



Enhancing Probiotic Stability through Alginate–Bengkuang (*Pachyrhizus erosus*) Flour Encapsulation of *Lactiplantibacillus plantarum* SN13T

Siti Auliarasulina¹, Sri Melia^{2*} and Indri Juliyarsi²

¹Magister Program of Animal Science Faculty, Universitas Andalas, Gedung Fakultas Peternakan Limau Manis Padang, West Sumatra, Indonesia, 25163

²Department of Animal Product Technology, Faculty of Animal Science, Universitas Andalas, Gedung Fakultas Peternakan Limau Manis Padang, West Sumatra, Indonesia, 25163

Abstract

To enhance the viability and effectiveness of *Lactiplantibacillus plantarum* SN13T as a probiotic, protection can be provided to the bacteria through encapsulation methods. This study aims to determine the best wall material formulation, consisting of alginate and bengkuang flour (*Pachyrhizus erosus*), in the encapsulation process of *L. plantarum* SN13T. In this research, the encapsulated bacteria (beads) will be tested for their characteristics, including moisture content, bead size and morphology, total lactic acid bacteria, resistance to simulated gastric juice (SGJ) and simulated intestinal juice (SIJ), as well as encapsulation efficiency. The treatments in this study are combinations of 1% alginate wall with bengkuang flour: A = without the addition of bengkuang flour (control), B = 1% bengkuang flour, C = 3% bengkuang flour, and D = 5% bengkuang flour. The results indicate that encapsulation of *L. plantarum* SN13T using alginate and bengkuang flour as wall materials, specifically the use of 1% alginate + 1% bengkuang flour produces the best encapsulation results, with a moisture content of 97.34%, bead size of 2.14 mm, total lactic acid bacteria colony count of 12.88 log CFU/mL, resistance to SGJ of 95.23%, and resistance to SIJ of 95.76%. This demonstrates that encapsulation with a formulation composition of 1% alginate + 1% bengkuang flour provides optimal results for the viability and effectiveness of *L. plantarum* SN13T. Bengkuang flour, which contains starch, is recommended as a wall material for the encapsulation process of probiotic bacteria.

Keywords: Alginate, Encapsulation, Lactic Acid Bacteria, Wall, and Probiotics.

Introduction

Probiotics are living microorganisms that, when consumed in sufficient quantities, provide health benefits to their host [1]. Probiotics play a role in producing vitamins, maintaining intestinal health, producing antimicrobial compounds, supporting lipid metabolism (lowering cholesterol), and preventing carcinogenic compounds [2]. One type of probiotic potentially used is *Lactiplantibacillus plantarum* SN13T, a starter culture of lactic acid bacteria (LAB) screened from stingless honey bees native to West Sumatra. *L. plantarum* SN13T was selected from 23 LAB isolates; this isolate, obtained from honey produced by the species *Tetrigona binghami*, has acid tolerance of 82.75%, bile salt tolerance of

94.44%, and the highest antimicrobial activity against pathogenic bacteria, with better resilience compared to antibiotics [3]. However, probiotics face significant challenges in surviving and providing their benefits to the host because they must endure harsh conditions during production, storage, and digestion, such as exposure to oxygen, heat, low pH, and high bile salt concentrations [4, 5]

To improve the viability and effectiveness of *L. plantarum* SN13T as a probiotic source, protection can be provided to the bacteria through encapsulation methods. Encapsulation is a process in which a material is coated with another substance, where the encapsulated material is usually called the core,

*Corresponding authors: Sri Melia, E-mail: srilmelia75@ansci.unand.ac.id, Tel.: 8126761782

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active ingredient, internal phase, or filler. Meanwhile, the substance used for encapsulation is called the wall, shell, external phase, or carrier [6]. Encapsulation can protect probiotics in acidic pH in the digestive tract [7] and maintain their stability during processing and storage under extreme conditions [8]. Several methods can be used in encapsulation, one of which is extrusion. The extrusion method involves mixing the core and wall material and utilizing pressure to drip the polymer solution into an ionizing solution by passing the solution through a nozzle so that hydrogel forms instantly [9].

In the encapsulation process, the selection of wall material plays an important role because the wall material provides a physical barrier against various stresses during the encapsulation process. The wall materials used in bacterial microencapsulation may consist of a single type or a combination of several materials. This is related to the desired microencapsulation characteristics for maintaining microencapsulation stability, the coated core material, and the method used in encapsulation [10]. One type of wall material that can be used is a combination of alginate and bengkuang starch.

Alginate is a natural carbohydrate polymer and heteropolysaccharide widely used in encapsulation [11]. Alginate is a polysaccharide consisting of mannuronic acid (M) and guluronic acid (G), which forms a gel with an 'egg box' structure when reacted with calcium ions (Ca^{2+}), and is capable of trapping water and particles such as probiotics within it [12]. Alginate is suitable as an encapsulation agent due to its biocompatibility, low toxicity, and mild gelling properties. The mild gelation process of alginate allows encapsulation of sensitive biological materials without compromising their integrity [13].

Meanwhile, bengkuang (*Pachyrhizus erosus*) starch has the potential to be developed as a wall (encapsulating) material, due to its high starch content. Bengkuang contains 73.47% starch, with 70.4% amylopectin and 29.6% amylose [14]. Although it does not form a gel ionically like alginate, bengkuang starch is rich in hydroxyl groups (-OH) and is water absorbent. These groups can form hydrogen bonds with carboxylate (-COO) and hydroxyl groups on alginate. Inulin and starch molecules will fill the spaces between alginate chains and reinforce the formed gel [15]. Starch is used in encapsulation because of its biocompatibility, ability to create a stable matrix, ease of gel formation and water binding, and effectiveness in improving encapsulated microorganisms' viability [16]. Using bengkuang in the form of flour as a wall material for encapsulation also aligns with efforts to diversify value-added local food products. Furthermore, bengkuang is a regional food product from Padang, West Sumatra, whose potential can be further developed [17].

Alginate and bengkuang flour are polysaccharides with bioadhesive substrates that can be used as wall materials for encapsulation [18]. Alginate, as a wall material, has good gel-forming abilities, but its limited water absorption can cause water to be released and affect stability during storage [19]. The addition of bengkuang flour, which is a source of starch, can improve the stability of the matrix as a wall material, thereby allowing the core material to be protected more effectively. Combining bengkuang flour with alginate as a wall material is expected to produce beads with good characteristics and the ability to protect the target probiotic. This study aims to determine the best wall material formulation, consisting of alginate and bengkuang flour (*Pachyrhizus erosus*), in the encapsulation process of *L. plantarum* SN13T.

Material and Methods

The materials used in this study were *L. plantarum* SN13T, which was isolated from stingless honey from West Sumatra [3], bengkuang flour, and alginate.

Bacterial Culture Preparation

The bacterial strain used in this study was *L. plantarum* SN13T, which was isolated from stingless honey bees originating from West Sumatra (Melia *et al.* [3]). The bacterial culture was prepared according to the modified method of [20]. All equipment was sterilized in an autoclave at 121°C for 15 minutes under 15 lbs of pressure. The LAB isolate was rejuvenated (enriched) three times in MRS broth for 24 hours at 37°C under anaerobic conditions in an incubator. The culture was centrifuged at 3000 rpm for 10 minutes at 25°C to obtain a pellet, and the supernatant was discarded. The pellet was then washed with 5 ml of sterile distilled water and centrifuged again at 3000 rpm for 10 minutes at 25°C to obtain a pellet, after which the supernatant was discarded.

Preparation of Bengkuang Flour

The process of making bengkuang flour is carried out based on modifications by [21]. Peeled bengkuang is stripped of its skin and thoroughly washed. Next, it is sliced using a slicer to a thickness of 1–2 mm. The bengkuang slices, cut to 1–2 mm, are then dried with a food dehydrator at 60°C for 6 hours. Once the bengkuang has turned into dry chips, it is ground using a grinder or blender until fine, then sieved using an 80-mesh sieve. The fine flour is collected and ready for use.

Probiotic Encapsulation Using the Extrusion Method

Encapsulation of *L. plantarum* SN13T is carried out using the extrusion method as referred to by [9], through several stages: First, the dispersed phase is prepared by mixing bengkuang flour in 100 ml of sterile distilled water while stirring at 400 rpm, then

adding 1% alginate to each treatment and bengkuang flour according to the treatment (A = without bengkuang flour, B = 1% bengkuang flour, C = 3% bengkuang flour, and D = 5% bengkuang flour) until homogeneous. Next, 1% probiotic biomass is added and homogenized for 5 minutes at 400 rpm. This solution is then dripped into a 1.5% calcium chloride solution while stirring at 200 rpm, using a nozzle, syringe, or dropper to form microcapsules. The microcapsules are left in the solution for 20–30 minutes until solid, then washed with physiological NaCl solution and stored in a refrigerator at 4°C.

Moisture Content

Moisture content was determined according to the AOAC method [22]. First, the dish was placed in an oven at 110°C for one hour. The dish was cooled in a desiccator to remove moisture and then weighed (A). A 5 g sample was weighed into the pre-dried dish (B) and oven-dried at 105°C for 8 hours. The sample was then cooled in a desiccator for 30 minutes and weighed (C). These steps were repeated until a constant weight was achieved. Moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{B - C}{B - A} \times 100\%$$

Note: A = weight of base flask (gram), B = weight of flask + sample weight (gram), C = weight of flask + dry sample (gram).

Bead Size and Morphology

The average diameter and morphology of 10 randomly selected beads were measured using an optical microscope (Olympus, Japan) at 100x magnification, equipped with a micrometer scale [23].

Calculation of Total Lactic Acid Bacteria

A 1.0 g sample was first diluted by adding it to 9 mL of de Man Rogosa Sharpe Broth, then mixed thoroughly using a vortex mixer. The resulting solution was diluted serially up to 10⁸. After that, the diluted sample was plated on petri dishes containing de Man Rogosa Sharpe Agar using the spread method, and spread evenly with a hockey-shaped stick. The petri dishes were incubated for 48 hours at 37°C. The number of colonies was counted using the Colony Forming Unit (CFU) method, according to [24].

$$\frac{\text{CFU}}{\text{ml}} = \text{Number of colonies} \times$$

$$\frac{1}{\text{Dilution factor}} \times \frac{1}{\text{Sample weight}} \times 10$$

Simulated gastric juice (SGJ) Test

SGJ is prepared by adding 0.3 g of pepsin into 100 ml of sodium chloride solution (0.2% w/v) and adjusting the pH to 2.0 using 0.1 N HCl [25]. Next, 1 g of the sample stored in the refrigerator is placed

into a tube containing 9 ml of SGJ and incubated at 37°C for 120 minutes. Secondly, the pH of the sample is adjusted to 7, and 10 ml of SIJ is added, followed by incubation at 37°C for 5 hours [26]. This is followed by dilution up to 10⁻⁶. Then, plating is performed using the spread method onto MRS Agar media, followed by incubation at 37°C for 48 hours, and the number of bacterial colonies that survive is counted using the Colony Forming Unit (CFU) method.

$$\frac{\text{CFU}}{\text{ml}} = \text{Number of colonies} \times$$

$$\frac{1}{\text{Dilution factor}} \times \frac{1}{\text{Sample Weight}} \times 10$$

The formula determines the calculation of LAB viability:

Reduction in colony number (%):

$$\frac{\text{Total colony control} - \text{Total SGJ colonies}}{\text{Total Koloni BAL Kontrol}} \times 100\%$$

Simulated Intestinal Juice (SIJ) Test

To prepare SIJ, 0.6% bile salt is added to an autoclaved 0.05 M KH₂PO₄ solution. The sampling process is done by adding 0.1 g of microcapsules from each sample into 9.9 mL of SIJ solution [27]. Then, incubation is carried out for 5 hours at 37°C. Next, serial dilution is performed up to 10⁻⁶, and the sample is plated on de Mann Rogosa Sharpe (MRS) Agar (Neogen Culture Media) using the spread plate method, followed by incubation at 37°C for 48 hours. Count the number of bacteria that survive using the plate count method in Colony Forming Units (CFU). The comparison between the cell numbers before and after incubation will be expressed as viability (%). The higher the viability percentage, the more resistant the bacteria are to bile salts. The total number of colonies that grow is calculated using the following formula:

$$\frac{\text{CFU}}{\text{g}} = \text{Number of colonies} \times$$

$$\frac{1}{\text{Dilution factor}} \times \frac{1}{\text{Sample Weight}} \times 10$$

Decrease in colony number (%)

$$\frac{\text{Total colony control} - \text{Total SIJ colonies}}{\text{Total Koloni BAL Kontrol}} \times 100\%$$

Viability (%) = 100 – decrease in colony number (%)

Encapsulation Efficiency

Encapsulation efficiency (EE) measures how many cells remain viable during encapsulation and how well those cells are entrapped after the encapsulation process [28]. The EE value is calculated using Equation 2, where N₁ is the number

of cells released from the resulting beads, while N0 is the initial number of cells before encapsulation.

Encapsulation efficiency (%)

$$\frac{N1 \left(\frac{\text{CFU}}{\text{mL}} \right)}{N0 \left(\frac{\text{CFU}}{\text{mL}} \right)} \times 100\%$$

Statistical analysis

The tests were performed in triplicate, and the data are presented as mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA with SPSS (Statistical Package for the Social Sciences), IBM SPSS Statistics version 26 (2016). Differences with $p < 0.05$ were considered statistically significant.

Results and Discussion

Moisture Content

Based on the data in Table 1, it can be seen that the moisture content of the beads resulting from the encapsulation of *L. plantarum* SN13T ranges from $95.30 \pm 0.67\%$ to $98.30 \pm 0.5\%$. Statistical analysis shows that the combination of alginate and bengkuang flour as wall materials significantly affects ($P < 0.05$) the moisture content of the encapsulated *L. plantarum* SN13T produced by the extrusion method. The lowest moisture content was found in treatment D, $95.30 \pm 0.67\%$, while the highest was found in treatment A, $98.30 \pm 0.50\%$ and did not contain bengkuang flour. This indicates a decrease in moisture content as the proportion of bengkuang flour in the wall material formula increases.

This decrease in moisture content can be explained by the characteristics of the starch and fiber contained in bengkuang flour, which are known to have physical and chemical properties that support the formation of a more stable matrix resistant to water penetration. According to [29], one of the primary mechanisms in reducing moisture content is the formation of hydrogen bonds between alginate and starch molecules. Both of these biopolymers have hydroxyl (-OH) groups on their polymer chains, facilitating intermolecular hydrogen bond formation. These bonds are the dominant mechanism in the interaction between alginate and starch, and play an important role in forming a homogeneous matrix and strengthening the biocomposite structure.

In addition, alginate has negatively charged carboxylate groups (-COO^-) that can interact with positively charged ions or other polar groups. In ionic conditions, such as calcium ions (Ca^{2+}), alginate undergoes an ionic gelation process, forming a three-dimensional network capable of entrapping starch molecules. This process increases the strength and stability of the resulting gel structure [30]. Thus, the interaction between alginate and starch, which is dominated by hydrogen bonds with additional

contributions from ionic bonds and interactions, enables the formation of beads with improved physical and functional properties. [31] also stated that the bonding of alginate and starch enhances encapsulation by creating a denser and less porous matrix, thereby reducing moisture permeability. This tighter structure minimizes water diffusion, thus lowering the water content in the encapsulation, leading to increased stability and encapsulation efficiency.

The presence of amylose and amylopectin in starch also plays a role in reducing water content. [14] stated that bengkuang flour has a substantial starch content, namely 73.47% with amylopectin at 70.4% and amylose at 29.6%. A high amylose content causes the starch to become drier and less sticky, thus increasing water absorption. Higher amylose content results in greater expansion and water absorption capacity due to amylose's ability to form stronger hydrogen bonds than amylopectin [32]. Moreover, adding a high amount of starch can increase the viscosity of the solution before gelation and affect the water-binding mechanism. This is also confirmed by [33], who mentioned that high viscosity due to polysaccharide content can strengthen the matrix and decrease moisture levels. Therefore, the more bengkuang flour added, the denser the hydrogel structure formed and the lower the final water content of the encapsulation product.

Size and Morphology of Beads

Based on the data in Table 2, it can be seen that the diameter of beads resulting from the encapsulation of *L. plantarum* SN13T ranges from 1.80 ± 0.04 mm to 2.38 ± 0.08 mm. Statistical analysis showed that the combination of alginate and bengkuang starch as wall materials had a significant effect ($P < 0.05$) on the bead diameter produced by the extrusion method in the encapsulation of *L. plantarum* SN13T. The smallest diameter was found in treatment D, 1.80 ± 0.04 mm, while the largest was found in treatment A, 2.38 ± 0.08 mm, and contained no bengkuang starch. This indicates a reduction in bead diameter as the proportion of bengkuang starch increases in the wall material formula.

Adding starch sources such as bengkuang starch can affect viscosity and stability, influencing bead size. The higher the addition of bengkuang starch, the stronger the bonds formed with alginate, resulting in a thicker structure and smaller bead size. As shown in the results, the highest addition of bengkuang starch at 5% (D) produced beads with the smallest size, 1.80 ± 0.04 mm. This shows that increasing the amount of bengkuang starch added results in smaller bead diameters.

In their study, [34] stated that adding starch makes the encapsulation mixture more viscous, resulting in beads with a stronger and denser

structure, making them less likely to deform. [35] also added that the greater the amount of starch added, the longer it takes for substances within the beads to be released; furthermore, the resulting beads tend to be smaller in size. This indicates that the amount of starch directly affects the bead size. The results of this study are similar to those of [36], in which the size of alginate and alginate–chitosan capsules ranged from 2.0 to 3.8 mm and from 3.0 to 4.4 mm, respectively. [37] stated that probiotic encapsulation using the extrusion method can produce beads ranging in size from 0.5 to 3 mm.

The morphology of the encapsulated beads can be seen in Figure 1, where the resulting beads appear clear in color. It is evident that most beads are generally imperfectly round, and some are oval-shaped. However, the beads appear to have a smooth texture. According to [38], sodium alginate beads generally have a smooth surface due to their ability to form capsules.

Total Lactic Acid Bacteria Colony Count

Table 3 shows that the total colonies of lactic acid bacteria (LAB) in bead form from encapsulation ranged between 12.83 ± 0.02 Log CFU/mL and 12.95 ± 0.02 Log CFU/mL. Statistical analysis indicated that the combination of alginate and bengkuang flour as wall materials had a significant effect ($P < 0.05$) on the total number of encapsulated LAB colonies of *L. plantarum* SN13T using the extrusion method. The lowest total LAB colony count was found in treatment A, at 12.83 ± 0.02 Log CFU/mL, while the highest was observed in treatment D, at 12.95 ± 0.02 Log CFU/mL. This demonstrates increased bacterial viability with more bengkuang flour in the wall formulation. In other words, the higher the proportion of bengkuang flour used, the greater the number of bacterial colonies that can survive in the encapsulation system.

Bengkuang flour plays a crucial role in supporting the growth and survival of lactic acid bacteria, as its nutrients act as a natural substrate and prebiotic. Thus, the higher the addition of bengkuang flour in the formulation, the more inulin it provides as a prebiotic source for LAB to enhance their growth. The study results showed that 5% bengkuang flour (D) was the highest addition of a total colony count of 90.3 ± 4.19 CFU/mL. [39] stated that several main compounds in bengkuang flour, such as polysaccharides and inulin, have been proven to function effectively as prebiotics—compounds that cannot be digested by humans but are utilized by probiotic bacteria for growth and development. As a soluble fiber, Inulin can increase the population of beneficial bacteria in the digestive system and support the microflora balance. Additionally, the carbohydrates in bengkuang serve as an energy source and facilitate the growth of lactic acid bacteria within the encapsulation medium. [40] reported that

adequate substrate intake is essential for encapsulation systems because the limited space and nutrients within the beads can restrict the metabolic activity of bacteria.

Previous data indicated that adding bengkuang flour decreases the moisture content. This lower moisture content correlates with reduced water activity, which slows down cell degradation and improves stability during storage. This is consistent with [41], who stated that matrices with lower moisture content prevent cell death due to contaminant microbial activity or enzymatic reactions. Furthermore, the increase in lactic acid bacteria colonies alongside the decrease in moisture content may be due to the fermentation ability of LAB in starch-rich environments. According to [42], the amylolytic capability of LAB enables them to produce amylase enzymes, which catalyze starch hydrolysis and convert it into simple sugars suitable for fermentation.

According to CODEX [43], a good probiotic source must contain at least 10^6 CFU/mL viable LAB. Meanwhile, the Indonesian National Standard [44] requires a minimum of 10^7 CFU/mL viable cells in probiotic sources. Based on the research results, the total LAB colonies ranged from $68.7 \pm 3.72 \times 10^{11}$ CFU/mL to $90.3 \pm 4.19 \times 10^{11}$ CFU/mL, indicating that the resulting beads meet the standards to be considered as a probiotic source.

Simulated gastric juice (SGJ)

Table 4 shows that the resistance of encapsulated SGJ beads ranges from $89.71 \pm 3.89\%$ to $95.23 \pm 1.20\%$. Statistical analysis shows that the combination of sodium alginate and bengkuang (bengkuang) flour as wall materials has a significant effect ($P < 0.05$) on probiotic resistance in simulated gastric juice. The lowest resistance to simulated gastric juice was found in treatment D, at $89.71 \pm 3.89\%$, while the highest resistance to SGJ was observed in treatment B, at $95.23 \pm 1.20\%$.

The encapsulated *L. plantarum* SN13T in treatment A, which utilized a 1% alginate addition, showed a resistance value of $90.87 \pm 0.60\%$. This is because beads produced solely by alginate tend to be sensitive to low pH, which can lead to acidic conditions damaging the bacteria encapsulated inside the beads. This aligns with the opinion of [45], who stated that beads produced from alginate are stable in neutral to slightly basic conditions. Still, at low pH, the carboxylate groups ($-\text{COO}^-$) become protonated to $-\text{COOH}$, which can lead to gel shrinkage, disruption of ionic cross-links (such as Ca^{2+} bridges in the "egg box" structure), and increased porosity, allowing the acidic medium to penetrate and damage the encapsulated bacteria. [46] noted that a decrease in pH causes protonation of the alginate, weakening its ionic bonds with Ca ions and reducing the integrity of the gel structure, leading to damage to

the encapsulation matrix. [47] also stated that the pH sensitivity of alginate causes shrinkage and swelling under gastrointestinal conditions. The resulting capsule's porosity increases, and the beads break down toward the end phase before reaching the intestine.

In treatment B, adding 1% alginate and 1% bengkuang flour, the highest resistance to SGJ was recorded at $95.23 \pm 1.20\%$. This is because the combination of alginate and starch in the right proportions can form a more stable encapsulation matrix resistant to acidic environments. The right amount of starch reinforces the physical structure of the formed beads. This is in line with [48], who noted that blending alginate with starch improves the physical and mechanical properties of the gel, potentially reducing pore size and enhancing intermolecular hydrogen bonding, thus increasing encapsulation efficiency and stability of the entrapped substances. This interaction results in thicker and denser capsule walls, decreasing the possibility of gastric acid penetrating the capsule core. This effect directly reduces matrix disintegration in gastric fluids and increases probiotic viability under low pH conditions. Furthermore, [49] explained that at low pH, the composite matrix formed from the alginate-starch mix acts as a diffusion barrier to hydrogen ions (H^+), thereby slowing acid penetration and maintaining internal pH stability within the capsule for a more extended period. This protection also provides an effective physical barrier, which prevents damage to encapsulated bacterial cell membranes.

However, in treatment D, adding 1% alginate and 5% bengkuang flour, the lowest resistance to SGJ was observed at $89.71 \pm 3.89\%$. This is because the increased addition of bengkuang flour containing starch at higher concentrations causes the starch to dominate the matrix, reducing the uniform distribution of alginate. This imbalance can cause matrix degradation, weaken encapsulation, and allow acid solution to diffuse into the matrix, disrupting the growth of lactic acid bacteria (LAB), resulting in cell death and further reduced SGJ resistance. This is consistent with [50], who stated that higher starch concentrations can cause phase separation or insolubility, especially if the system is poorly plasticized. Excess starch may form aggregates or self-associate, reducing the effectiveness of polymer bonding with alginate. [30] also noted that this may increase gel density but weaken the uniformity of Ca^{2+} cross-links because starch is not ionically bound, which may decrease mechanical strength. Therefore, formulations with excessively high starch content from bengkuang flour can weaken the encapsulation matrix's physical and chemical structure, reducing resistance to SGJ.

In previous studies, *L. plantarum* SN13T isolated from the honey of the *Tetrigona binghami* species

had an acid tolerance of 82.75% [3]. This shows increased SGJ resistance when alginate and bengkuang flour are used as wall materials.

Simulated intestinal juice (SIJ)

Based on the data in Table 5, it can be seen that the resistance to SIJ beads resulting from encapsulation ranges from $81.32 \pm 1.27\%$ to $95.76 \pm 1.60\%$. Statistical analysis showed that using alginate and bengkuang flour as wall materials significantly affects ($P < 0.05$) SIJ from *L. plantarum* SN13T encapsulated by the extrusion method. The lowest SIJ resistance value was found in treatment D, $81.32 \pm 1.27\%$, while the highest was found in treatment B, at $95.76 \pm 1.60\%$.

The encapsulated *L. plantarum* SN13T's resistance to SGJ in treatment A, which involved the addition of 1% alginate, showed a value of $89.72 \pm 0.66\%$. Alginate forms a hydrogel matrix that physically protects the bacteria, limiting their direct exposure to bile salts. [45] stated that the "egg box" structure produced by Ca^{2+} ion cross-linking helps create a diffusion barrier against components of bile salts. However, the drawback is that the alginate gel can swell or partially degrade in bile, making it possible for bile salts to penetrate.

In treatment B, adding 1% alginate and 1% bengkuang flour resulted in the highest resistance to SIJ at $95.76 \pm 1.60\%$. This is because the right combination of alginate and starch can form a more stable encapsulation matrix that is more resistant to bile salts. Adding the appropriate proportion of starch can reinforce the physical structure of the formed beads by thickening the encapsulation wall. This is consistent with [48], who stated that mixing alginate with starch improves the physical and mechanical properties of the gel, potentially reducing pore size and increasing intermolecular hydrogen bonding, thereby improving encapsulation efficiency and the stability of trapped substances. This will minimize porosity and slow the diffusion of bile salts into the microcapsules.

However, adding bengkuang flour to 3% (C) and 5% (D) can reduce SIJ resistance to $87.97 \pm 0.98\%$ and $81.32 \pm 1.27\%$. This is because the increased addition of bengkuang flour, which contains starch at higher concentrations, causes the starch component to dominate the matrix system. This can interfere with the even distribution of alginate, thus hindering the formation of a compact and organized three-dimensional network. This is in line with [50], who stated that excessively high starch concentrations can cause insolubility, aggregate formation, or self-association, which can reduce interpolymer bonding with alginate, thereby weakening the physical and chemical structure of the encapsulation matrix. This imbalance leads to degradation of the matrix structure and results in capsules with lower mechanical strength. Consequently, it allows SIJ to

diffuse into the capsules and causes damage to *L. plantarum* SN13T cells, ultimately reducing resistance to SIJ.

In previous studies, *L. plantarum* SN13T derived from honey isolates of *Tetrigona binghami* had a bile salt tolerance of 94.44% [3]. This shows that only treatment B (1% alginate and 1% bengkuang flour) could approach and even surpass that value with a SIJ resistance result of 95.76%.

Encapsulation Efficiency

Based on the data in Table 6, it can be seen that the encapsulation efficiency ranges from $66.84 \pm 2.64\%$ to $73.08 \pm 0.99\%$. Statistical analysis shows that using alginate and bengkuang flour as wall materials significantly affects ($P < 0.05$) on the encapsulation efficiency of *L. plantarum* SN13T using the extrusion method. The lowest encapsulation efficiency value was found in treatment D, $66.84 \pm 2.64\%$, while the highest was in treatment B, $73.08 \pm 0.99\%$.

Encapsulation efficiency is one of the indicators of the success of the encapsulation process, indicating the percentage of probiotic cells successfully trapped within the wall material matrix compared to the initial amount used [51]. This study's results show that using 1% alginate as the wall material (treatment A) provided high efficiency (72.96%). This is in line with previous studies that report alginate as the most commonly used hydrophilic polymer for probiotic encapsulation because it can form a firm gel in the presence of calcium ions (Ca^{2+}), creating a three-dimensional network structure that effectively protects probiotic cells [49].

Treatment B, a combination of 1% alginate and 1% bengkuang flour, resulted in the highest efficiency (73.08%). This indicates that adding bengkuang flour at the appropriate concentration does not interfere with gel matrix formation; it can provide additional protection. Bengkuang flour contains starch and dietary fibers such as inulin and oligofructan, which act as prebiotics [52]. Starch as a wall material in the right amount can fill the pores of the gel and reduce its permeability, thus helping to retain probiotic cells within the capsule. In addition, the prebiotic components can also serve as substrates for probiotics after the capsule is released, creating a synbiotic effect [53].

However, in treatments C and D, which used bengkuang flour at higher concentrations (3% and 5%), the encapsulation efficiency decreased significantly (68.59% and 66.84%). This decline may

be due to the increased viscosity of the wall solution due to adding a large amount of bengkuang flour. High viscosity can hinder the diffusion of Ca^{2+} ions into the alginate matrix during the gelation process, resulting in less optimal gel structure formation [54]. Consequently, some probiotic cells are not properly trapped, leading to leakage during bead formation. This was also reported by [55], who stated that the success of encapsulation is highly influenced by the concentration of polymers and added components, as these can affect the compactness of the gel and the distribution of cells within it. Additionally, when more starch is added, encapsulation efficiency decreases due to the potential for excessive density, which limits the interaction between phenolic compounds and the starch matrix. This reduces encapsulation effectiveness and microcapsule stability during storage [56].

Conclusion

The conclusion from the study on the encapsulation of *Lactiplantibacillus plantarum* SN13T using the extrusion method with alginate and bengkuang flour as wall materials is that the use of 1% alginate + 1% bengkuang flour yields the best encapsulation results, with a moisture content of 97.34%, bead size of 2.14 mm, total LAB colonies of $12.88 \log \text{CFU/mL}$, resistance to SGJ at 95.23%, and resistance to SIJ at 95.76%. This indicates that encapsulation with a 1% alginate + 1% bengkuang flour formulation provides optimal results for the viability and effectiveness of *L. plantarum* SN13T.

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Ethical of approval

This research has passed the ethical review by the Faculty of Pharmacy Commission Team of Andalas University with Number: 46/UN.16.10.D.KEPK-FF/2025 with the title Encapsulation of *Lactiplantibacillus plantarum* SN13T Using the Extrusion Method with Alginate and Bengkuang Flour (*Pachyrhizus erosus*) and its Application to Ice Cream.

Conflict of Interest

Not applicable

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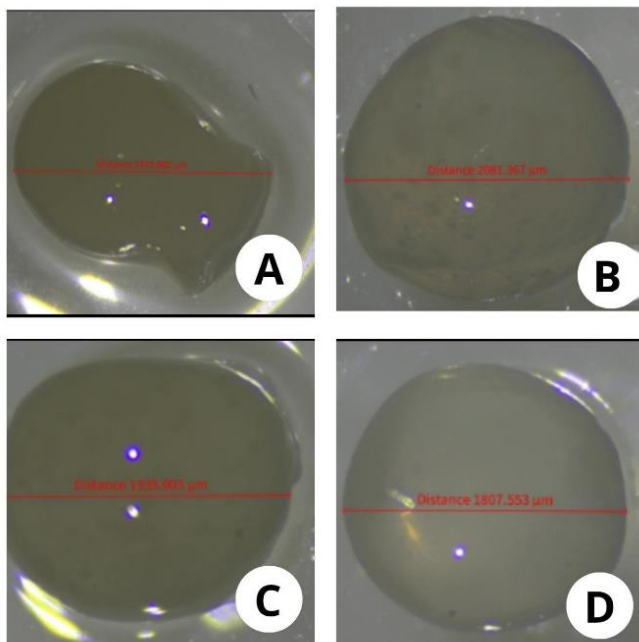


Fig 1. Morphology of Beads Resulting from the Encapsulation of *L. plantarum* SN13T

TABLE 1. Moisture Content of *L. plantarum* SN13T Encapsulation

Treatment	Moisture content (%)
A	98.30 ± 0.50 ^c
B	97.34 ± 0.52 ^c
C	96.34 ± 0.85 ^b
D	95.30 ± 0.67 ^a

Note: Different superscripts indicate significant differences.

TABLE 2. Diameter of Bead Size from Encapsulation of *L. plantarum* SN13T

Treatment	Diameter beads (mm)
A	2.38 ± 0.08 ^d
B	2.14 ± 0.05 ^c
C	1.93 ± 0.02 ^b
D	1.80 ± 0.04 ^a

Note: Different superscripts indicate significant differences.

TABLE 3. Total Lactic Acid Bacteria of Encapsulation of *L. plantarum* SN13T

	Total Lactic Acid Bacteria Colony (Log CFU/mL)
A	12.83 ± 0.02 ^a
B	12.88 ± 0.01 ^b
C	12.89 ± 0.02 ^b
D	12.95 ± 0.02 ^c

Note: Different superscripts indicate significant differences.

TABLE 4. Resistance to SGJ of *L. plantarum* SN13T Encapsulation

Treatment	Simulated gastric juice (%)
A	90.87 ± 0.60 ^a
B	95.23 ± 1.20 ^b
C	94.88 ± 1.62 ^b
D	89.71 ± 3.89 ^a

Note: Different superscripts indicate significant differences

TABLE 5. Resistance to SGI (%) of *L. plantarum* SN13T Encapsulation

Treatment	Simulated intestinal juice (%)
A	89.72 ± 0.66 ^b
B	95.76 ± 1.60 ^c
C	87.97 ± 0.98 ^b
D	81.32 ± 1.27 ^a

Note: Different superscripts indicate significant differences.

TABLE 6. Average Encapsulation Yield Efficiency of *L. plantarum* SN13T

Treatment	Encapsulation Efficiency (%)
A	72.96 ± 1.06 ^b
B	73.08 ± 0.99 ^b
C	68.59 ± 1.47 ^a
D	66.84 ± 2.64 ^a

Note: Different superscripts indicate significant differences.

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