Viability of the probiotic *Lactobacillus plantarum* microencapsulated in skim milk and maltodextrin: implications for nutritional and functional uses

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Abstract. Probiotic lactic acid bacteria provide numerous physiological benefits, but their viability is often compromised during processing, storage, and gastrointestinal transit. This necessitates innovative techniques to preserve their functionality and enhance their nutritional contribution. This study evaluates the viability of microencapsulated *Lactobacillus plantarum* isolated from North Sumatra River buffalo milk. Probiotics were encapsulated using skim milk and maltodextrin as carriers, followed by spray drying. The viability of microencapsulated and free cells of *L. plantarum* was tested under simulated gastrointestinal conditions and during storage at 4 ºC and room temperature for 30 days. Subsequently, the data were analyzed using ANOVA, revealing that skim milk and maltodextrin significantly enhanced probiotic survival, with retention rates of 92.19% (8.79 log CFU/g) and 90.53% (8.61 log CFU/g), respectively. Microencapsulated *L. plantarum* exhibited lower population reductions than free cells under simulated gastric (pH 2) and bile salt conditions, maintaining populations of 7.69 log CFU/g and 7.43 log CFU/g, respectively. After 30 days, viable counts remained at 10^7 CFU/g at 4 °C and 10^5 CFU/g at room temperature. These findings indicate that microencapsulation with skim milk and maltodextrin effectively protects *L. plantarum*, preserving its potential as a functional probiotic.

1 Introduction

Probiotic bacteria, particularly strains from lactic acid bacteria (LAB), are known for their health benefits, including reducing oxidative stress, enhancing immune function, and improving gut health. These benefits contribute to overall nutritional health, as probiotics can enhance nutrient absorption, such as calcium and magnesium, and improve metabolic. processes in the gut. As a result, they are widely used in nutraceuticals, products derived from food that provide health benefits beyond basic nutrition. Although most studies focus on LAB derived from cow's milk or other sources, buffalo milk offers unique nutritional advantages, including higher levels of fat, protein, calcium, and bioactive compounds. These components may enhance the viability and functionality of LAB, making buffalo milk a promising medium for probiotic research. In addition, probiotic LAB has been isolated from river buffalo milk from North Sumatra, Indonesia.

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LAB, commonly found in milk and fermented foods, produces bioactive substances such as peptides, exopolysaccharides (EPS), and bacteriocins. These compounds support the immune system, provide antioxidant effects, and inhibit harmful bacteria [1]. EPS, for instance, serves as prebiotics, promoting the growth of beneficial gut microbiota. This potential for positive health effects has led to the incorporation of LAB as probiotics in various foods and dietary supplements.

Probiotics in foods are typically transient and unable to establish or replicate in the human gastrointestinal (GI) tract. Therefore, regular consumption of probiotics is necessary to maintain their associated health benefits [2]. Beyond fermented foods, LAB can also inhabit various environments, including gastrointestinal (GI) tracts, oral cavities, human and animal vaginal tracts, silages, and composts.

Maintaining probiotic viability during shelf life and GI transit is essential for delivering health benefits. This requires viable counts between 10^6 and 10^7 Colony-forming units (CFU)/mL at the time of consumption. Techniques such as alginate encapsulation, the use of cryoprotectants, ultrasonic processing, and spray drying have been developed to improve probiotic stability under storage and GI conditions [3]. Optimizing these methods and selecting robust strains ensures consistent probiotic efficacy throughout the product's shelf life. As a result, there is increasing interest in encapsulating viable probiotic organisms within a physical barrier to shield them from harsh environmental conditions. Microencapsulation has emerged as an effective solution to address the loss of viability and functionality of probiotic cultures.

Recent studies have evaluated food-grade materials such as maltodextrin, gelatin, and skim milk for their potential to enhance probiotic survival during spray drying. Skim milk has shown particular effectiveness due to its milk proteins and lactose, which form a protective matrix around encapsulated cells. Maltodextrin also supports probiotic viability and serves as a prebiotic. It is a preferred wall material in microencapsulation because of its affordability, neutral aroma, and ability to reduce oxidation. These findings highlight the critical role of selecting suitable encapsulating agents to enhance the stability and efficacy of probiotics in various applications [4].

Based on the information above, *L. plantarum* is known as a probiotic with numerous health benefits. However, its viability is frequently reduced during processing, storage, and GI transit, limiting its functional efficacy. While microencapsulation has proven effective in enhancing probiotic stability, the use of specific materials such as skim milk and maltodextrin for improving the survival of *L. plantarum* under these conditions remains inadequately explored. This gap is particularly evident for isolates derived from North Sumatra River buffalo milk, a unique and underutilized source of probiotics with significant potential.

This study builds on previous findings that demonstrated the potential of *L. plantarum* as a probiotic. Specifically, we aim to evaluate the impact of skim milk and maltodextrin as encapsulating agents on the viability of *L. plantarum* during simulated gastrointestinal transit and storage at both refrigerated and room temperatures over 30 days. By optimizing encapsulation materials, we aim to enhance the stability and functional efficacy of L. *plantarum* for use in food and nutraceutical applications.

2 Materials and methods

2.1 Microorganism

Two isolates of *L. plantarum* were obtained from LAB and isolated from North Sumatra River buffalo milk then selected for its probiotic characteristic and used in the test

2.2 Microencapsulation by spray drying

Microencapsulation was performed according to the specified procedures. Microparticles were created using skim milk and maltodextrin (with a total solid content of 20%), which were inoculated with biomass of *L. plantarum* and then subjected to spray drying at inlet and outlet temperatures of 120 ºC and 70 ºC, respectively. The resulting microcapsules containing *L. plantarum* were collected and stored in sterile plastic bottles. To assess the viability of *L. plantarum* after spray drying, cell counts were measured both before and after the process. The viable cell counts of *L. plantarum* under simulated gastrointestinal conditions were evaluated for both the free (control) and microencapsulated form [5].

2.3 Enumeration of microencapsulated bacteria

The entrapped bacteria in skim milk and maltodextrin were counted according to established procedures [5]. To enumerate *L. plantarum*, the samples were serially diluted using buffer peptone water (0.1 g 10 ml^{-1}) and plated on MRS agar. The plates were then incubated at 37 ºC for 48 hours. Following the incubation period, the viable probiotic cell counts were determined and expressed as log colony-forming units per gram (log CFU g^{-1}).

2.4 Viability of intestinal condition of microencapsulated bacteria in simulated high acid gastric conditions and bile solution intestinal condition

The encapsulated and free bacteria were introduced into MRSB that had been adjusted to simulate gastric juice (pH 2) using aliquots of 1 M HCl. The samples were incubated at 37 ºC for 3 hours. A 10 ml aliquot was then diluted to a 1/10 ratio and subsequently serially diluted to achieve an appropriate concentration for plating. The plates were incubated anaerobically at 37 ºC for 48 hours. To prepare the intestinal juice (bile solution), a solution of oxgall (Difco) was created by dissolving 10 g of oxgall in 90 ml of distilled water, which was then used to make a 0.5% bile salt concentration. All solutions were sterilized at 121 ºC for 15 minutes. The encapsulated and free bacteria were added to the MRSB adjusted to a 0.5% bile salt concentration and incubated at 37 ºC for 6 hours. Again, a 10 ml aliquot was diluted to a 1/10 ratio and serially diluted for plating, with the plates incubated anaerobically at 37 ºC for another 48 hours [6].

2.5 Assessment of survival during storage

To determine the stability of the microencapsulated *L. plantarum* during storage, the samples were placed in sterile plastic bottles then stored at refrigerated (4 ºC) and room temperature for 30 days. After the storage periods (30 days), samples were taken and viability cells were determined using standard plating techniques on MRS agar.

This study used a Completely Randomized Design (CRD) method with 4 treatments: L20 skim milk, L20 maltodextrin, S3 skim milk, and S3 maltodextrin, with 5 replications. Data was analysed using Two Way ANOVA and continued with Duncan's test.

3 Results and discussion

In this study, skim milk and maltodextrin were used as a microencapsulation material. The effectiveness of skim milk and maltodextrin on the bacteria *L. plantarum* microencapsulation (LM) were presented in this result.

3.1 Survival of *L. plantarum* after spray drying

After spray drying, *L. plantarum* microencapsulation (LM) exhibited greater viability in skim milk compared to maltodextrin (Table 1).

Table 1. Survival of *L. plantarum* in skim milk and maltodextrin (20% TS) after Spray drying

Strain *L. plantarum*	Encapsulating material	Cell counts (log CFU ml^{-1})		Cell death (log before-log after)
		Before	After	
L20	Skim milk	9.51 ± 0.66	8.79 ± 0.54^{a}	0.71^{a}
	Maltodextrin	9.50 ± 0.46	8.64 ± 0.60^{b}	0.86^{b}
S3	Skim milk	9.56 ± 0.69	8.78 ± 0.51^{a}	0.77^{a}
	Maltodextrin	9.52 ± 0.47	8.59 ± 0.47^{b}	0.94^{c}

^{a,b,c} cell counts in rows with different superscripted letters are significantly different ($p>0.05$).

The survival percentages of *L. plantarum* in skim milk and maltodextrin LM were 92.19% and 90.53%, respectively, corresponding to probiotic concentrations of 8.79 and 8.64 log CFU/g. Both skim milk and maltodextrin are effective encapsulation materials due to their protective properties during spray drying. As noted by [7], skim milk prevents cellular damage by stabilizing the cell membrane, which helps the cells rehydrate easily after drying and shields them with proteins that form a protective coating. The survival of the two strains varied in skim milk and maltodextrin post-spray drying. The findings surpassed those of [18], who reported a survival rate of 77.73% and a probiotic count of 7.27 log CFU/g for *L. acidophilus* La-5. Previous studies have also shown that spray drying is an effective method for producing powders from skim milk-based media that contain large quantities of viable bacteria from various species [3].

In terms of nutritional benefits, the encapsulation process preserves not only the viability of *L. plantarum* but also enhances the potential for the probiotic to positively influence gut health, digestion, and nutrient absorption, making it a valuable addition to functional food products. The use of skim milk, which is rich in proteins and calcium, contributes to the nutritional value of the encapsulated probiotics, providing added benefits to consumers beyond the probiotic effects alone.

3.2 Viability of *L. plantarum* during storage

The effect of different microencapsulating materials on the viability of *L. plantarum* throughout storage at refrigerated (4°C) and room temperature is shown in Figure 1. The viability of *L. plantarum* microencapsulated with skim milk and maltodextrin which were stored in room temperature was significantly lower than at 4 ºC for 30 days of storage. In addition, throughout the entire storage period at 4°C, the number of viable probiotic cells remained above the recommended threshold for probiotic foods (10^7 CFU/g).

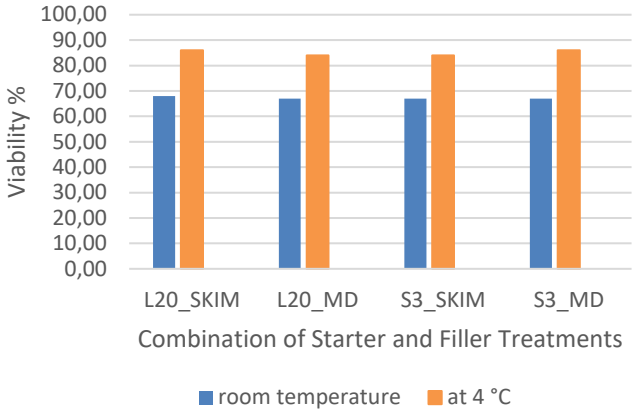

Fig. 1. Viability of *L. plantarum* during storage (30 days) in refrigerated (4 °C) and room temperature

The bacterial counts of the microencapsulated bacteria decreased by 1 and 2 log cycles after being stored for 30 days at 4 °C and room temperature, respectively. After the 30 days, the remaining probiotic count was 10^7 and 10^5 CFU/g when stored at 4ºC and room temperature, respectively. Similar findings have been reported in studies using sodium alginate and carrageenan for encapsulation [2]. Encapsulation helps prolong the shelf-life probiotic bacteria by shielding them from environmental stresses such as temperature fluctuations. At 4°C, encapsulated LM maintained high viability (10^7 CFU/g) after 30 days, demonstrating the efficacy of encapsulation in preserving probiotic viability under refrigerated conditions. At room temperature, viable cell counts below the recommended levels (10^5 CFU/g), the lower viability underscores the challenge of maintaining probiotic stability without encapsulation.

Moreover, the stability of the probiotic during storage is essential to ensure that the functional and nutritional benefits are delivered to consumers. Probiotic bacteria contribute to the synthesis of essential vitamins, such as B12 and K, and support gut health. Therefore, maintaining high cell viability throughout storage directly impacts the nutritional value of probiotic-rich foods.

Previous research highlights the importance of storage conditions for preserving the viability of dried probiotic cells [9]. It has been observed that cells damaged during the drying process are often the first to lose viability during storage. However, since storage itself can be a stressful situation, even cells that were not initially damaged may lose their viability over time [10]. Storing probiotics at refrigeration temperatures is an effective method for preserving cell viability over extended periods [11].

3.3 Survival of *L. plantarum* in simulated highly acidic gastric conditions and bile solution

The viability of *L. plantarum* (LM) was assessed under harsh conditions, revealing significant reductions in colony-forming units per gram (CFU/g). The results of the viability of LM in gastric juice are presented in Figure 2. The viability of LM cells in gastric juice was decreased after 3 hours of incubation, by 0.93 log CFU/g, while free cells experienced a more significant decline of 1.17 log CFU/g. Encapsulating *L. plantarum* in skim milk and maltodextrin enhanced its viability compared to non-encapsulated cells.

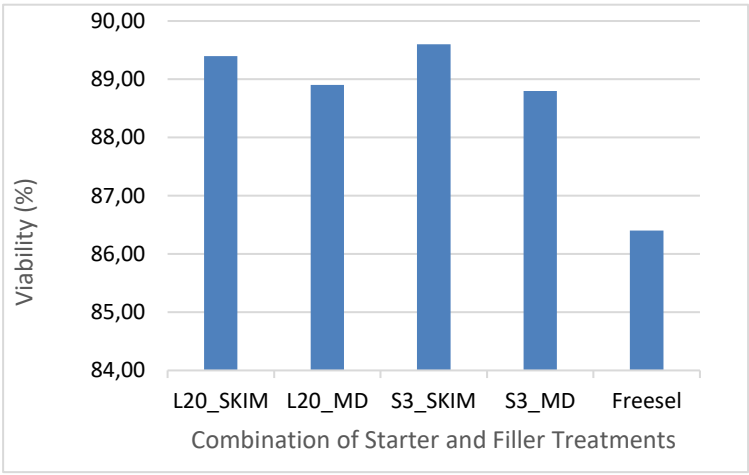

Fig. 2. Viability of *L. plantarum* microencapsulation using skim milk and maltodextrin material compared to free cell in simulated acid condition (pH 2) 3 h incubation

A primary objective of microencapsulation is to improve the survival rate of probiotic cells when exposed to the low pH conditions of the human stomach, which is crucial for their effectiveness as functional ingredients [12]. Our findings demonstrate that encapsulating L. plantarum with skim milk and maltodextrin significantly enhances its survival rate compared to free cells.

Research shows that the viability of probiotic bacteria in the human stomach is influenced by environmental pH and varies by strain. [13] observed a significant increase in viable numbers of encapsulated *L. acidophilus* in alginate-$CaCO_3$ microcapsules after just 2 hours of exposure to simulated gastric fluid. These results emphasize the need to select appropriate encapsulation materials to improve probiotic effectiveness in the gastrointestinal tract. Enhancing cell viability can help maximize the health benefits of probiotics, potentially leading to more effective functional foods and dietary supplements.

The ability of encapsulated *L. plantarum* to survive in the acidic environment of the stomach is critical for its ability to exert its health benefits, including enhancing nutrient absorption and supporting gut microbiota balance. Additionally, the inclusion of skim milk in the encapsulation material offers added nutritional benefits, such as calcium and protein, which are essential for bone health and muscle function, thus contributing to the overall nutritional profile of the functional food.

The viability of LM in bile salt solution is shown in Figure 3. The results indicate that encapsulated L. plantarum experienced a decrease of 1.11 log CFU/g after 6 hours of incubation, compared to a 1.74 log CFU/g decrease for free cells. Encapsulation in skim milk and maltodextrin significantly improves *L. plantarum*'s resistance to challenging conditions such as acidic pH and bile salts. Previous studies have confirmed that encapsulation can protect probiotic bacteria in bile solutions [14]. These findings align with previous research, which showed that microencapsulation improves the viability of *L. plantarum* in intestinal juice. For example, [6] reported similar results in protecting probiotic bacteria using skim milk and maltodextrin, while [15] found that encapsulating *L. acidophilus* ATCC-4356 in sodium alginate and carrageenan microcapsules significantly boosted its viability in simulated gastrointestinal conditions.

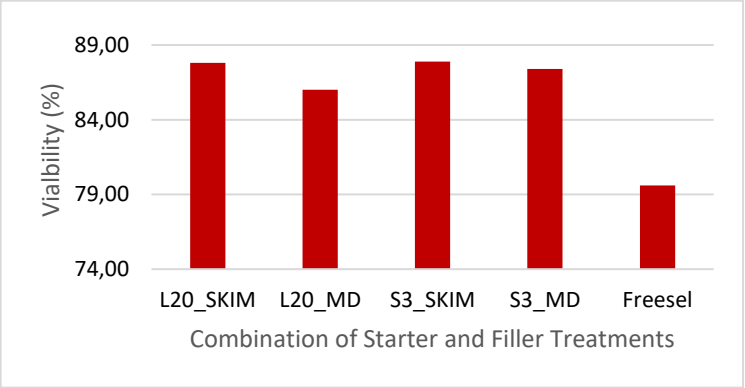

Fig. 3. Survival of *L. plantarum* microencapsulation compared to free cells simulated in bile salt solution/intestinal condition 6 h incubation

The resistance of probiotic bacteria to gastrointestinal conditions is influenced not only by the bacterial strain but also by the encapsulation technique, the addition of protective substances, or the food matrix used to deliver the probiotics [15]. In this study, skim milk and maltodextrin have shown potential as effective encapsulating materials for producing capsules that are resistant to the gastrointestinal tract.

4 Conclusion

The use of skim milk and maltodextrin as encapsulating material for *Lactobacillus plantarum* bacteria isolated from North Sumatra river buffalo milk has significantly increased viability after spray drying, incubation in simulated gastrointestinal conditions, and storage at refrigerated (4 ºC) and room temperature for 30 days. The viability of microencapsulated *L. plantarum* remained above the recommended threshold for probiotic food level (10^6 CFU/g). After 30 days of storage at 4 ºC the remaining viability was 10^7 CFU/g, while decreases in probiotic bacteria under gastrointestinal conditions were measured at 0,93 log CFU/g for gastric juice and 1.11 log CFU/g for intestinal juice simulations. These findings suggest that skim milk and maltodextrin can be effectively utilized in producing functional foods that support gut health.

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