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Producing Dry *Lactobacillus plantarum* NHC6 Starter Using Branch Matrix

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Abstract

Background: A Lactic Acid Bacteria (LAB) starter is an instant LAB culture prepared to initiate lactic acid production in fermentation. The manufacture of BAL starter requires alternative media as a substitute for MRS media, which costs a lot when used for industrial production. The dry starter has advantages compared to the liquid starter. An encapsulant matrix is needed to protect cells, which provides insulation for bacterial cells against the drying process, which can damage cells; a good media is needed and can be an encapsulation matrix in manufacturing a dry LAB starter. *Lactobacillus plantarum* NHC6 bacteria is a lactic acid bacterium that has the potential to be developed. **Research aim**: producing Dry Starter using Rice Bran Waste as a matrix. **Method:** Making encapsulation matrix using rice bran. The bran media consisted of 10% (w/v) rice bran added with 5% (w/v) glucose and 1% (w/v) ammonium sulfate. As much as 10% (w/v) of *L. plantarum* NHC6 culture was inoculated in bran media in the late log phase. The liquid starter was then incubated again for 14 hours at 37°C. After that, the drying process was carried out using a spray dryer at an inlet temperature of 170°C. **Result:** The number of live cells in the bran starter decreased after the spray drying. Starter storage at 28°C and 4 °C has a significantly different effect. **Conclusion:** The rice bran matrix can be an encapsulating agent and protect *L. plantarum* NHC6 cells from high temperatures during the spray drying method in manufacturing a dry starter.

Keywords: dry starter; rice bran encapsulation; Lactobacillus plantarum.

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Introduction

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria that are homofermentative anaerobes, do not have catalase, are cocci or rod-shaped, and produce L-(+)-lactic acid (Behera et al., 2018). This bacterium is essential in food fermentation because it is used as a starter culture to preserve food and as a probiotic (Bintsis, 2018; Riani et al., 2020; Asnita & Meryandini, 2023). LAB, widely used as a starter and probiotic, belongs to the Lactobacillus group.

LAB starter can be described as an instant LAB culture containing single or combination types of bacteria that have been prepared to be able to initiate lactic acid production in the raw material fermentation process (Parente et al., 2017). Spray drying is one way to make a dry starter, but it causes high microbial death rates due to dehydration and high

temperatures (Vivek et al., 2023). Protection against microbes when dried in the spray drying process can be done by adding protective ingredients such as trehalose, prebiotics, soluble fiber, maltodextrin, Arabica gum, or tapioca acid (Erdiandini et al., 2015; Vivek et al., 2023).

The use of dairy products as a matrix in making dry starters causes problems for humans with lactose intolerance, galactosemia, allergies to milk proteins, and high cholesterol levels. To overcome this problem, non-dairy-based probiotic foods can be a good alternative. Some non-dairy products that LAB can ferment include oats and wheat bran (Min et al., 2017). The LAB isolate with the code NHC6, which is *Lactobacillus plantarum*, is isolated from pineapple juice, which has been proven to have great potential as a probiotic (Riani et al., 2020).

MRS media (de Man Rogosa Sharpe) is a nutrient-rich medium designed based on LAB's specific needs and is a particular medium for culturing fermentative bacteria, especially LAB (De Man et al., 1960). However, the high price of MRS is an inhibiting factor for the industry (Ayiyi & Ibrahim, 2022). The search for alternative media that is cheaper and capable of providing nutrition for LAB growth is an alternative (Wulan et al., 2017). One alternative medium, acidic tapioca waste, can be used as a microencapsulation matrix for LAB starter (Erdiandini et al., 2015).

Another alternative microencapsulation matrix is rice bran. Rice bran is a by-product of grinding grain into rice with a high carbohydrate content and other nutrients such as protein, fat, minerals, and vitamins (Bhosale & Vijayalakshmi, 2015). The nutritional content and availability of rice bran are sufficient to make rice bran a potential alternative medium for making *Lactobacillus plantarum* NHC6 starter. Rice bran has many benefits as a functional food (Gul et al., 2015), and *Lactobacillus plantarum* NHC6 isolate, which has potential as a probiotic, can be an innovative combination to be developed into a dry starter.

Method

Hot Water-Soluble Rice Bran Extraction

50 g of rice bran was dissolved in 500 mL of distilled water and then heated until boiling. After boiling, the mixture is allowed to stand until the rice bran fraction, which is not soluble in water, rises to the surface. The water-insoluble rice bran fraction above the surface is separated from the water-soluble rice bran fraction. The water-soluble rice bran fraction is then poured into an aluminum baking pan with a heat-resistant plastic bottom. Then, dried in an oven at 60°C. The dried rice bran is sifted again until it passes through a 100-mesh sieve. Then, a proximate analysis was carried out on the rice bran. In the form of water content, ash content, carbohydrate content, protein content, fat content, and crude fiber content (AOAC, 1995).

Measurement of the Growth Curve of L. plantarum NHC6

The *L. plantarum* NHC6 isolate was rejuvenated on MRSA for 24 hours at 37°C. Growth curves were made in 100 mL Duran bottles with a culture volume of 100 mL, using MRS Broth (MRSB) media and 1% (w/v) rice bran media with the addition of 5% glucose (w/v) and 1% ammonium sulfate (w/v). v) (Nurlaela et al., 2017). MRSB media was inoculated with 1 mL (1%) (v/v) of bacterial culture, which had been subcultured in MRSB and had reached an OD value of 0.8 – 1 (Wulan et al., 2017). Rice bran media was inoculated with bacterial culture in MRS as much as 1 mL (1%) (v/v) at the end log phase of the growth curve on MRSB media. Growth curve cultures were incubated at 37°C for 24 hours. Observations were made at 0, 4, 8, 12, 16, 20 and 24 hours. The parameters observed were bacterial colonies that grew after incubation at 37°C for 48 hours. The results of cup calculations using the spread technique for MRSA are expressed in log CFU/ml units (Erdiandini et al., 2015).

Dry Starter Production

Rice bran media consists of 10% (w/v) rice bran added with 5% (w/v) glucose and 1% (w/v) ammonium sulfate. A total of 450 mL of rice bran media was mixed with 50 mL (10%) (w/v) of *L. plantarum* NHC6 culture in rice bran media, which was in the late log phase based

on the growth curve (Erdiandini et al., 2015), namely 14 hours of incubation. The liquid starter was then incubated again for 14 hours at 37°C. After that, the drying process was carried out using a spray dryer at an inlet temperature of 170°C. The dry starter obtained was stored in an LDPE ziplock plastic container for 28 days at 28°C (room temperature) (RH 76.85%) and 4°C (54.95%).

Starter Viability Measurement

Before spray drying, the liquid *L. plantarum* NHC6 starter was counted for the number of cells using the spreading technique on MRSA media, with serial dilutions up to 10-5. Then, 100 µL of solution was spread on solid MRS and incubated at 37°C for 48 hours. After spray drying, storage viability was measured on days 0, 7, 14, 21 and 28. 1 g of dry starter was dissolved in 9 mL of sterile physiological salt (dilution 10-1) and then homogenized with a rotator. The starter solution was made into serial dilutions up to 105, and then 100 µL of the solution was spread on solid MRS media and incubated at 37°C for 48 hours. The parameter for this test is the number of colonies that grow and is expressed in log CFU/g.

Next, starter characterization was carried out. Starter characterization is carried out by measuring the total dissolved solids in the liquid starter and measuring the water content in the dry starter. The parameters for measuring total dissolved solids are the total amount of solids contained in the starter at 0 hours of culture age and 14 hours before spray drying. Then, the parameter for measuring water content is the percentage of water content in the starter before and after spray drying.

Many data are presented in the form of tables and graphs. Data that require a comparison of means are tested using the t-test. The experimental design used in this research was a Randomized Block Design (RAK) with two factors: storage temperature and storage time. Each factor was tested for influence using One-way ANOVA analysis of variance. If the results of the ANOVA analysis show significant differences in the groups and meet the requirements, continue with a further or post hoc test, namely the Duncan test. The interaction between the two factors was analyzed using two-way ANOVA. Tests were conducted using the statistical analysis software SPSS (Statistical Package for the Social Sciences) version 25.

Result

Soluble Rice Bran in Hot Water

The water-soluble rice bran obtained has a fine texture with the size of the flour grains passing through a 100-mesh sieve. The elemental and nutrient content of water-soluble rice bran is presented in Table 1.

Table 1. Results of proximate analysis of water-soluble rice bran

Element	Percentage Content (%)
Water	10,16 ± 0,11
Ash	12,92 ± 0,06
Crude Fat	5,47 ± 0,01
Crude Protein	10,44 ± 0,06
Crude Fiber	57,28 ± 0,69
Carbohydrates (by difference)	3,71 ± 0,59

Growth curve of L. plantarum NHC6

Growth curves were made on two different types of growth media. The two media are MRSB media and 1% (w/v) rice bran media enriched with 5% (w/v) glucose and 1% (w/v) ammonium sulfate. Measurements of the growth of NHC6 isolates from both media are shown in the form of growth curves (Figure 1). Bacterial growth is measured by calculating the number of bacterial colonies that grow based on the Total Plate Count (TPC) method.

Based on the curve image obtained, there was a difference in the number of cells at hour 0 in MRSB media and 1% rice bran media. In MRSB media, the initial cell number was 1 x 10^7 CFU/mL, while in 1% rice bran media, the initial cell number was only 1 x 105 CFU/mL.

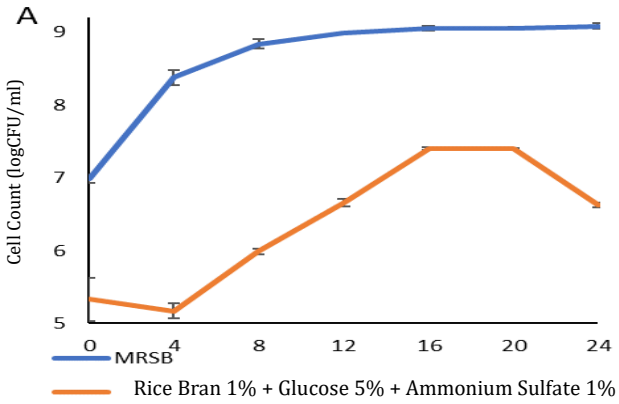

Figure 1. Growth curve of NHC6 isolates in MRSB medium and 1% rice bran + 5% glucose + 1% ammonium sulfate.

Dried L. plantarum NHC6 starter

Liquid starter culture is produced using media from rice bran, which is concentrated until the composition is 10% (w/v) rice bran, 5% (w/v) glucose, and 1% (w/v) ammonium sulfate. Thus, more dissolved solids are in the starter medium than in the culture medium, measuring the growth curve (1% rice bran). The dissolved solids are used as a fermentation substrate for *L. plantarum* NHC6 in the starter culture. So, the difference in total dissolved solids in the initial and final fermentation conditions was measured. The results of measuring total dissolved solids in a liquid starter aged 0 hours and 14 hours of fermentation are presented in Table 2.

Table 2. Total dissolved solids in liquid starter before and after fermentation

Starter Age	Total Dissolved Solids (g/mL)
0 Hours	1,05 ± 0,026[a]
14 Hours	0,78 ± 0,019[a]

Information: [a] Numbers accompanied by the same letter in the same column indicate results that are not significantly different based on the paired sample t-test at the 5% test level.

The starter, at the age of 14 hours, experienced a decrease in dissolved solids by a difference of 0.27 g/mL, namely to 0.78 ± 0.02 g/mL. Even though there was a decrease, the changes that occurred were not significantly different (P>0.05).

The dry starter from the spray drying process is yellowish brown with grains resembling flour (Figure 2). Dry starter powder can break down well, but small lumps of flour will form. The water content of the liquid starter and dry starter is compared in Table 3.

Figure 2. Dried *Lactobacillus plantarum* NHC6 starter with 10% rice bran matrix + 5% glucose + 1% ammonium sulfate resulting from spray drying.

The spray drying process resulted in a significant decrease in water content (P<0.05). This change resulted in a difference of 67.9% with the percentage value of dry starter moisture content, namely 16.1% (Table 3).

Table 3. Starter moisture content before and after spray drying

Treatment	Water Content (%)
Before Spray Drying	84,0 ± 0,100[a]
After *Spray Drying*	16,1 ± 0,148[b]

Information: [a] Numbers accompanied by different letters in the same column show significantly different results based on the paired sample t-test at the 5% test level.

This difference means that the drying process using spray drying removes up to ± 67.9% of the water in the starter. The dry starter obtained observed under a light microscope. Observation with a light microscope with 100 times magnification shows chunks of rice bran matrix with a scale of 20 µm. The appearance of the structure of water-soluble rice bran grains and lactic acid bacteria starter before and after drying by spray drying is shown in Figure 3.

Figure 3A. shows rice bran grains, which appear porous on the surface and have a compact structure. The pure rice bran chunks looked intact and solid before being inoculated with bacterial culture. Figure 3B. shows the appearance of a liquid starter, namely *L. plantarum* NHC6 starter, in rice bran media before drying. *L. plantarum* NHC6 cells in the form of bacilli/stems were scattered in the rice bran medium. The rice bran matrix chunks appear more hollow and less dense than pure rice bran chunks.

Figure 3C. shows the appearance of the dried *L. plantarum* NHC6 starter, which has been dissolved in sterile distilled water. The rice bran chunks were seen to be more hollow and smaller in size as if they had been broken up, but the *L. plantarum* NHC6 cells did not appear to be scattered; instead, it was thought that the bacterial cells were only trapped in the pores of the rice bran matrix.

Viability of dry L. plantarum NHC6 starter

A comparison of bacterial cell viability before and after the spray drying process is presented in Table 4. The number of live cells in the rice bran starter decreased after the spray drying, namely to 1 x 107 CFU/g. The spray drying method reduced cell viability in the starter by 27.43% and was significantly different (P<0.05).

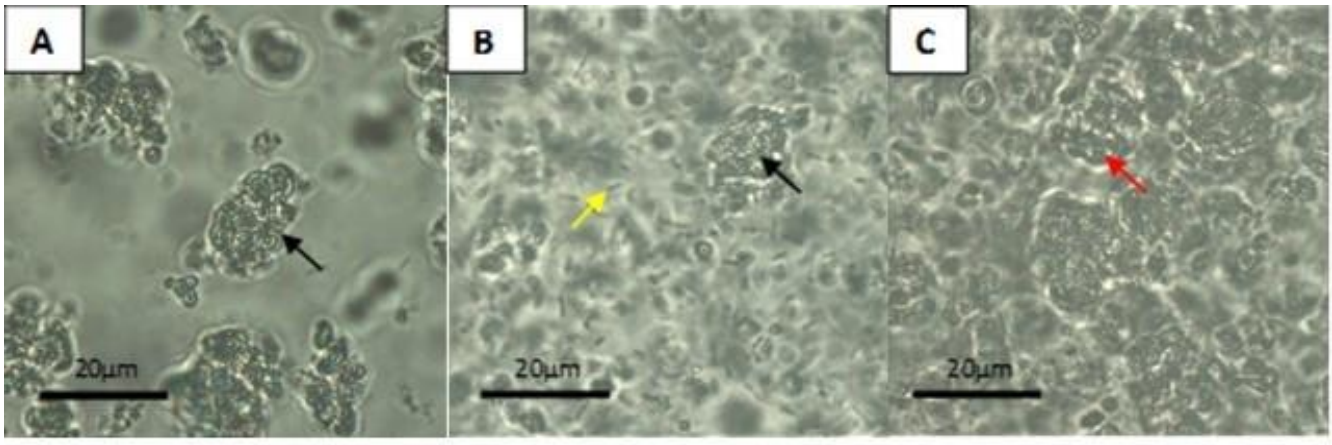

Figure 3. View using a light microscope with 100 times magnification of A) water-soluble rice bran granules (black arrow), B) 14-hour-old liquid starter (yellow arrow bacterial cells and black rice bran arrow), and C) dry starter resulting from the spray drying process dissolved with sterile distilled water (red arrow of rice bran containing bacterial cells).

Table 4. Viability of bacteria before and after the spray drying starter process

Traetment	Number of Cells	Percentage
Before *Spray Drying* (starter cair)	10,364 ± 0,015[a] log CFU/g	100,00%
After *Spray Drying* (starter kering)	7,521 ± 0,047[b] log CFU/g	72,57%

Information: [a] Numbers accompanied by different letters in the same column indicate significantly different results based on the paired sample t-test at the 5% test level.

The viability of bacterial cells in the starter per seven days can be seen in Table 5. The storage temperature factor, storage at 28°C or 4°C, has a significantly different effect (P<0.05). It can be seen that storage at a temperature of 4°C has a higher viability with a higher number of cells than those stored at a temperature of 28°C. The storage time factor, storage at 28°C, experienced a decrease in the number of cells every seven days until the 28th day of storage, whereas in storage at 4°C, there was a decrease and increase in the number of cells during 28 days of storage.

Table 5. Viability of bacteria in starter during 28 days of storage

Time (Days)	Number of Cells (log CFU/g)		Percentage	
	Storage 28°C	Storage 4°C	Storage 28°C	Storage 4°C
7	7,252 ± 0,109^{Aa}	7,681 ± 0,018^{Ba}	96,43%	102,13%
14	6,823 ± 0,003^{Ab}	7,278 ± 0,048^{Bb}	91,72%	97,77%
21	6,221 ± 0,069^{Ac}	7,312 ± 0,036^{Bb}	82,72%	97,22%
28	6,180 ± 0,027^{Ac}	7,504 ± 0,027^{Bc}	82,16%	99,77%

Information: [A] Numbers accompanied by different letters in the same row indicate significantly different results based on ANOVA analysis of variance at the 5% test level.

[a] Numbers accompanied by different letters in the same column indicate significantly different results based on ANOVA analysis of variance and Duncan's post hoc test at the 5% test level.

These two storage factors were proven to influence the viability of bacteria in the starter during 28 days of storage based on a Two-way ANOVA analysis of variance (P<0.05). Storage at a temperature of 28°C experienced a decrease in viability of up to 17.84% until the 28th day of storage. In comparison, storage at a temperature of 4°C could maintain the viability of bacteria in the starter with a viability percentage range of 97.22% - 102.13%.

Visualization of the dynamics of dry starter viability at temperatures of 28°C and 4°C for 28 days is presented in Figure 4. Figure 4 shows a line graph comparing the movement of cell numbers between two different storage temperatures. Storage at 4°C has fluctuating line movements, and the number of cells for 28 days increases and decreases but can maintain the number of bacterial cells in the dry starter as much as 1 x 107 CFU/mL. Storage at 28°C experienced a line movement that continued to decrease for 28 days until finally, it could only maintain the number of bacterial cells in the dry starter as much as 1 x 106 CFU/mL.

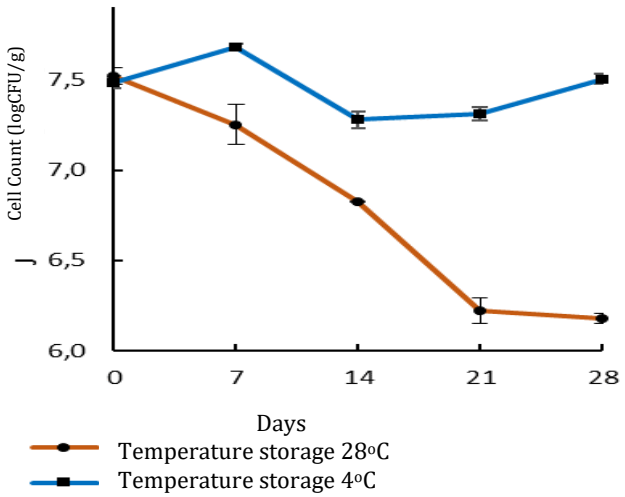

Figure 4. Viability of dry starter during 28 days of storage at room temperature (28°C) and 4°C based on cell number measurements (log CFU/g).

Storage at 4°C can maintain bacterial viability in dry starter above 95% or even exceed 100%. Storage at a temperature of 28°C can maintain the viability of bacteria in the starter up to 80%; however, based on the graph formed it can be predicted that it will continue to decline after 28 days of storage.

Discussions

Soluble Rice Bran in Hot Water

The element with the highest levels contained in water-soluble rice bran is carbohydrates, followed by ash and protein. The protein contained in water-soluble rice bran can be hydrolyzed into simple peptides, which can be broken down into amino acids to be utilized by LAB as a nitrogen source for their growth (Utami et al., 2019). The small amount of carbohydrates is sufficient to become a substrate for LAB, as shown by the growth curve in Figure 1. Hatti-Kaul et al (2018), reported using cellobiose or oligosaccharide as a carbon source for LAB. Sapwarobol et al (2021), reported that oligosaccharides from rice bran can increase the growth of Lactobacillus and Bifidobacterium.

Growth of L. plantarum NHC6 in rice bran media

Lactic acid bacteria (LAB) can grow well on cereal substrates because carbohydrates are the main constituents of the composition of cereals (Ziarno and Cicho ´nska, 2021). Carbohydrates calculated based on Table 1. are carbohydrate components soluble in acid. Rice bran is a by-product of rice that contains polysaccharides, phytosterols, minerals, and trace minerals with a high crude fiber content (Manzoor et al., 2023). This crude fiber cannot be digested by human digestion but by fermentative bacteria, namely BAL. Lactic Acid Bacteria use carbohydrate components as an energy source for growth and as a fermentation substrate.

The growth of *L. plantarum* NHC6 in MRSB media was faster and produced more cells than in 1% rice bran media. MRS media is a standard media and is commonly used in culturing LAB. The nutrients in MRS media have been designed based on research to become an optimum medium for LAB growth. The composition of MRS media is rich in simple sugars and simple proteins and supplemented with essential minerals at predetermined levels (De Man et al., 1960). The simple form of substance in the MRS composition that has been designed makes it easier for *L. plantarum* NHC6 to grow and develop in MRSB media. Water soluble rice bran was proven to contain a source of nutrients to be used as a fermentation substrate (Table 1.), and *L. plantarum* NHC6 was proven to be able to grow in 1% rice bran media enriched with 5% glucose and 1% ammonium sulfate (Figure 1.). The nutrients in rice bran are complex substances, so *L. plantarum* NHC6 takes longer to use for growth. The complex substances in question are insoluble dietary fiber, soluble dietary fiber, and carbohydrates (Manzoor et al., 2023).

Apart from the different types of nutrients in the two media, differences in cell mass production are influenced by other factors, such as pH. The pH of the MRSB media is around 5.5, while the pH of the rice bran media is around 6.5. The difference in the number of cells at hour 0 in MRSB and 1% rice bran media was due to the absorption ability of the rice bran matrix from the beginning of injection of *L. plantarum* NHC6 bacteria into 1% rice bran media. This proves that without specific treatment, for example, a drying process, the rice bran matrix can become an encapsulation agent for bacterial cells. The number of cells measured at hour 0 on 1% rice bran media was lower than the number of cells on MRSB media, even though the amount of culture inoculum added was the same, namely 1%. The number of cells counted in the 1% rice bran media is an imperfect calculation result. It is thought that some of the inoculum cells added to the 1% rice bran media were not wholly dispersed in the liquid media, but rather, some of the cells were absorbed by the rice bran matrix. 1% rice bran media is in the form of a suspension with imperfect solubility like MRSB

media. Even though the rice bran matrix is soluble in water, a precipitate still forms when it is not homogenized. Counting the number of cells uses a serial dilution technique with physiological salt and is carried out using the plate spread method. It is suspected that the distributed suspension only contained a portion of the actual number of cells. This imperfect calculation applies throughout measuring the growth curve in 1% rice bran media.

The decrease in the number of cells from hour 0 to hour 4 in 1% rice bran media (Figure 1.) is thought to be due to the absorption of bacterial cells by the rice bran matrix, as previously described. Apart from that, the decrease in the number of cells occurred because some of the bacteria in the media suspension died, and the bacterial cells that were still alive could not divide because they were adapting to the rice bran medium. The success of *L. plantarum* NHC6 in adapting to nutritional sources from rice bran is shown by exponential growth from the 4th to the 16th hour. Exponential growth is caused by bacteria being able to adjust their metabolism to utilize the nutrients in the rice bran medium, even though cell growth takes longer to reach the final exponential phase.

L. plantarum NHC6 reached the stationary phase in both media types at different times and durations (Figure 1.). The stationary phase experienced in MRSB media lasts until the 24th hour, while the stationary phase in rice bran media tends to be shorter and begins to enter the death phase at the 20th to 24th hour. This is because the bacteria have used up all the nutritional sources available in the rice bran media, and the rice bran media no longer provides components that the bacteria can digest, so the bacteria can no longer continue growth but experience cell death.

Dried L. plantarum NHC6 starter

Dry LAB starters have been widely developed because of their advantages, such as saving energy, being cheap, and having a longer shelf life. However, they still have the disadvantage of reducing microbes. The spray drying method is one of the methods chosen to dry the LAB starter. The principle of spray drying, which utilizes high temperatures, poses a big risk, namely damage to the death of BAL cells so that it can reduce functional BAL cells in the dry LAB starter obtained (Vivek et al., 2023), so the choice of matrix for encapsulation is essential.

Concentration of the rice bran media is carried out to enrich the nutrients in the rice bran media and increase the total solids in the starter. The reduction in total dissolved solids in the starter at the 0th hour and 14th hour of culture in rice bran media proves that *L. plantarum* NHC6 has used rice bran as a fermentation substrate (Table 2.). Based on the difference, *L. plantarum* NHC6 was calculated to have utilized 0.271 g/ml of solids during 14th hours of incubation at 37°C. In addition, the total dissolved solids in the starter were increased so that the rice bran matrix, thought to be the encapsulation matrix for *L. plantarum* NHC6, was more available in the starter. The drying speed and continuous production of the spray drying process means that probiotics can survive even after drying at higher temperatures (150–200°C) (Vivek et al., 2023).

A dried *L. plantarum* NHC6 starter with concentrated rice bran media was successfully obtained by spray drying (Figure 2.). The resulting solid has a powdery structure with few lumps. These lumps are formed due to water-binding compounds in the dry starter, even though spray drying has turned the starter into a dry powder. The water-binding compounds in the dry starter are simple sugars and carbohydrates from rice bran and free glucose. The presence of these constituents causes water to remain in the dry starter. The amount of remaining water is known based on the measured water content (Table 3.). The presence of water remaining in the dry *L. plantarum* NHC6 starter plays an important role in the starter's success as a functional dry LAB starter.

Rice bran is seen as an intact chunk of the matrix with pores on its surface (Figure 3A.), so it can become a matrix for encapsulation. The ability of lactic acid bacteria to adapt to nutrition, environment, and adhesion makes them adaptable to various food matrices (Bintsis, 2018).

L. plantarum NHC6 culture was scattered around the rice bran matrix (Figure 3B.). The rice bran matrix chunks appear to be starting to break up into small chunks with a matrix structure that is not compact. It can be seen that the chunks have been eroded due to fermentation, forming cavities and small gaps on the surface. Then, microscope light cannot penetrate chunks of rice bran matrix. These conditions indicate that *L. plantarum* NHC6 cells have attached to the matrix surface.

L. plantarum NHC6 cells were not distributed around the rice bran matrix (Figure 3C.). This is thought to be because the bacterial cells are in the pores on the surface of the rice bran matrix. Chunks of rice bran matrix that are impenetrable to microscope light show the presence of *L. plantarum* NHC6 cells trapped in the pores of the rice bran matrix.

Apart from being caused by fermentation activities and the impact of the spray drying process, changes in the structure of the rice bran matrix chunks, which became increasingly hollow and fragmented, were caused by the activity of the *L. plantarum* NHC6 isolate. The rice bran matrix is eroded because *L. plantarum* NHC6 digests the oligosaccharides that make up the rice bran matrix into short-chain fatty acids.

Viability of dried L. plantarum NHC6 starter

Measuring the viability of the dry *L. plantarum* NHC6 starter serves to see the success of making the dry starter obtained and helps determine the shelf life and storage recommendations. The dried *L. plantarum* NHC6 starter obtained from spray drying maintained a live cell count of 72.57%, with a calculated cell count reaching log 7 CFU/g. These results show that the dry starter obtained complies with the minimum standard of the LAB population that must be contained in the LAB starter. A LAB starter can be said to be a good starter if the LAB population contained in the starter reaches at least log 7 (Freire et al., 2017). This suitability proves that the rice bran matrix can be an encapsulation material and protect *L. plantarum* NHC6 from high temperatures in the spray drying process. Alves et al. (2016), used Arabic gum or maltodextrin matrix to encapsulate *Lactobacillus casei* NRRL B-442 and experienced a reduction of 4-6 logs or around 50%.

The Dried *L. plantarum* NHC6 starter was stored in LDPE (low-density polyethylene) ziplock plastic containers for 28 days at two different temperature conditions. LDPE plastic containers were chosen based on their properties suitable for temporarily storing dry LAB starters. Plastic made from LDPE is quite thick and stiff, resistant to chemical compounds, and not easily penetrated by water vapor. It also has high gas permeability, one of which is oxygen (Nurminah, 2002). Viability measurements were carried out to determine the best conditions for storing dry *L. plantarum* NHC6 starter and to determine its stability. The storage time and temperature factors show that the dry *L. plantarum* NHC6 starter has a shelf life depending on the storage temperature conditions. The two tested factors had an interaction in influencing the viability of dry *L. plantarum* NHC6 starter based on Two-way ANOVA analysis ($P<0.05$). Storage at a temperature of 4°C has a better effect on viability resistance than storage at a temperature of 28°C. These differences were proven to be significantly different based on statistical analysis with the One-way ANOVA test ($P<0.05$) (Table 5.).

A temperature of 4°C is the best condition for storing dry *L. plantarum* NHC6 starter because it can maintain cell viability with more bacterial populations compared to storage at 28°C. Storage in cold temperatures causes LAB growth to slow (Ayuti et al., 2016). Inhibited LAB growth can inhibit the production of its metabolite, namely lactic acid. At room temperature storage (28°C), the viability of *L. plantarum* NHC6 in the starter decreased further until the 28th day. In this case, it is suspected that the bacteria can still metabolize and produce lactic acid. Over time, lactic acid will accumulate and can result in death for BAL (Ayuti et al., 2016). The lactic acid, thought to accumulate, causes the environmental pH to decrease or the environmental conditions to become more acidic. The presence of water can be represented by the percentage of air humidity (RH%). Cold conditions cause dry environmental conditions, which inhibit the metabolism of bacteria in the starter. Environments with higher humidity are assumed to be wetter conditions.

Storage at 4°C can maintain the viability of *L. plantarum* NHC6 bacteria in dry starter above 95% and can even exceed 100% (Figure 4.). This occurs because the *L. plantarum* NHC6 starter is dried when it is in the exponential phase. In the exponential phase, bacterial cells actively divide, and each grain of rice bran matrix is assumed to contain a different number of cells. Storage at 28°C can maintain the viability of bacteria in the starter up to 80%; however, based on the graph formed it can be predicted that it will continue to decline after 28 days of storage. Based on this viability measurement, the dry *L. plantarum* NHC6 starter with rice bran can be said to have met the requirements to be used as a probiotic food product with a non-dairy substrate. This statement follows standards: effective probiotic food products contain at least a live LAB cell population of at least log 6–log 7 per 10-3 kg or 10-6 m3 (Tripathi & Giri, 2014).

Conclusions

Rice bran enriched with glucose and ammonium sulfate can function as a growth medium and encapsulation agent for *L. plantarum* NHC6. The dry *L. plantarum* NHC6 starter with the resulting rice bran matrix meets the requirements as a functional starter. *L. plantarum* NHC6 starter with rice bran can survive for 28 days better in storage at 4°C.

Declaration statement

The authors reported no potential conflict of interest.

References

Alves, N. N., Messaoud, G. B., Desobry, S., Costa, J. M. C., and Rodrigues, S. (2016). Effect of Drying Technique and Feed Flow Rate On Bacterial Survival And Physicochemical Properties Of A Non-Dairy Fermented Probiotic Juice Powder. *Journal of Food Engineering, 189* (1), 45–54. http://dx.doi.org/10.1016/j.jfoodeng.2016.05.023

Asnita, D. dan Meryandini, A. (2023). Bakteri Asam Laktat Kandidat Probiotik dari Susu Kuda Bima. *Jurnal Sumberdaya Hayati*, 9 (2), 49-54. https://doi.org/10.29244/jsdh.9.2.49-54

Association of Official Analytical Chemists (AOAC). (1995). *Official Methods of Analysis*. 16th ed ed. Arlington (VA): AOAC International.

Ayivi, R. D. and Ibrahim, A. A. (2022). Review: Lactic Acid Bacteria: An Essential Probiotic And Starter Culture For The Production Of Yoghurt. *International Journal of Food Science and Technology*, 57, 7008–7025. https://doi.org/10.1111/ijfs.16076

Ayuti, S.R., Nurliana, N., Yurliasni, Y., Sugito, S. and Darmawi, D. (2016). Dinamika Pertumbuhan Lactobacillus casei dan Karakteristik Susu Fermentasi Berdasarkan Suhu Dan Lama Penyimpanan. *Jurnal Agripet*, 16(1), 23. https://doi.org/10.17969/agripet.v16i1.3476

Bhosale, S. and Vijayalakshmi, D. (2015). Processing and Nutritional Composition of Rice Bran. *Current Research in Nutrition and Food Science*, 3(1), 74–80. http://dx.doi.org/10.12944/CRNFSJ.3.1.08

Bintsis, T. (2018). *Review* Lactic Acid Bacteria As Starter Cultures: An Update In Their Metabolism And Genetics. *AIMS Microbiology*, 4(4), 665–684. https://doi.org/10.3934%2Fmicrobiol.2018.4.665

Erdiandini, I., Sunarti, T.C. and Meryandini, A. (2015). Seleksi Bakteri Asam Laktat Dan Pemanfaatannya Sebagai Starter Kering Menggunakan Matriks Tapioka Asam. *Jurnal Sumberdaya Hayati*, 1(1), 26–33. https://doi.org/10.29244/jsdh.1.1.26-33

Freire, A.L., Ramos, C.L., da Costa Souza, P.N., Cardoso, M.G.B. and Schwan, R.F. (2017). Non-dairy Beverage Produced By Controlled Fermentation With Potential Probiotic Starter Cultures Of Lactic Acid Bacteria And Yeast. *International Journal of Food Microbiology*, 248, 39–46. https://doi.org/10.1016/j.ijfoodmicro.2017.02.011

Gul, K., Yousuf, B., Singh, A.K., Singh, P. and Wani, A.A. (2015). Rice bran: Nutritional Values And Its Emerging Potential For Development Of Functional Food - A review. *Bioactive Carbohydrates and Dietary Fibre*, 6(1), 24–30. http://dx.doi.org/10.1016/j.bcdf.2015.06.002

De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960). A Medium For The Cultivation of *Lactobactilli*. *Journal of Applied Bacteriology*, 23(1), 130–135. https://doi.org/10.4236/als.2019.74012

Hatti-Kaul, R., Chen. L., Dishisha, T. and El Enshasy, H. (2018). Lactic Acid Bacteria: From Starter Cultures to Producers of Chemicals. *FEMS Microbiology Letters*, *365* (20), 1-20. https://doi.org/10.1093/femsle/fny213

Manzoor A., Pandey, V.K., Dar, A.H., Fayaz, U., Kshirod, K., Dashe, K.K. , Shamsf, R., Ahmada, S., Bashi, I., Fayaz, J., Singhg, P., Khanc, S.A., Ganaiec , T.A. (2023). Rice bran: Nutritional, Phytochemical, and Pharmacological Profile and its Contribution to Human Health Promotion. *Food Chemistry Advances*, 100296(2). https://doi.org/10.1016/j.focha.2023.100296

Min, M., Bunt, C., Mason, S., Bennett, G. and Hussain, M. (2017). Effect of Non-Dairy Food Matrices on the Survival Of Probiotic Bacteria During Storage. *Microorganisms*, 5(4), 43–50. https://doi.org/10.3390%2Fmicroorganisms5030043

Miskiyah, N., Juniawati, N. and Yuanita, L. (2020). Mutu Starter Kering Yoghurt Probiotik di Berbagai Suhu Selama Penyimpanan. *Jurnal Penelitian Pascapanen Pertanian*, 17(1), 15–23. http://dx.doi.org/10.21082/jpasca.v17n1.2020.15-23

Nurlaela, S., Sunarti, T.C. and Meryandini, A. (2017). Formula Media Pertumbuhan Bakteri Asam Laktat *Pediococcus Pentosaceus* Menggunakan Substrat Whey Tahu. *Jurnal Sumberdaya Hayati*, 2(2), 31–38. https://doi.org/10.29244/jsdh.2.2.31-38

Nurminah, M. (2002). *Penelitian Sifat Berbagai Bahan Kemasan Plastik Dan Kertas Serta Pengaruhnya Terhadap Bahan Yang Dikemas*. Medan (ID) : Fakultas Pertanian, Universitas Sumatera Utara.

Parente, E., Cogan, T.M. and Powell, I.B. (2017). Starter Cultures: General Aspects. In: *Cheese: Chemistry, Physics and Microbiology: Fourth Edition*. Academic Press.

Riani, C.R., Nuraida, L. and Meryandini, A. (2020). Isolasi Bakteri Asam Laktat Asal Jus Nanas sebagai Kandidat Probiotik. *Jurnal Teknologi dan Industri Pangan*, 31(2), 103–112. https://doi.org/10.6066/jtip.2020.31.2.103

Sapwarobol S., Saphyakhajorn, W. and Astina J. (2021). Biological Functions and Activities of Rice Bran as a Functional Ingredient: A Review. *Nutrition and Metabolic Insights*, 14, 1–11. https://doi.org/10.1177%2F11786388211058559

Tripathi, M.K. and Giri, S.K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, 9(1), 225–241. https://doi.org/10.1016/j.jff.2014.04.030

Utami, T., Kusuma, E.N., Satiti, R., Rahayu, E.S. and Cahyanto, M.N. (2019). Hydrolyses of meat and soybean proteins using crude bromelain to produce halal peptone as a complex nitrogen source for the growth of lactic acid bacteria. *International Food Research Journal*, 26(1), 117–122.

Vivek, K., Mishra. S., Pradhan, R. C., Nagarajan, M., Kumar, P.K., Singh, S.S., Manvi, D. and Gowda, N.N.N. (2023). A Comprehensive Review on Microencapsulation of Probiotics: Technology, Carriers and Current Trends. *Applied Food Research* 3, 100248, 1-16. https://doi.org/10.1016/j.afres.2022.100248

Wulan, R., Meryandini, A. and Sunarti, T.C. (2017). Potensi Limbah Cair Industri Tapioka sebagai Media Pertumbuhan Starter Bakteri Asam Laktat *Pediococcus pentosaceus* E.1222. *Jurnal Sumberdaya Hayati*, 3(1), 27–33. https://doi.org/10.29244/jsdh.3.1.27-33

Ziarno M and Cicho ´nska., P. (2021). Review Lactic Acid Bacteria-Fermentable Cereal- and Pseudocereal-Based Beverages. *Microorganisms,* 9 (2532), 1-24. https://doi.org/10.3390%2Fmicroorganisms9122532