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ENHANCING PROBIOTIC VIABILITY IN FERMENTED MILK STARTER USING MALTODEXTRIN AND WHEY PROTEIN ISOLATE MICROENCAPSULATION TECHNIQUES

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ABSTRACT

Probiotic microencapsulation encapsulates probiotics with a hydrocolloid layer to protect the probiotic cells of *Pediococcus acidilactici* BK01 and enhance the viability of probiotic cultures in fermented milk. Microencapsulation of probiotics can extend the shelf life of cultures and increase the viability of the digestive tract. The study examined the effectiveness of wall material using maltodextrin (MD) and whey protein isolate (WPI): Control (pure culture), MD (1:1.5), MD and WPI (1:1.5). The results demonstrated that the combination of maltodextrin and whey protein wall material had a significant difference ($P<0.05$) on total lactic acid bacteria (LAB), probiotic viability, gastric acid and bile salt viability, microencapsulation morphology, and moisture content. The combination of MD and WPI provided protection and improved the survival of probiotic cultures with a total LAB colony count of 13.57 x 10^9 CFU/ml, gastric acid and bile salt viability of 87.64% and 78.17%, moisture content of 9.20%, and a smoother and more compact morphological shape. In its application as a fermented milk starter, it exhibited a pH value of 4.92, titratable acidity of 1.57%, total LAB 12.08 x 10^9 CFU/mL, and moisture content of 85.48%. This study indicates that the combination of MD+WPI (1:1.5) has potential as a fermented milk starter because of its ability to increase the survival of the probiotic *Pediococcus acidilactici* BK01.

Keywords: Microencapsulation, *Pediococcus acidilactici* BK01, Maltodextrin, Whey Protein Isolate, Starter

INTRODUCTION

The development of food products that contain probiotics is increasing. Probiotics are live microorganisms that provide health benefits to their hosts when consumed in sufficient quantities **(Hill *et al.* 2014)**. Therefore, a general health claim can be made as long as the benefit has been proven, the consumer is going to consume a significant amount, and the microorganisms are alive. In addition, one of the criteria for probiotics that must be met is the ability to colonise the intestine by adhesion to the intestinal epithelium. Probiotic viability in products should be at least 10^7 CFU/g to offer health benefits, such as balancing gut microflora, preventing diarrhoea, enhancing the immune system and anticarcinogenic properties, and reducing the risk of lactose intolerance **(Shah, 2007).**
Any probiotic must have the ability to survive stomach acid and pancreatic juice in order to survive in the environment and multiply in large numbers. This provides them the opportunity to dominate and eliminate pathogenic bacteria. Probiotics support the gastrointestinal system in two ways: by battling pathogenic organisms by producing toxins, and by establishing an acidic environment in which most pathogens cannot survive, they nourish the gastrointestinal tract, especially when administered at relatively high doses **(Irokanulo and Akalegbere, 2020).** *Pediococcus acidilactici* BK01 is a probiotic strain currently under development for wider health applications and improved survival in the gastrointestinal tract. *P. acidilactici* BK01 is a probiotic isolated from bekasam that can survive stomach acid and bile salt conditions and has antimicrobial activity against *Escherichia coli* 0157: H7, *Staphylococcus aureus* ATCC25923, and *Listeria monocytogenes* CFSAN0044330 **(Melia *et al.*, 2019)**.
The viability of probiotics can be maintained through protective measures such as microencapsulation. The materials utilised for microencapsulation were selected based on their desired protective characteristics and release properties (Mohamadzadeh et al., 2024). Microencapsulation is the process of covering probiotics with a hydrocolloid layer to protect probiotic cells and increase the viability of probiotic cultures in fermented milk **(De Prisco *et al.*, 2016).** Microencapsulation is an effective method for improving the viability and stability of probiotics during processing and storage and to protect probiotics from reaching the gastrointestinal tract **(Dolly *et al.*, 2011)**. Polysaccharides and proteins are potential coating materials **(Liao *et al.*, 2017; Rossi *et al.*, 2021; Pato *et al.*, 2022)**. One of these is maltodextrin, which has the advantage of binding solid power, is easily soluble in water, and can form textures **(Purnamayati *et al.*, 2016)**. Whey proteins have the advantages of heat stability, the ability to form gels and films, dispersibility, and good emulsifying properties **(Foegeding and Bavis, 2011)**. A prior study examined the encapsulation of *L. plantarum* 299v using an equal mixture of maltodextrin (MD) and resistant starch (RS) **(Sun et al., 2023).** Numerous techniques exist for encapsulating active components within a matrix. However, the selection of an appropriate method depends on the nature of the material to be encapsulated, its intended application, and the desired mechanism for releasing the active agent. A critical distinction among microencapsulation approaches lies in the manner in which the active ingredient interacts with or is entrapped by the encapsulating substance. This interaction can be physical, chemical, or a combination thereof. The choice of the method depends on these factors and the specific requirements of the application. **(Rossi et al. (2024).**
Based on these considerations, this study used a combination of two wall materials, namely maltodextrin and whey protein isolate in the process of microencapsulating *P. acidilatici* BK01 bacteria to be further utilized as a starter in fermented milk processing.

MATERIALS AND METHODS

Materials

The material used in this study was *P. acidilactici* BK01 obtained from the Animal Product Technology Laboratory, Faculty of Animal Husbandry, Universitas Andalas, Padang. Maltodextrin (*Landisa*®) and whey protein isolate (*Puro*®) were the dressing materials used. As much as 1800 ml of goat milk was obtained from a dairy goat farm in Korong Gadang, Kuranji District, Padang City. In addition, the materials used were MRS Agar (*Neogen Culture Media*®), MRS Broth (*Neogen Culture Media*®), sterile distilled water, oxgall (Merck, Germany) 0.3%, HCL, NaOH 0.1 N, *Phenolphthalein* indicator, and buffers 4.01 and 6.86.

Methods

This research used the Completely Randomized Design experimental method with three treatments and six replicates. The treatments involved different material compositions (Maltodextrin (MD) and whey protein isolate (WPI)) for encapsulating *P. acidilactici* BK01: Control (pure culture), MD (1:1.5 ratio), and MD combined with WPI (1:1.5 ratio). Subsequently, the freeze-drying microencapsulation process was conducted and applied as a fermented milk starter. The parameters measured in this study included lactic acid bacteria (LAB) count, viability of microencapsulated LAB in gastric acid, viability of microencapsulated

LAB in bile salt, morphology of microencapsulated LAB, moisture content, and the quality of fermented milk using this microencapsulated starter (pH, titratable acidity, total LAB count, and moisture.

Preparation of Bacterial Cultures

Probiotic *P. acidilactici* BK01 was rejuvenated with 30 ml of isolate into 270 ml MRS Broth and then incubated for 24 hours at 37ºC. The culture was then centrifuged at 1107 g rpm for 10 minutes at 25ºC. The pellet was then washed with 5 ml sterile distilled water solution and centrifuged again to obtain probiotic bacterial cells **(Rajam *et al.,* 2012)**.

Procedure of microencaptulation.

200 ml of sterile distilled water was stirred using a magnetic stirrer to dissolve MD and WPI. Maltodextrin and WPI were dissolved according to the treatment and stirred until homogeneous. Then, the pellets (bacterial cells) were mixed into the wall material solution at a ratio of 1:1.5 (w/b). Add sterile distilled water up to 15% (b/v), which is 83 ml. Homogenize for 5 minutes before drying **(Rajam *et al.,* 2012)**.

Microencapsulation of Probiotics by Freeze Drying Method

The mixture of probiotic bacteria suspension and wall material solution, prepared according to the treatment protocol, was subjected to freezing at -20°C. Subsequently, the frozen mixture underwent vacuum drying at a pressure of 0.036 Psi for approximately 5 days or until complete dehydration was observed. This process was conducted at the Pekanbaru Industrial Product Development and Standardization Center.

Preparation of Fermented Milk Starter

Goat milk (1800 ml) was pasteurized at 85°C for 15 min. Subsequently, the milk temperature was reduced to ambient temperature. *P. acidilactici* BK01, which was microencapsulated using the freeze-drying method, was introduced into goat milk at a concentration of 1% (w/v) and incubated at 37°C for 18 h (incubator Memmert IPP55).

Measurement Lactic Acid Bacteria.

The total LAB colonies were determined using the method described by **Purwati *et al*. (2005)**. One sample of 1 gram was dissolved in 9 ml MRS Broth. The result of dilution was taken as 100 µL, placed into an Eppendorf tube containing 900 µl MRS Broth solution, and vortexed. Dilutions were made to 10^{-6}. The dilution results were obtained using the spread method and levelled with a hockey stick. The samples were incubated at 37°C for 48 h.

Probiotic Viability in Gastric Acid.

The viability of gastric acid test was assessed using the method described by **Sunaryanto and Marwoto (2013)**. One gram of the microencapsulated powder was inoculated into 9 ml MRS Broth and incubated at 37°C for 24 h. The inoculation results were taken at 1 ml and inoculated into a test tube containing 9 ml MRS Broth pH 3 (HCL 5N) and the control, and then incubated for 90 min. Dilutions were made up to 10^{-6}. The plant was then flattened using the spread method with a hockey stick. Incubate the sample at 37ºC for 48 hours. The formula calculates LAB viability:

Decrease in number of colonies (%):

$$\frac{\text{Total Colonies of Control LAB - Total Colonies of pH Adjusted LAB}}{\text{Total Colonies of Control LAB}} \times 100\%$$

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

Probiotic Viability in Bile Salt

The microencapsulated viability in the bile salt test was conducted using the method of **Sunaryanto and Marwoto (2013)**. One gram of microencapsulated powder was inoculated in 9 ml MRS Broth and incubated at 37ºC for 24 hours. The result of inoculation was 1 ml and inoculated into a test tube containing 9 ml MRS Broth with oxgall 0.3% and control, and then incubated for 5 h. Dilutions were made up to 10^{-6}. It was then planted using the spread method and levelled with a hockey stick. Incubate the sample at 37ºC for 48 hours. The formula calculated calculation of LAB viability:

Decrease in number of colonies (%):

$$\frac{\text{Total Colonies of Control LAB - Total Colonies of Oxgall Setting LAB}}{\text{Total Colonies of Control LAB}} \times 100\%$$

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

Morphology of microencapsulated probiotic *Pediococcus acidilactci* BK01

The microencapsulated morphology was tested according to the method described by **Rajam *et al.* (2012)** using a *Scanning Electron Microscope* (SEM) (Leo 435 VP, Leo Electronic System, Cambridge, UK). Freeze-dried samples were mounted on specimen holders, coated with gold (2 min, 2 mbar), and observed at 15 kV and 9.75 x 10^{-5} torr vacuum.

Moisture Content Assay

Water content was tested according to the **AOAC (2005)** method, and the cup was heated at 110ºC for 1 h. The cup was cooled in a desiccator to remove moisture and weighed; a 5 gram sample was placed in a porcelain cup and oven at 105ºC for 8 h. The sample was then cooled in a desiccator for 30 min and the final weight was measured. Moisture content was calculated by subtracting the weight of the sample before and after drying and dividing by the weight of the sample multiplied by 100%.

pH and Titratable Acidity Assay of Fermented Milk Starter

The pH was determined using a calibrated digital pH meter (HANNA Instrument), with calibration performed using buffer solutions of pH 4.01 and 6.86 (AOAC 2005). To assess titratable acidity, fermented milk was combined with 10 mL of sterile distilled water and subsequently titrated with 0.1N NaOH, using phenolphthalein as an indicator (AOAC, 2005).

Data Analysis

Statistical analysis of the acquired data was performed using the SPSS software. In cases where the treatment demonstrated a significant effect (P<0.05), further evaluation was conducted using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results showed that the combination of maltodextrin and whey protein isolate had a significant difference (P<0.05) on the total lactic acid bacteria, gastric acid and bile salt viability, and moisture content in the microencapsulation of probiotic *P. acidilactici* BK01. The averages of total LAB, gastric acid viability, bile salt viability, and water content of each treatment are presented in Table 1.

Table 1 Microencapsulation characteristics of probiotic *P. acidilactici* BK01 by freeze-drying method

Comparison of Wall Materials	Total LAB (10^9 CFU/ml)	Gastric Acid Viability (%)	Bile Salt Viability (%)	Moisture Content (%)
Control (Pure culture)	16.36±1.84^{c}	60.07±10.93^{a}	56.98±7.69^{a}	95.76±0.10^{c}
MD (1:1.5)	11.57±0.91^{a}	75.17±11.58^{b}	67.98±9.79^{b}	12.02±1.16^{b}
MD and WPI (1:1.5)	13.57±1.76^{b}	87.64±5.35^{c}	78.17±9.15^{c}	9.20±1.17^{a}

Note: Values are Mean ± SD of three measurements, ^{abc} Different superscripts in the same columns indicate significant differences (P<0.05). MD: Maltodextrin, WPI: Whey Protein Isolate.

Total Lactic Acid Bacteria of Microencaptulation

The data presented in Table 1 demonstrate a statistically significant difference (P<0.05) of MD and WPI on the total LAB colony count in the microencapsulation of the probiotic *P. acidilactici* BK01. The combination of MD and WPI was found to mitigate the reduction of probiotics during the freeze-drying microencapsulation process, yielding a concentration of 13.57 x 109 CFU/ml. This effect can be ascribed to the ability of MD to shield probiotics from low temperatures and cell dehydration, wherein it establishes an amorphous matrix structure. **Kurtmann *et al.* (2009)** posit that carbohydrates at high concentrations produce an amorphous glass matrix. Furthermore, the elevated viscosity of MD results in decreased probiotic mobility, thus limiting reactions and enhancing core material resilience**.**

Tyutkov *et al*. (2022) also noted that ice crystal formation during freezing may cause mechanical damage to the MD and probiotic matrix, leading to cell death via leakage of cellular contents**. Ger and Santivarangkna (2015)** proposed that high viscosity in carbohydrates can inhibit reactions, thereby bolstering core resistance. In addition, WPI forms a compact film that protects the probiotic *P. acidilactici* BK01 under freeze-drying conditions. A more robust film layer of WPI can be utilised as an encapsulation wall material for probiotics **(Perez Gago and Krochta, 2001)**. During the freeze-drying process, water is removed through sublimation, thereby mitigating damage to microorganisms and demonstrating that whey protein possesses superior encapsulation properties. WPI is a globular protein that can protect probiotics from adverse mechanical properties during freeze-drying owing to the predominantly hydrophobic nature of most whey proteins. **Rajam *et al*. (2012)** assert that whey protein can protect probiotics from unfavourable environments, attributing this to the hydrophobic nature of whey protein itself. In a study conducted by **Bhagwat *et al*. (2020)**, utilising a mixture of whey protein and maltodextrin resulted in high survival rates of 85-90% for *Enterococcus* probiotics during the spray drying process.

Viability in Gastric Acid

Analysis of the data in Table 1 showed a significant difference ($P<0.05$) between maltodextrin and whey protein isolate with respect to gastric acid viability. A control (pure culture) resulted in improved gastric acid viability for the microencapsulated probiotic *P. acidilactici* BK01 when using maltodextrin as the wall material as well as whey protein isolate. The results indicated viability percentages of 60.07% for pure culture (control), 75.17% for B. MD (1:1.5), and 87.64% for C. MD and WPI (1:1.5). Notably, microencapsulated MD and WPI (1:1.5) exhibited superior viability compared to MD (1:1.5) and pure culture. This heightened viability was ascribed to WPI resistance to acidic conditions (gastric acid). Moreover, the observed decline in stability of free probiotic cells (pure culture/control) during gastric acid simulation underscored the necessity for encapsulation. **Rajam *et al*. (2012)** posit that free probiotic cells are prone to stability loss during gastric acid passage. Conversely, whey protein microencapsulation, achieved through freeze or spray drying techniques, demonstrated improved viability under gastric acid stimulation.

In contrast to maltodextrin, whey proteins exhibit emulsifying and hydrophobic characteristics. This is corroborated by **Gunasekaran *et al*. (2007)**, who asserted that whey protein can expand in water due to its hydrophilic groups within the stomach's acidic environment, where the pH is lower than the isoelectric point of WPI. This results in a positive charge, enabling electrostatic interactions that trigger the release of the wall material and nuclei through diffusion. However, when the gastric pH matches the isoelectric point of whey protein, the release of microencapsulated material slows down. In a prior investigation, **Limbachiya *et al*. (2022)** employed spray-drying techniques to microencapsulate *Lactobacillus fermentum* MTCC8711, assessing WPI as a coating substance. Their findings revealed a 79.41% cell survival rate under acidic gastric conditions (pH 3) when using a 10% WPI concentration.

Viability in Bile Salt

A statistically significant difference ($P<0.05$) in bile salt viability was observed between the MD and WPI groups (Table 1). Microencapsulation of *P. acidilactici* BK01 using MD wall material and WPI as a control enhanced bile salt viability. The results showed pure culture (control) at 56.98%, MD (1:1.5) at 67.98%, and microencapsulated MD and WPI (1:1.5) at 78.17%. MD and WPI (1:1.5) microencapsulation demonstrated superior viability compared to MD (1:1.5) and pure cultures. This improved performance can be attributed to the stability of the wall material in MD and WPI microencapsulation, where the whey protein's capacity to create a more robust and insoluble film restricts cell release in simulated gastric acid and bile salt environments. In contrast, the pure culture exhibited decreased bile salt viability due to a continuous loss of viability under bile salt conditions. According to **Rajam *et al*. (2012)**, denatured WPI can form a solid and insoluble film that limits cell release under bile salt conditions.

The viability in bile salt for the MD (1:1.5) was significantly lower at 67.98% compared to the MD and WPI (1:1.5), demonstrating a statistically significant difference ($P<0.05$). This disparity can be attributed to the high solubility of maltodextrin, which results in a more rapid release of the core. **De Andrade et al. (2019)** demonstrated that incorporating whey powder into an MD matrix effectively enhances the survival of Lactobacillus plantarum CCMA0359 in both acidic gastric conditions and bile salt environments during storage at 70C in refrigeration. Their findings revealed that Lactobacillus plantarum CCMA0359 exhibited an 86.3% survival rate, whereas Lactobacillus brevis CCMA1284 showed a 69.7% survival rate when exposed to bile salts. It is important to highlight that under bile salt conditions, a difference is observed in probiotic viability rather than the treatment itself.

Moisture Content

The analysis presented in Table 1 reveals a statistically significant difference ($P<0.05$) between MD and WPI in terms of moisture. Moisture levels in the microencapsulated probiotic ranged from 9.20% to 12.02% when using MD and WPI. Notably, the MD and WPI combination (1:1.5) as an encapsulant reduced the moisture content to 9.20%, which is lower than the 12.02% observed with MD (1:1.5) alone. This phenomenon can be attributed to WPI behavior of WPI during freeze-drying: it adsorbs onto the ice/liquid interface during freezing, and as ice crystals sublimate in the drying phase, surface cavities form. Thus, WPI use of WPI as an encapsulant enhances porosity, facilitating greater ice sublimation and yielding a product with reduced moisture content. These findings corroborate the work of **Dolly *et al*. (2011)**, who reported that increasing the WPI as a wall material led to enhanced porosity, resulting in higher ice sublimation and lower moisture levels. It is important to note that Table 1 illustrates the significant differences rather than the significant effects of these variables.

The microencapsulation of the probiotic *P. acidilactici* BK01 utilising MD wall material (1:1.5) resulted in a notably high water content of 12.02%, attributable to its enhanced hydrophilicity in comparison to the hydrophobic WPI. This heightened hygroscopicity is a consequence of the inherent hydrophilic properties of MD. Furthermore, the temperature during the microencapsulation process significantly influences the hygroscopic nature of the resultant powder; elevated processing temperatures correlate with decreased hygroscopicity in the maltodextrin powder. This phenomenon aligns with the observations of **Tonon *et al*. (2008)**, who reported low hygroscopicity in maltodextrin when employed as a microencapsulation wall via spray-drying techniques. The established relationship between temperature and powder hygroscopicity underpins these findings. Moreover, **Wang *et al*. (2019)** asserted that whey protein exhibits superior efficacy in preserving probiotics against deterioration during storage under high-humidity conditions, as hygroscopic substances are prone to moisture absorption from the atmosphere. **Rajam *et al*. (2012)** employed freeze-drying to microencapsulate *Lactobacillus plantarum* MTCC 5422, utilising WPI and sodium alginate, which yielded a moisture content of 3.87%, while their denatured counterparts produced a moisture content of 4.34%.

Morphology of microencapsulated probiotic *Pediococcus acidilactci* BK01

Morphology of microencapsulated LAB using MD and WPI based on scanning electron microscopy (SEM) images. Figure A. MD (1:1.5) and figure B. MD and WPI (1:1.5) illustrate the morphology of freeze-drying microencapsulated powder with 3000X, 5000X, and 10000X magnification. Figure 1 demonstrates that the morphology of microencapsulation with MD (1:1.5) dressing material differs from MD and WPI (1:1.5). The microencapsulated powder with the MD wall material alone exhibited a uniform, rigid, and rounded shape, with a non-smooth surface. **Choi *et al*. (2010)** posited that surface dents were caused by the presence of polysaccharides. The morphology of the microencapsulation also exhibited a porous sponge-like structure. This phenomenon is attributed to the microencapsulation resulting from the formation of ice crystals during sublimation **(Anandharamakrishnan *et al*., 2007).**

The MD and WPI (1:1.5) microencapsulated powder exhibited spherical morphology with depressions on the outer surface, devoid of cracks or fractures. The MD and WPI wall materials demonstrated good mechanical strength in protecting the probiotic *P. acidilactici* BK01 from undesirable oxidation and release. **Limbachiya *et al*. (2022)** reported that the morphology of WPI observed through SEM revealed a round shape without breaks on the outer surface, thus providing protection for probiotics against oxidation reactions. The smooth microencapsulated surface, free of cracks, indicates a smooth surface texture of WPI and prevents the loss of the coating material. Furthermore, the absence of cracks on the whey protein surface impedes the damage or loss of the core material **(Rajam *et al*., 2012).**

Fermented Milk Starter

The results showed that the microencapsulation of *P. acidilactici* BK01 using a combination of MD and WPI as a fermented milk starter had a significant difference ($P<0.05$) in total LAB, titratable acidity, and pH value. However, there was no significant effect ($P>0.05$) on the water content of *P. acidilactici* BK01 fermented milk starter. The averages of total LAB, pH, titratable acidity, and moisture content of the fermented milk starter are presented in Table 2.

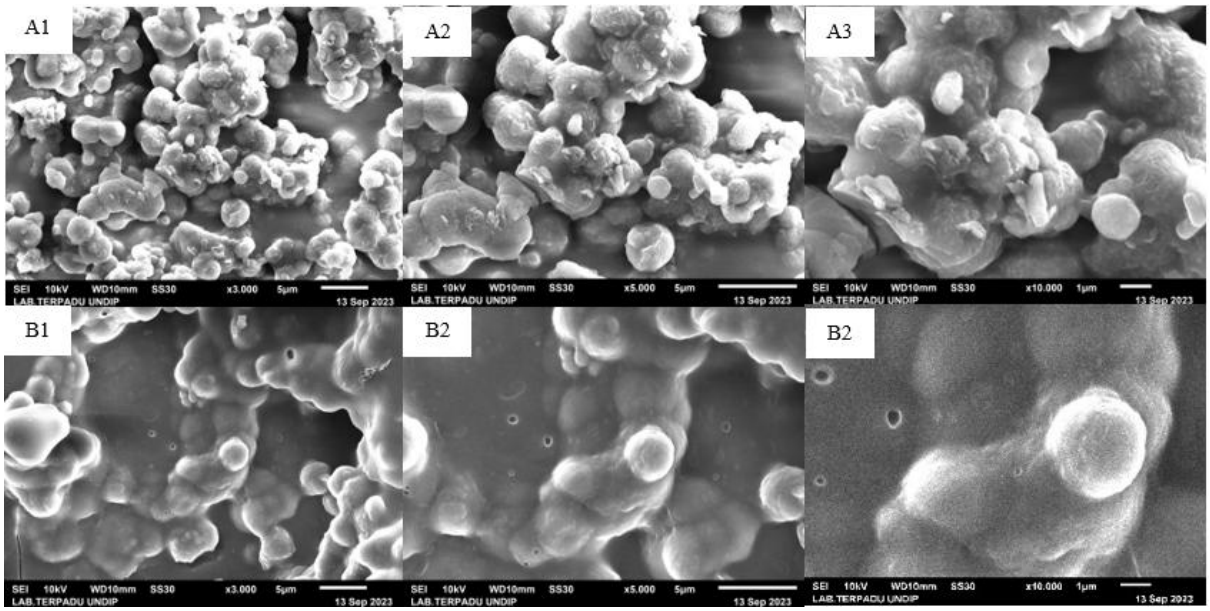

Figure 1 *Scanning Electron Microscope* (SEM) morphology of microencapsulated probiotic *Pediococcus acidilactci* BK01 MD and WPI wall material by freeze-drying method**.** Wall material: A1. (MD 1:1.5) 3000X magnification, A2. (MD 1:1.5) 5000X magnification, A3. (MD 1:1.5) 10.000X magnification, B1. (MD and WPI 1:1.5) 3000X magnification, B2. (MD and WPI 1:1.5) 5000X magnification, B3. (MD+WPI 1:1.5) 10.000X magnification.

Table 2 pH, titratable acidity, total lactic acid bacteria, and water content of fermented milk starter using microencapsulated *P. acidilactici* BK01.

***Pediococcus acidilactici* BK01**	**pH Value**	**Titratable Acidity (%)**	**Total LAB (10^9 CFU/mL)**	**Moisture Content (%)**
Control (pure cultures)	4.80±0.13[a]	1.62±0.07[b]	14.27±0.80[b]	82.91±3.73
MD (1:1.5)	5.05±0.11[b]	1.46±0.09[a]	10.90±3.35[a]	84.94±1.59
MD and WPI (1:1.5)	4.92±0.36[a]	1.57±0.05[b]	12.08±2.90[ab]	85.48±0.89

Note: [ab] Different superscripts in the same columns indicate significant differences (P<0.05). MD: Maltodextrin, WPI: Whey Protein Isolate

The statistical analysis presented in Table 2 revealed a significant difference (P<0.05) in pH and titratable acidity of *P. acidilactici* BK01 fermented milk starter when using MD and WPI (1:1.5). The pH of fermented milk starter ranged from 4.80–5.50. The MD and WPI (1:1.5) combination resulted in a lower pH than MD (1:1.5) alone, as WPI acts as a protective agent for probiotics during freeze–drying, thus preserving the LAB colony count in the fermented milk starter. Lactic acid bacteria metabolize lactose to produce lactic acid during fermentation, leading to a decrease in pH. **Melia *et al*. (2022)** attributed the pH reduction in fermented milk to the increased lactic acid production by *L. Plantarum* SN13T. This is supported by **Wu et al. (2011)**, who noted that organic acids produced during lactic acid bacteria fermentation lower the pH. The pH values observed in this study were lower than those reported for *P. acidilactici* BK01 fermented whey product (5.10–5.20) by **Melia *et al.* (2021)**, but higher than the 4.17–4.47 range reported by **Melia *et al.* (2022)** for *P. acidilactici* BK01 fermented milk with added red ginger.

Titratable acidity in *P. acidilactici* BK01 fermented milk starter ranged from 1.46–1.62% (Table 2). Fermented milk starter using MD and WPI (1:1.5) have a higher titratable acidity when compared to titratable acidity MD (1:1.5). The WPI is hydrophobic, which can protect probiotics during the freeze-drying microencapsulation process. The protection provided by probiotics increases titratable acidity. According to **Melia *et al.* (2022)**, the production of organic acids during fermentation increases with a higher count of LAB colonies, resulting in elevated titratable acidity. As the number of LAB colonies increases, there is a corresponding decrease in pH and an increase in titratable acidity. **Sebastian *et al*., (2018)** noted a relationship between declining pH levels and increasing total acid titration throughout the fermentation process.

The research revealed that the titratable acidity of the control fermented milk starter using pure culture showed no significant difference (P>0.05) on MD and WPI fermented milk starter (1:1.5) but significantly difference (P<0.05) MD fermented milk starter (1:1.5) (Table 2). The study found that total LAB colonies in *P. acidilactici* BK01 fermented milk starter ranged from 10.90–14.27 x 10^9 CFU/mL. Pure culture fermented milk starter exhibited higher counts compared to MD (1:1.5) and MD and WPI (1:1.5). Microencapsulation can decrease bacterial viability owing to cell membrane and protein structural damage during the process. **Dianawati *et al.* (2016)** indicated that cell viability loss during microencapsulation results from cell denaturation and changes in membrane damage. Furthermore, the hydrophobic nature of the WPI dressing material shields probiotics from poor mechanical properties during freeze-drying. The total LAB count of *P. acidilactici* BK01 starter in this study higher than the findings of **Melia *et al.* (2022)**, which reported 3.0–9.0 x 10^9 CFU/mL in fermented milk with added *L. plantarum* SN13T. MD and WPI (1:1.5) showed nearly identical results those as of the control (pure culture). **Najgebauer-Lejko (2014)** suggests that probiotic health benefits are realized when lactic acid bacteria concentrations reach 10^6–10^9 CFU/mL.

There was no significant difference (P>0.05) in the moisture content of *P. acidilactici* BK01 fermented milk starter when comparing MD and WPI. The water levels in the fermented milk starter samples ranged from 82.91% to 85.48%. A slight, non-significant increase in moisture was observed in fermented milk starter using MD (1:1.5) and a blend of MD and WPI (1:1.5). This can be attributed to the addition of WPI, which enhances water retention in fermented milk. According to **Lee and Lucey (2010)**, water holding capacity is influenced by increased protein content, as milk proteins are amphiphilic in nature. Notably, the moisture content in this study was lower than that reported for *Lactobacillus fermentum* NCC2970 fermented goat milk products, which ranged from 85.05% to 86.16% **(Melia *et al*., 2019)**.

CONCLUSION

The study concluded that microencapsulation using maltodextrin (MD) and whey protein isolate (WPI) wall material in a 1:1.5 ratio resulted in a lactic acid bacteria colony count of 13.57 x 10^9 CFU/ml, viability in gastric acid of 87.64%, and viability in bile salts of 78.17%. When freeze-dried, the wall material consisting of MD and WPI (1:1.5) exhibited SEM morphological characteristics with a smoother, rounder structure and no fractures on the outer surface, which could protect probiotics from oxidation during storage. Fermented milk starter using the microencapsulated with MD and WPI (1:1.5) had a pH value of 4.92, titratable acidity of 1.57%, total lactic acid bacteria count of 12.08 x 10^9 CFU/mL, and a moisture content of 85.48%. Microencapsulated probiotic powder of *P. acidilactici* BK01 with maltodextrin and whey protein isolate can effectively protect probiotic cells.

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REFERENCES

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. (2007). Effects of process variables on the denaturation of whey proteins during spray drying. *Drying technology*, *25*(5), 799-807. https://doi.org/10.1080/07373930701370175

AOAC. (2005). Official Methods of Analysis. Association of Official Analytical Chemists. Benjamin Franklin Station, Washington DC.

Bhagwat, A., Bhushette, P., & Annapure, U. S. (2020). Spray drying studies of probiotic *Enterococcus* strains encapsulated with whey protein and maltodextrin. *Beni-Suef university journal of basic and applied sciences*, *9*, 1-8. https://doi.org/1 0.1186/s43088-020-00061-z

Chairunnisa, H., Balia, R. L., & Utama, G. L. (2006). Penggunaan starter bakteri asam laktat pada produk susu fermentasi "*Lifihomi*" (Utilization of Lactic Acid Bacteria In Fermented Milk Product "*Lifihomi*"). *Jurnal Ilmu Ternak*, *6*(2), 102-107. https://doi.org/10.24198/jit.v6i2.2276

Choi, K. O., Ryu, J., Kwak, H. S., & Ko, S. (2010). Spray-dried conjugated linoleic acid encapsulated with Maillard reaction products of whey proteins and maltodextrin. *Food Science and Biotechnology*, *19*, 957-965. https://doi.org/10.1007/s10068-010-0134-7

De Andrade, D. P., Ramos, C. L., Botrel, D. A., Borges, S. V., Schwan, R. F., & Ribeiro Dias, D. (2019). Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. *International Journal of Food Science & Technology*, *54*(7), 2355-2362. https://doi.org/10.1111/ijfs.14114

De Prisco, A., & Mauriello, G. (2016). Probiotication of foods: A focus on microencapsulation tool. *Trends in food science & technology*, *48*, 27-39. https://doi.org/10.1016/j.tifs.2015.11.009

De Sousa, V. M. C., dos Santos, E. F., & Sgarbieri, V. C. (2011). The importance of prebiotics in functional foods and clinical practice. *Food and nutrition sciences*, *2*(2), 133-144. http://dx.doi.org/10.4236/fns.2011.22019

Dianawati, D., Mishra, V., & Shah, N. P. (2016). Survival of microencapsulated probiotic bacteria after processing and during storage: a review. *Critical reviews in food science and nutrition*, *56*(10), 1685-1716. https://doi.org/10.1080/10408398.2013.798779

Dolly, P., Anishaparvin, A., Joseph, G. S., & Anandharamakrishnan, C. (2011). Microencapsulation of *Lactobacillus plantarum* MTCC 5422 by spray-freeze-drying method and evaluation of survival in simulated gastrointestinal conditions. *Journal of microencapsulation*, *28*(6), 568-574. https://doi.org/10.3109/02652048.2011.599435

Foegeding, E. A., & Davis, J. P. (2011). Food protein functionality: A comprehensive approach. *Food hydrocolloids*, *25*(8), 1853-1864. https://doi.org/10.1016/j.fo odhyd.2011.05.008

Ger P and Santivarangkna C. (2015). Advances in probiotic technology. CRC Press, New York, USA

Gunasekaran, S., Ko, S., & Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *Journal of food engineering*, *83*(1), 31-40. https://doi.org/10.1016/j.jfoodeng.2006.11.001

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... & Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature reviews Gastroenterology & hepatology*, *11*(8), 506-514. https://doi.org/10.1038/nrgastro.2014.66

Irokanulo, E. O., & Akalegbere, M. A. (2020). Probiotics for Gastrointestinal Health and General Wellbeing. *International Journal of Probiotics and Prebiotics*, *15*(1), 22–29. https://doi.org/10.37290/IJPP2641-7197.1522-29

Kurtmann, L., Carlsen, C. U., Skibsted, L. H., & Risbo, J. (2009). Water activity-temperature state diagrams of freeze-dried *Lactobacillus acidophilus* LA-5: Influence of physical state on bacterial survival during storage. *Biotechnology Progress*, *25*(1), 265-270. https://doi.org/10.1002/btpr.96

Kusumawati N. (2002). Selection of Indigenous Lactic Acid Bacteria as Probiotic Genus with the Ability to Maintain Fecal Microflora Balance and Reduce Rat Blood Serum Cholesterol. Thesis. Bogor. Bogor Agricultural University.

Lee, W. J., & Lucey, J. A. (2010). Formation and physical properties of yogurt. *Asian-Australasian journal of animal sciences*, *23*(9), 1127-1136. https://doi.org/10.5713/ajas.2010.r.05

Liao, L. K., Wei, X. Y., Gong, X., Li, J. H., Huang, T., & Xiong, T. (2017). Microencapsulation of Lactobacillus casei LK-1 by spray drying related to its stability and in vitro digestion. *LWT-food science and technology*, *82*, 82-89. https://doi.org/10.1016/j.lwt.2017.03.065

Melia, S., Juliyarsi, I., Kurnia, Y. F., Pratama, Y. E., & Azahra, H. (2022). Examination of titratable acidity, pH, total lactic acid bacteria and sensory properties in whey fermented with probiotic *Pediococcus acidilactici* BK01. *Advances Animal Veterinary Science*, *10*(1), 114-119. http://dx.doi.org/10.17582/journal.aavs/2022/10.1.114.119

Melia, S., Ferawati., Zulkarnain, I., Purwanto, H., Yuherman., Jaswandi., & Purwati, E., (2019). Quality, viability, and antibacterial properties of *Lactobacillus fermentum NCC2970* in probiotic fermented goat milk at 4°C. *Asian Journal Microbiology Biotechnology Environment Science*., 21(2), 237-242. https://doi.org/10.32530/jaast.v3i1.70

Melia, S., Purwati, E., Kurnia, Y. F., & Pratama, D. R. (2019). Antimicrobial potential of *Pediococcus acidilactici* from Bekasam, fermentation of sepat rawa fish (*Tricopodus trichopterus*) from Banyuasin, South Sumatra, Indonesia. *Biodiversitas Journal of Biological Diversity*, *20*(12). https://doi.org/10.13057/biodiv/d201210

Melia, S., Juliyarsi, I., & Kurnia, Y. F. (2022). Physicochemical properties, sensory characteristics, and antioxidant activity of the goat milk yogurt probiotic *Pediococcus acidilactici* BK01 on the addition of red ginger (*Zingiber officinale var. rubrum rhizoma*). *Veterinary World*, 15(3): 757. https://doi.org/10.14202%2Fvetworld.2022.757-764

Mohamadzadeh, M., Fazeli, A., & Shojaosadati, S. A. (2024). Polysaccharides and proteins-based bionanocomposites for microencapsulation of probiotics to improve stability and viability in the gastrointestinal tract: A review. *International Journal of Biological Macromolecules*, 129287. https://doi.org/10.1016/j.ijbiomac.2024.129287

Najgebauer-Lejko, D. (2014). Effect of green tea supplementation on the microbiological, antioxidant, and sensory properties of probiotic milks. *Dairy Science Technology*, *94*(4), 327-339. https://doi.org/10.1007/s13594-014-0165-6

Pato, U., Yusmarini, Riftyan, E., Rossi, E., Hidayat, R., Anjani, S. F., ... & Surono, I. S. (2022). Probiotic Properties of Lactobacillus fermentum InaCC B1295 Encapsulated by Cellulose Microfiber from Oil Palm Empty Fruit Bunches. *Fermentation*, *8*(11), 602. https://doi.org/10.3390/fermentation8110602

Perez-Gago, M. B., & Krochta, J. M. (2001). Denaturation time and temperature effects on solubility, tensile properties, and oxygen permeability of whey protein edible films. *Journal of Food Science*, *66*(5), 705–710. https://doi.org/10.1111/j.1365-2621.2001.tb04625.x

Limbachiya, P., Jayesh, H. K, & Vimal, M. R. (2022). Exploration of Spray Drying for Microencapsulation of Probiotic with Whey Protein Isolate to Improve Survivability During Gastrointestinal. *International Journal of Current Microbiology and Applied Science*, *11*(12), 40-56. https://doi.org/10.20546/ijcmas.2022.1112.006

Purnamayati, L., Eko, N. D., & Retno, A. K. (2016). Physical characteristics of spirulina phycocyanin microcapsules at different concentrations of dressing material. *Journal of Agricultural Product Technology*, Vol 9: 1-8.

Purwati. E., Syukur. S., & Hidayat. Z. (2005). *Lactobacilus sp*. isolation from biovicophitomega as probiotics. Indonesian Institute of Sciences, Jakarta.

Rajam. R., Karthik, P., Parthasarathi, S., Joseph, G. S., & Anandharamakrishnan C. J. J. O. F. F. (2012). Effect of whey protein–alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal conditions. *Journal of Functional Foods*, *4*(4), 891-898. https://doi.org/10.1016/j.jff.2012.06.006

Rossi. E., Restuhadi. F., Efendi, R., & Dewi, Y. K. (2021). Physicochemical and microbiological properties of yogurt made with microencapsulation probiotic starter during cold storage. *Biodiversitas: Journal of Biological Diversity*, *22*(4): 2012-2018. https://doi.org/10.13057/biodiv/d220450

Rossi, E., Restuhadi, F., Efendi, R., Yusmarini, R., Riftyan, E., Winata, B. P., ... & Pato, U. (2024). Physicochemical, microbiological, and sensory characteristics of yoghurt produced by back-sloping fermentation. *Adv. Anim. Vet. Sci*, *12*(6), 1099-1106. https://dx.doi.org/10.17582/journal.aavs/2024/12.6.1099.1106

Sebastian, A., Barus, T., Mulyono, N., Yanti. (2018). Effects of fermentation and sterilization on quality of soybean milk. *International Food Research Journal*., *25*(6): 2428- 2434.

Shah, N. P. (2007). Functional cultures and health benefits. *International Dairy Journal*., 17: 1262–1277. https://doi.org/10.1016/j.idairyj.2007.01.014.

Sunaryanto, R and Marwoto, B. (2013). Isolation, identification and characterisation of lactic acid bacteria from buffalo milk curd. Indonesian *Journal of Science and Technology, 14*(3), 228-233.

Sun, W., Nguyen, Q. D., Süli, B. K., Alarawi, F., Szécsi, A., Gupta, V. K., ... & Bujna, E. (2023). Microencapsulation and application of probiotic bacteria *Lactiplantibacillus plantarum* 299v strain. *Microorganisms*, *11*(4), 947. https://doi.org/10.3390/microorganisms11040947

Suter, I. K. (2013). Functional food and its development prospects. In Food Technology. One-day Seminar on the theme "The Importance of Natural Food for Long-term Health" (pp. 1-17).

Tonon, V. R., Brabet, C., & Hubinger D. M., (2008). Influence of process conditions on the physicochemical properties of acai (*Euterpe Oleraceae Mart*.) powder produced by spray drying. *Journal of Food Engineering*, *88,* 411–418. https://doi.org/10.1016/j.jfoodeng.2008.02.029

Tyutkov, N., Zhernyakova, A., Birchenko. A., Eminova, E., Nadtochii, L., & Baranenko, D. (2022). Probiotics viability in frozen food products. *Food Bioscience*, 50, 101996. https://doi.org/10.1016/j.jconrel.2012.06.003

Wang, C., Wang, M., Wang, H., Sun, X., Guo, M., & Hou, J. (2019). Effects of polymerized whey protein on survivability of *Lactobacillus acidophilus LA5* during freeze drying. *Food Science and Nutrition*, 7(8), 2708-2715. https://doi.org/10.1002/fsn3.1130

Wu, S. C., Su, Y. S., Cheng, H. Y. (2011). Antioxidant properties of *Lactobacillus* fermented and non-fermented Graptopetalum paraguayense E. Walther at different stages of maturity. *Food Chemistry*, 129 (3), 804-809. https://doi.org/10.1016/j.foodchem.2011.05.025