC powder from palm leaves and encapsulant gel MCC palm leaves is relatively the same, namely peak 1 = 1765 nm, peak 2 = 972.5 nm and peak 3 = 3112 nm with an average of 1,949.8 nm. For the encapsulant gel sample, peak 1 = 2035 nm, peak 2 = 3687 nm,

and peak 3 = 26.07 nm with an average of 1,916.02nm (Figures 1 and 2).

PSA measurements are good if the Polydispersity Index (Pdl) is 0.3 to 0.4. Pdl value shows particle size uniformity during measurement with a value of 0.1 to 1. The higher the Pdl value, the more non-uniform the particle size of the sample. In this study, Pdl was obtained as 0.52, indicating heterogeneous particle distribution. From the results of the particle size analysis of MCC, the diameter size above 1000 nm was received; these measurement results suggest that the sample is micro-sized, not including nano size. Pdl values close to zero indicate homogeneous particle distribution, while Pdl values exceeding 0.5 indicate that particles have a high degree of heterogeneity. This is thought to occur first due to the inhomogeneity of the sample during preparation. Inhomogeneity in the sample can occur due to brown motion in the sample. Secondly, due to a lack of operating variation [18].

3.2. Encapsulant Viscosity

Viscosity is the viscosity of a fluid caused by the friction force between the molecules that make up the fluid. The results of viscosity measurements using a viscometer can be seen in Table 2.

Table 2. Viscosity of Encapsulant

Sample	Replication	Viscosity (cps)
Encapsulant	1	6755.6
	2	6448.5
Palm Leaf	3	7448.0
Average		6884.03

The data in Table 2 shows that the viscosity of MCC encapsulant from palm leaf waste averaged 6884.03 cps. This indicates that the encapsulant meets the requirements for a good gel preparation

category. A good gel is a gel that is not too liquid and not too thick. This opinion follows (SNI No.16 th. 1996) that a good gel formula has a viscosity value between 2,000-50,000 cps.

3.3 Test of *Lactobacillus Fermentum* Resistance to Low pH

3.3.1 Total Bacteria *Lactobacillus Fermentum*

Total lactic acid bacteria is the number of lactic acid bacteria that grow after encapsulation. The variance results showed that the treatment of storage duration and the interaction between temperature and storage duration had a significant effect ($P>0.05$) on the total lactic acid bacteria produced. Still, the storage temperature treatment had no significant effect ($P<0.05$) on the total *Lactobacillus fermentum* bacteria produced. The average total bacteria of *L. fermentum* can be seen in Table 3, while the graph is presented in Figure 3..

Table 3. Average Total *Lactobacillus fermentum* InaCC B1295 at pH 2 with Various Temperature and Storage Time (CFU/ml)

Storage Time (B)	Total BAL (CFU/ml)		
	Room Temperature (A1)	Temperature 4°C (A2)	Temperature -18°C (A3)
Hari ke-0 (B1)	9.85 ^a	9.89 ^a	9.88 ^a
Day-7 (B2)	9.53 ^b	9.54 ^b	9.69 ^{ab}
Day-14 (B3)	9.38 ^c	9.41 ^c	9.46 ^c
Day-21 (B4)	9.25 ^d	9.28 ^d	9.25 ^d
Day-28 (B5)	9.21 ^e	9.27 ^{de}	9.19 ^{ef}
Day-35 (B6)	9.18 ^{ef}	9.22 ^{ef}	9.10 ^f
Day-42 (B7)	9.13 ^f	9.16 ^f	9.08 ^f

Notes: Numbers followed by small letters in the same row and column indicate significant differences ($P<0.05$) according to the DNMR test at the 5% level.

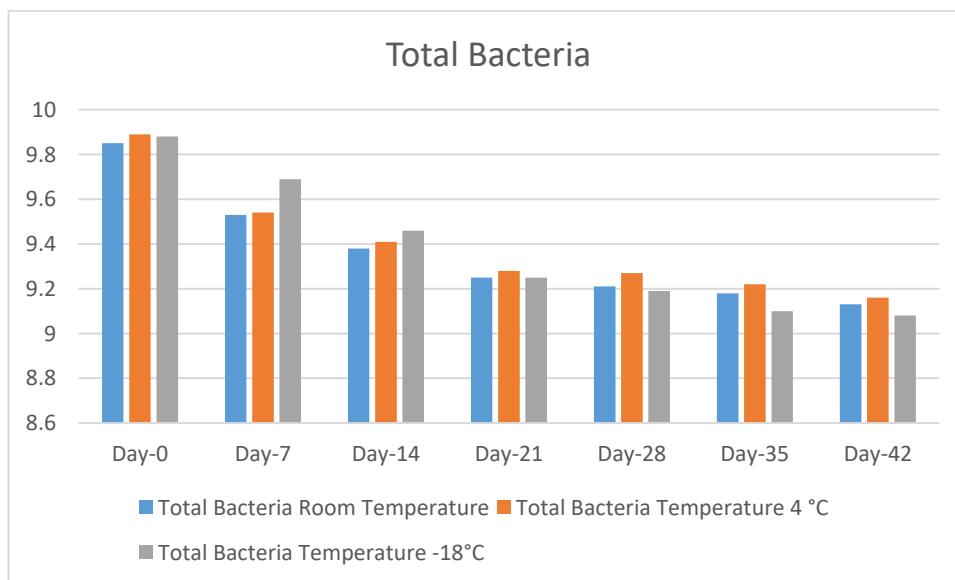


Figure 3. Total *Lactobacillus fermentum* InaCC B1295 at pH=2 with Various Temperatures and Storage Time

The data in Table 3 and Figure 3 show that the total lactic acid bacteria produced significantly differs from one treatment to another at the storage time and its interaction. In general, the interaction between temperature and storage time caused a decrease in total lactic acid bacteria. Table 4 shows that the total LAB encapsulated with palm leaf MCC ranged from 9.08-9.89 CFU/ml. The longer the storage time of probiotic bacteria encapsulated with MCC hydrogel from palm leaves, the lower the total probiotic bacteria that grow. This decrease is due to the longer storage time, the MCC hydrogel layer on the encapsulant that envelops bacterial cells has decreased stability so that the bonds that occur between MCC begin to stretch and bacteria quickly come out of the dressing, which results in bacteria experiencing direct contact with an acidic environment that causes cell damage to bacteria and affects bacterial growth. The results of this study agree with [19], which states that the

robustness of the encapsulant matrix formed will decrease with the length of storage.

The data in Table 3 shows that the temperature treatment is not significantly different from the total lactic acid bacteria produced. This means encapsulating bacteria with MCC hydrogel from palm leaves has the same good survival at room temperature storage, 4°C, and freezing temperatures. This is because MCC hydrogels contain cellulose fibrils composed of anhydroglucopyranose units connected with β -1,4-glycosidic bonds to form an unbranched macromolecular chain and have monomers arranged linearly then between the polymers. There are hydrogen bonds that connect one polymer to another. This causes MCC hydrogels to have a compact structure and strong physical properties, able to form networks and have a strong binding ability to protect probiotic bacteria from environmental factors that affect bacterial growth such as changes in

temperature, pH, and salt content. The properties of cellulose fibers are high tensile strength, the ability to form strong networks, binding solid ability, and relatively colorless [20].

3.3.2 Viability of *Lactobacillus Fermentum* InaCC B1295 Bacteria

The viability of *Lactobacillus fermentum* bacteria is the survival of *Lactobacillus fermentum* bacteria in the encapsulant during

storage. The variance results showed that the treatment of storage time variation and temperature variation and the interaction between the two had no significant effect ($P>0.05$) on the viability of *L. fermentum* bacteria. The average percentage of viability of *L. fermentum* can be seen in Table 4, while the graph is presented in Figure 4.

Table 4. Average Viability Percentage of *Lactobacillus fermentum* InaCC B1295 at pH=2 with Variation of Temperature and Storage Time

Storage Time (B)	Viability BAL (%)		
	Room Temperature (A1)	Temperature 4°C (A2)	Temperature -18°C (A3)
Day-0 (B1)	99.83	99.92	99.49
Day-7 (B2)	99.31	99.34	99.31
Day-14 (B3)	99.20	99.58	99.34
Day-21 (B4)	99.53	99.24	99.35
Day-28 (B5)	99.31	99.21	99.28
Day-35 (B6)	99.20	99.17	99.45
Day-42 (B7)	99.63	99.59	99.34

Notes: All treatments had no significant effect according to DNMRT test at 5% level.

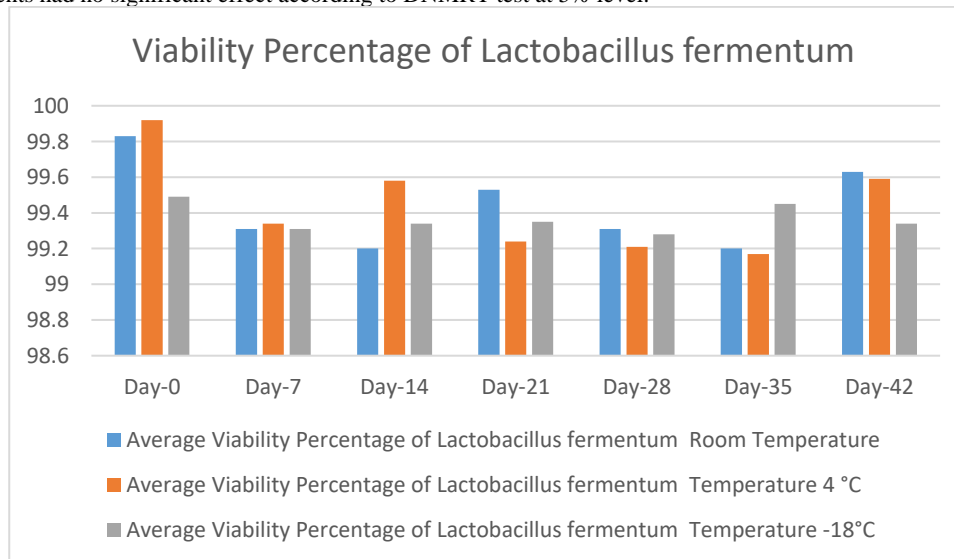


Figure 4. Viability of *Lactobacillus fermentum* InaCC B1295 at pH=2 with Various Temperature and Storage Time

The data in Table 4 and Figure 4 show that the viability of LAB is not significantly different from one treatment to another at temperature and length of storage and their interactions. These results indicate that encapsulation of bacteria with MCC hydrogel from palm leaves has the same good viability at room temperature storage, 4°C temperature, and freezing temperature for 42 days of storage. This is because the temperature difference does not experience direct contact with bacteria because MCC hydrogel, as an encapsulant dressing forms a strong bond enveloping bacterial cells, thus providing protection to bacteria from external influences such as changes in ambient temperature and low pH. Encapsulation aims to protect bacteria from harmful environmental factors such as temperature differences for bacteria [20]. This study is also in accordance with the research results by Ref. [19], which utilizes sodium alginate as a coating. The results showed that the viability of encapsulated cells was

better than cells without encapsulation during 8 weeks of frozen storage, with a decreased viability of *Lactobacillus plantarum* and *Streptococcus thermophilus* without encapsulation decreased by 12% and 25%, respectively.

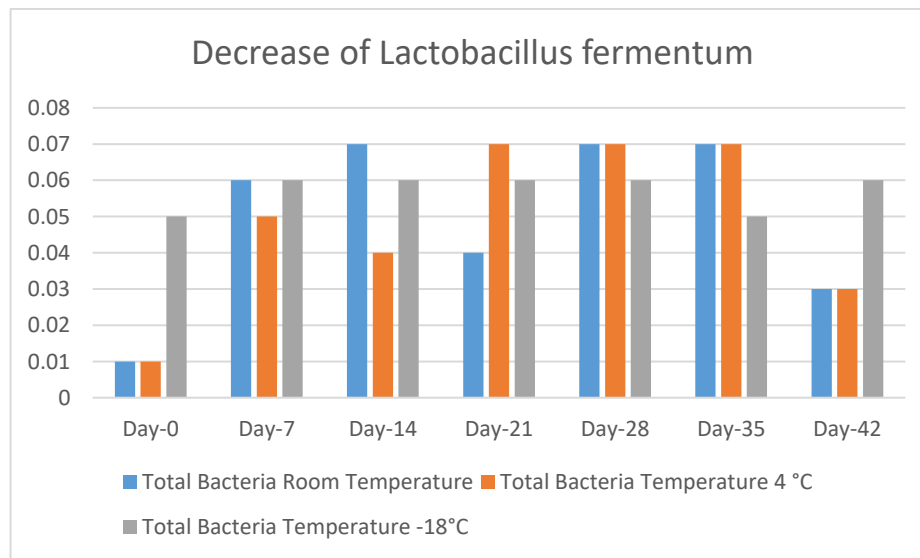
3.3.3 Decrease of *Lactobacillus fermentum* InaCC B1295 Against pH 2

The resistance of *Lactobacillus fermentum* bacteria to pH = 2 can be seen from the amount of bacterial decline after being tested at pH = 2. The decrease in *L. fermentum* bacteria is calculated to determine the number of bacteria that can survive at pH = 2. The variance results showed that the treatment of storage time variation and temperature variation and the interaction between the two had no significant effect ($P>0.05$) on the decrease in *Lactobacillus fermentum* bacteria at pH 2. The average decrease in *L. fermentum* to pH 2 can be seen in Table 5, and the graph is presented in Figure 5.

Table 5. Average Decrease of *Lactobacillus fermentum* InaCC B1295 at pH=2 with Various Temperature and Storage Time (CFU/ml)
Penurunan BAL (CFU/ml)

Treatment	Penurunan BAL (CFU/ml)		
	Room Temperature (A1)	Temperature 4°C (A2)	Temperature -18°C (A3)
Day-0 (B1)	0.01	0.01	0.05
Day-7 (B2)	0.06	0.05	0.06
Day-14 (B3)	0.07	0.04	0.06
Day-21 (B4)	0.04	0.07	0.06
Day-28 (B5)	0.07	0.07	0.06
Day-35 (B6)	0.07	0.07	0.05
Day-42 (B7)	0.03	0.03	0.06

Notes: All treatments had no significant effect according to the DNMRT test at 5% level.

**Figure 5.** Decrease of *Lactobacillus fermentum* InaCC B1295 at pH=2 with Various Temperature and Storage Time

The data in Table 5 and Figure 5 show that the LAB decrease is not significantly different between one treatment and another treatment at temperature and storage time and their interactions at pH = 2 conditions. This fact shows that bacterial encapsulation with MCC from palm leaves has almost the same good resistance when stored at room temperature, 4°C, and freezing temperature for 42 days of storage tested at pH=2. This is due to the difference in temperature, and a bad environment does not cause direct contact with bacteria because the mixing between MCC and PVA that envelops bacteria produces strong physical bonds for encapsulants, produces a strong gel, has a strong binding ability, and high flexibility so as to protect bacteria from environmental factors that affect bacterial resistance such as temperature, pH and salt content [20]. This follows research by Ref. [19], which utilized sodium alginate as an encapsulant. The results showed that the encapsulant was resistant and could protect LAB from freezing temperatures for 8 weeks of storage with a decrease in *Lactobacillus plantarum* and *Streptococcus thermophilus* of only 2% and 14%, respectively. It can be seen from the results of the study [19] that the decrease was greater than in this study. This is because alginate, as an encapsulant base material, is easily torn when exposed to freezing temperatures (below 0°C), so the possibility of LAB cell damage is greater. Damage due to the freezing process results in changes in cell morphology, cell structure, changes in LAB cell function, and changes in LAB

genetic stability or regrowth capacity, while in this study, cellulose-based encapsulant base material is used, which has a compact structure and strong physical properties, can form networks, and has a solid binding ability, to protect bacteria from environmental factors that affect bacterial growth such as changes in temperature and pH.

The data in Table 5 shows the decrease in LAB obtained from the difference between the number of LAB before and after testing under acidic conditions (PH=2) at various temperatures and storage times and their interactions. The higher the difference between the decrease in LAB before and after testing, the less effective the encapsulant is in protecting bacteria. The value of the difference in the decrease of bacteria encapsulated with MCC hydrogel from palm leaves for storage time from day 0 to day 42 at room temperature, 4°C temperature, and freezing temperature has an average of 0.05 CFU/ml, 0.04 CFU/ml and 0.05 which means that encapsulation of bacteria with MCC is effective in protecting bacteria from acidic conditions (pH = 2) until the storage time of day 42. This is because MCC hydrogel as an encapsulation dressing, forms a gel layer that envelops bacterial cells, thus protecting bacteria from external influences such as changes in ambient temperature and low pH. This follows [21], which states that encapsulation aims to protect bacteria from harmful environmental factors.

4. CONCLUSION

The results indicated that storage time and the interaction between storage time and temperature significantly affected the total LAB count. However, neither storage temperature alone nor its interaction with storage time significantly influenced the percentage reduction in the number of *Lactobacillus fermentum* InaCC B1295 encapsulated in MCC from oil palm leaves at pH 2. Encapsulated bacteria stored at various temperatures exhibited consistently strong resistance

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