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Resistance of Lactobacillus fermentum Inacc B1295 Encapsulated with Microcrystalline Cellulose from Palm Leaf Waste to Acidic Conditions at Varied Temperatures and Storage Time

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A B S T R A C T

Lactobacillus fermentum InaCC B1295 bacteria are probiotic bacteria that have weaknesses in defending themselves in a very acidic environment, in bile fluids, and at high temperatures so that physical protection was needed in the form of Microcrystalline Cellulose encapsulant from palm leaf waste. The purpose of this study was to determined the effect of the resistance of probiotic bacteria encapsulated with Microcrystalline Cellulose from palm leaf waste to acidic conditions at various temperatures and storage times, get the most appropriate temperature. This study used a factorial complete randomized design (CRD) consisting of two factors. Factor A was storage temperature at room temperature, 4°C, and freezer temperature (-18°C). Factor B was the storage time of 0, 7, 14, 21, 28, 35, 42 days. The results showed that the treatment of storage time and the interaction of storage time and storage temperature had a significant effect on total LAB, but the treatment of temperature and storage time and their interaction had no significant effect on the percentage and decrease in the number of Lactobacillus fermentum InaCC B1295 encapsulated using MCC from oil palm leaves at pH = 2, encapsulated bacteria from MCC of oil palm leaves stored at various temperatures produced the same good 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, can also produce acetic acid, succinic acid, CO_2, bekteriosin, and H2O2 which can be antimicrobial. [4]. *Lactobacillus fermentum has a weakness in maintaining itself in a very acidic environment, in bile fluids, and at high temperatures. The optimum pH value that Lactobacillus can tolerate is in the range of pH 3-5* [5]. The availability of probiotics with physical protection is necessary to maintain their life and fight against adverse environmental conditions, therefore one technique to maintain the survival of probiotics during processing until they reach the digestive system is encapsulation.

Encapsulation is a coating technique of a material that is able to 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 content of cellulose in palm leaves has the potential to 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, bekteriosin, and H2O2 which can be antimicrobial [4]. Lactobacillus fermentum has a weakness in maintaining itself in a very acidic environment, in bile fluids, and at high temperatures. The availability of probiotics with physical protection can be obtained by encapsulation.

Encapsulation is a technique of coating a material that is able to 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, has a low weight, is biodegradable and renewable [10]. These characteristics make MCC have 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

The purpose of this study was to determine the effect of the resistance of probiotic bacteria encapsulated with Microcrystalline Cellulose from palm leaf waste to acid conditions at various temperatures and storage time to get the most appropriate temperature.

2. Materials And Methods

2.1. Preparation of Tools and Materials

2.1.1 Equipmen Sterilization

The equipment used 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 test tubes 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]. MRS agar as much as 68.2 g was dissolved with 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 autoclave at 121°C pressure 1 atm for 15 minutes. Pour into a Petri dish that has been 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 carried out 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 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 was 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 (100ºC) for 1 hour, after which it is filtered and washed with water until clean, then dried in an oven at 60ºC for 4 hours. The dried fibers were put into a glass goblet, then 6% KOH was added as much as 1,000 ml and then soaked at room temperature for 12 hours. After that, the fibers were washed with water for three rinses. Furthermore, the washed fibers were then soaked using hypochlorite solution for 5 hours, 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 60ºC for 4 hours until the moisture content was ± 5% and then mashed using a blender until smooth. After that, it was sieved using an 80 mesh sieve so that leaf fiber powder was obtained. Furthermore, it was sent to Nano Center Indonesia Tangerang Banten to be processed into Microcrystalline Cellulose (MCC).

MCC processing was carried out by milling the sample using a Planetary Ball Mill machine at a speed of 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 by means of 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 Polyvinyl alcohol 8% solution refers to [15] which was modified. The preparation of polyvinyl alcohol (PVA) solution was carried out by weighing 96 g of PVA then added 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 5 ml cryovial, then stored 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 particle size (length, width, height and diameter) of MCC, viscosity of MCC hydrogel. 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 to determine the size or distribution of MCC particles and bacterial encapsulant characteristics using Particle Size Analyzer (PSA). Preparation of MCC powder samples from palm leaves was carried out by dispersing MCC powder from palm leaves into Deionized Water, then vortexed until the MCC powder was dispersed, then a sample of 1.0-1.5 ml was placed in a disposable cuvatte 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 by means of palm leaf MCC encapsulant gel dispersed into Deionized Water, then vortexed until homogeneous, then a sample of 1.0-1.5 ml was placed in a disposable cuvatte 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 for the flow of 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 from a to b was recorded using a stopwatch. Calculation of viscosity can be calculated by the formula as in the following equation.

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

Description:
η^{o} = Viscosity of comparison liquid (poise)
η = Viscosity of sample liquid (poise)
P^{o} = Pressure in the comparison liquid (dyne/cm2)
P = Pressure in sample liquid (dyne/cm2)
t^{o}= Comparison liquid flow time (secon)
t = Sample liquid flow time (secon)

c. Total Bacteria Lactobacillus Fermentum InaCC B1295

Total LAB counts used the spread surface plate method. The number of bacteria was analyzed after the medium was incubated for 24 hours at 37°C. Calculation of 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 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 hockeystick that had been sterilized by burning over a bunsen flame [16].

This inoculation process is carried out in a sterile room, namely laminar air flow. 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 calculation of total LAB is calculated by the formula as in the following equation.

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

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 hockeystick that had been sterilized by burning over a bunsen flame.

The inoculation process is carried out in a sterile room, namely laminar air flow. 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 [16]. The calculation of LAB viability is calculated by the formula as in the following equation.

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

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 37ºC. 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 obtained if Fcount ≥ Ftable, 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		

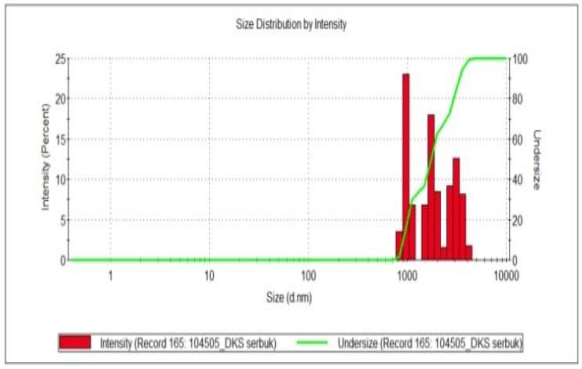

Figure 1. Testing results of MCC powder from oil palm leaves using PSA

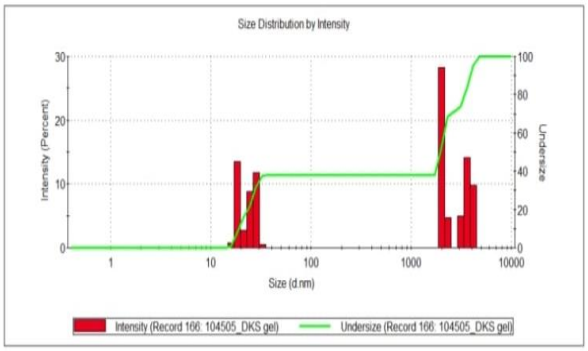

Figure 2. Test results of MCC encapsulant from palm leaves using PSA

From PSA testing, it is known that the size of MCC powder from palm leaves and encapsulant gel MCC palm leaves are relatively the same, namely peak 1 = 1765 nm, peak 2 = 972.5 nm and peak 3 = 3112 nm with an average = 1,949.8 nm while for the encapsulant gel sample, the size of peak 1 = 2035 nm, peak 2 = 3687 nm and peak 3 = 26.07 nm with an average = 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 the uniformity of particle size 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, this indicates heterogeneous particle distribution. From the results of the particle size analysis of MCC, the diameter size above 1000 nm was obtained, these measurement results indicate 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 particles have a high degree of heterogeneity. This is thought to occur firstly: 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 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	Deuteronomy	Viscosity value (cps)
Encapsulant Palm Leaf	1	6755,6
	2	6448,5
	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 shows 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 is in accordance with (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 the encapsulation process. The results of variance 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, but 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 Temperature (A)	Total Bacteria (CFU/ml)						
	Storage Time (B)						
	Day-0 (B1)	Day-7 (B2)	Day-14 (B3)	Day-21 (B4)	Day-28 (B5)	Day-35 (B6)	Day-42 (B7)
Room Temperature (A1)	9,85^{a}	9,53^{b}	9,38^{c}	9,25^{d}	9,21^{e}	9,18^{ef}	9,13^{f}
Temperature 4°C (A2)	9,89^{a}	9,54^{b}	9,41^{c}	9,28^{d}	9,27^{de}	9,22^{ef}	9,16^{f}
Temperature -18°C (A3)	9,88^{a}	9,69^{b}	9,46^{c}	9,25^{d}	9,19^{ef}	9,10^{f}	9,08^{f}

Notes: - Numbers followed by the same lowercase letter on the same line show a noticeable difference (P<0.05) according to the DNMRT test at the level of 5%.

-Numbers followed by the same lowercase letter in the same column are not real (P>0.05) according to the DNMRT test at the level of 5%.

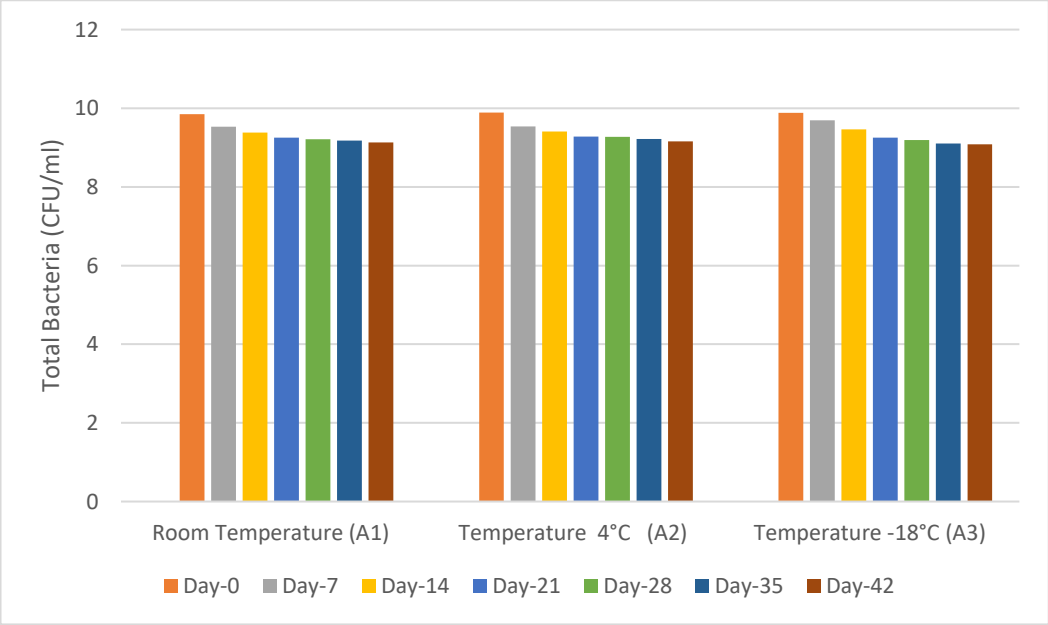

Figure 3. Total Lactobacillus fermentum InaCC B1295 at pH=2 with Various Temperature and Storage Time

The data in Table 3 and Figure 3 show that the total lactic acid bacteria produced is significantly different 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 easily 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 that the encapsulation of bacteria with MCC hydrogel from palm leaves has the same good survival at room temperature storage, 4°C temperature and freezing temperature. 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, so as 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, able to form strong networks, have strong binding ability, and are relatively colorless [20].

3.3.2 Viability of Lactobacillus Fermentum InaCC B1295 Bacteria

Viability of Lactobacillus fermentum bacteria is the survival of Lactobacillus fermentum bacteria in the encapsulant during storage. The results of variance showed that the treatment of storage time variation and storage 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 Temperature (A)	Viability (%)						
	Storage Time (B)						
	Day-0 (B1)	Day-7 (B2)	Day-14 (B3)	Day-21 (B4)	Day-28 (B5)	Day-35 (B6)	Day-42 (B7)
Room Temperature (A1)	99,83	99,31	99,20	99,53	99,31	99,20	99,63
Temperature 4°C (A2)	99,92	99,34	99,58	99,24	99,21	99,17	99,59
Temperature -18°C (A3)	99,49	99,31	99,34	99,35	99,28	99,45	99,34

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

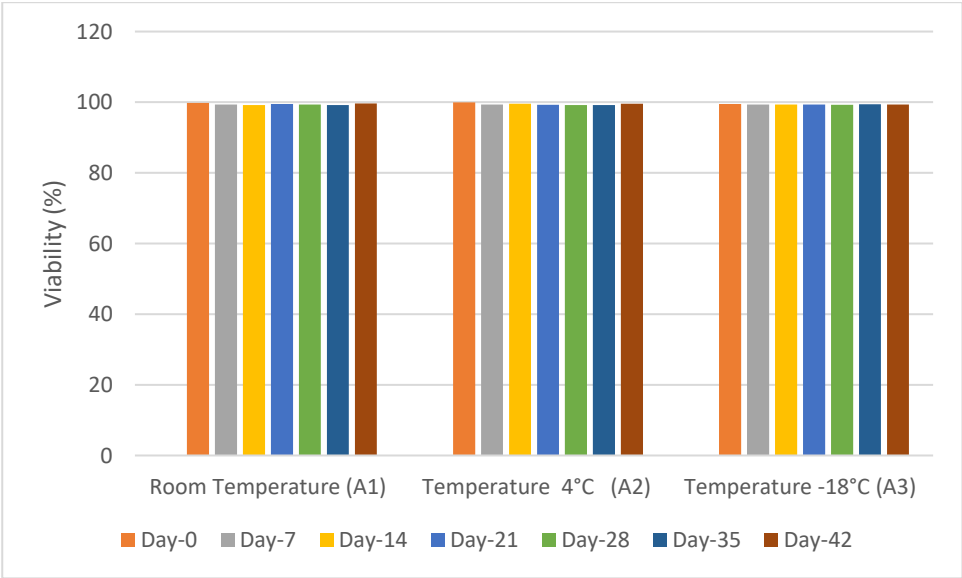

Figure 4. Viability of Lactobacillus fermentum InaCC B1295 at pH=2 with Various Temperature and Storage Time

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 results of research [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 decrease in viability of Lactobacillus plantarum and Streptococcus thermopillus 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 calculation of the decrease in L. fermentum bacteria is done to determine the number of bacteria that can survive at pH = 2. The results of variance showed that the treatment of storage time variation and storage 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)

Storage Temperature (A)	Decrease of Lactobacillus fermentum (CFU/ml)						
	Storage Time (B)						
	Day-0 (B1)	Day-7 (B2)	Day-14 (B3)	Day-21 (B4)	Day-28 (B5)	Day-35 (B6)	Day-42 (B7)
Room Temperature (A1)	0,01	0,06	0,07	0,04	0,07	0,07	0,03
Temperature 4°C (A2)	0,01	0,05	0,04	0,07	0,07	0,07	0,03
Temperature -18°C (A3)	0,05	0,06	0,06	0,06	0,06	0,05	0,06

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

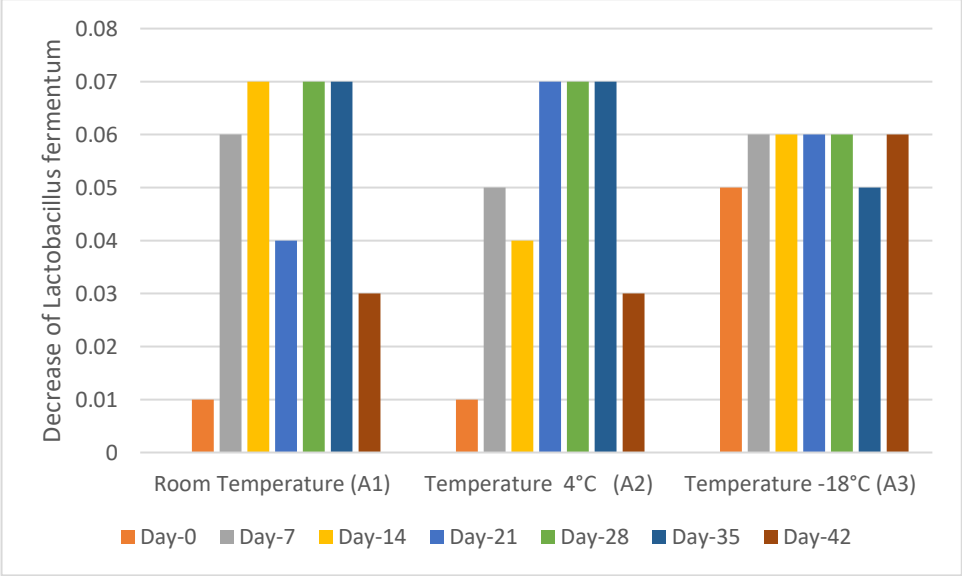

Figure 5. Decrease of Lactobacillus fermentum InaCC B1295 at pH=2 with Various Temperature and Storage Time

The data in Table 5 and Figure 5 show that the amount of 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 bad environment does not occur 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 is in accordance with research [19] which utilized sodium alginate as an encapsulant. The results showed that the encapsulant was resistant and able to protect LAB from freezing temperatures for 8 weeks of storage with a decrease in Lactobacillus plantarum and Streptococcus thermopillus of only 2% and 14%, respectively. It can be seen from the results of the study [19] that the decrease was greater than this study. This is because alginate as an encapsulant base material is easily torn when exposed to freezing temperatures (below 0°C) so that 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, is able to form networks, and has a strong binding ability, so as 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 the number of 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 providing protection to bacteria from external influences such as changes in ambient temperature and low pH. This is in accordance with [21] which states that encapsulation aims to protect bacteria from environmental factors harmful to bacteria.

4. CONCLUSION

The results showed that the treatment of storage time and the interaction of storage time and storage temperature had a significant effect on total LAB, but the treatment of temperature and storage time and their interaction had no significant effect on the percentage and decrease in the number of Lactobacillus fermentum InaCC B1295 encapsulated using MCC from oil palm leaves at pH = 2, encapsulating bacteria from oil palm leaf MCC stored at various temperatures produced the same good bacterial resistance.

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