International Journal of Technology v(i) pp-pp (YYYY)
Received Month Year / Revised Month Year / Accepted Month Year

International Journal of Technology

http://ijtech.eng.ui.ac.id

Enhancing Viability of Probiotic by Microencapsulation: A Case Study in Ice Cream

Abstract. Public awareness of digestive disorders have increased the interest to consume nutritious foods with probiotics. To ensure the effectiveness of probiotic bacteria, their resistance during processing and storage is essential. Microencapsulation by spray drying protects probiotic cells and maintains their viability. This study investigates the impact of spray dryer temperature and coating material on microencapsulated probiotic powder production and evaluates the viability of the microencapsulated probiotic when added to ice cream. Spray dryer temperature (130°C, 140°C, and 150°C) and coating agent ratios (maltodextrin:whey protein concentrate of 5:1 and 5:3) were varied, and results showed that the temperature and coating composition did not affect yield but influenced powder hygroscopicity and glass temperature, respectively. Probiotic microcapsule powder yield ranged from 16.77±0.32% to 21.65±3.34%. Encapsulated probiotic cells maintained high viability during 14-day storage in probiotic ice cream at a value of 80.56±0.80%, compared to 44.63±0.21% for non-encapsulated cells.

Keywords: maltodextrin; microencapsulation; probiotic; spray drying; whey protein concentrate

1. Introduction

In the modern era, unhealthy eating habits have become a leading cause of various health problems, particularly digestive issues. The rise in digestive system diseases can be attributed to the shift in modern lifestyles, which often involve the consumption of instant, high-fat, or low-protein foods (Heidarzadeh-Esfahani *et al*., 2021). These dietary choices have detrimental effect on the delicate balance of microflora in the small intestines (Aritonang *et al*., 2019). One effective approach to restore disturbed microflora in the small intestines is by incorporating probiotics into our diets, which actively suppress the growth of harmful bacteria within the digestive system (Yadav *et al.*, 2022). By consuming food and drinks rich in probiotics, the balance of digestive microbiome can be restored (Cunningham *et al*., 2021), leading to improved digestive health, enhanced immune function, gut maturation, improved energy metabolism, and nutrition uptake (Rusch *et al*., 2023). *Lactobacillus casei*, for example, is proven to help prevent diarrhea and constipation, provide relief from the symptom of irritable bowel syndrome, gingivitis, and assist in anti-inflammatory response (Maftei *et al*., 2024). However, for probiotics to be most beneficial, they should be consumed in a viable state since viability is proportional to probiotic effectiveness. Therefore, preserving the viability of probiotic bacteria is crucial during processing and storage. To achieve this, a preservation method, such as encapsulation, is required (Rajam and Subramanian, 2022).

Microencapsulation involves trapping probiotic cells within a protective capsule made of matrix material. This encapsulation process enhances the viability and prolongs shelf life of probiotic bacteria (Paula *et al.*, 2019), so that when the food products arrive at the consumers, the probiotics remain viable and beneficial. The encapsulated probiotics are stored inside the coating walls, and can be utilized for a specific duration, ensuring their viability when needed. Coating materials with low viscosity at high solid content are desired

because these materials yield higher concentration powders (Siccama *et al.*, 2021). Several coating materials have been reported, e.g. maltodextrin (MD), which is preferred because of its ability to dissolve in cold water (Sahlan *et al*., 2019), and whey protein concentrate (WPC) (de Araújo *et al*., 2020), a byproduct of cheese production (Dinika *et al*., 2019).

Considering that nutritious foods often have unfavorable taste, it is necessary to innovate and develop healthy food options that contain probiotics. One such innovation is probiotic ice cream. Ice cream, a frozen treat made from cream, sugar, and fat with various flavors, is a widely enjoyed dessert (Gozan *et al.*, 2020). By employing microencapsulation, the viability of probiotics in ice cream can be preserved throughout the ice cream-making process, storage, and consumption. Previous efforts in probiotic ice cream formulation have been done by Afzaal et al. (2019) using sodium alginate and carrageenan, but none have specifically used WPC, MD, or spray drying as the method of encapsulation. The spray-drying method is employed to process food materials because of its fast, flexible, and straightforward nature of the process (Shofinita *et al.*, 2023). This research aims to create microencapsulated probiotic powder that can be used as an additive in the production of probiotic ice cream.

2. Methods

2.1. Materials

Probiotic culture was obtained from fermentation drink "Yakult" (PT Yakult, Indonesia). Materials used in this study include maltodextrin (MD) of dextrose equivalent (DE) value 11 (Lihua Starch, China) and whey protein concentrate (WPC) (Davisco, USA).

2.2. Preparation of Probiotic Cultures

Lactobacillus casei was inoculated from fermentation drink. MRS broth and nutrient agar were used as media for inoculation. The probiotic inoculation process was started by growing the bacteria in a petri dish. The probiotics were then transferred to agar slant for another inoculation step. The presence of probiotics was checked using bacterial staining test method. Color test was carried out using Crystal giving *L. casei* bacteria a deep blue color. Finally, the probiotics were transferred into MRS broth before being mixed with the feed solution.

2.3. Preparation of Feed Solutions

Feed solutions were made by mixing coating material solutions with the probiotics. The coating material solution was prepared by mixing MD, WPC, and water at varied ratios. The solution was heated at 100^{o}C and stirred continuously until homogeneous. Then, 270 mL of coating material solution was added to 30 mL MRS Broth at room temperature. The number of probiotic cells was calculated by counting chamber method and the data was used as the initial number of probiotic cells.

2.4. Microencapsulation by Spray Drying

The feed solution was fed into a spray dryer (Procept 4M8-TriX Spray Dryer, Belgium) with varying inlet temperatures (130-150^{o}C). The feed and air flow rates used were 330 mL/hour and 0.3 m^3/min, respectively, with nozzle size of 1 mm. The microcapsule powder in the collecting vessel, as illustrated in Figure 1, was then weighed, and compared with the solid content in the liquid feed to determine the spray drying yield. The yield was calculated using Equation 1.

$$Yield\ (\%) = \frac{mass\ of\ the\ probiotic\ microcapsule\ powders}{solid\ mass\ of\ the\ feed\ solution} \quad (1)$$

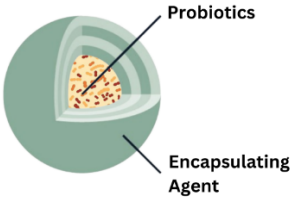

Figure 1 Illustration of a probiotic microcapsule.

2.5. Probiotic Cell Viability Analysis of Microcapsule Powder

Viability was analyzed according to Arepally *et al.* (2019). The number of probiotic cells in microcapsule powder was calculated by disolving 1 gram of the powder into a 10 mL volumetric flask using distilled water. Calculation of the number of probiotic cells was done by the counting chamber method. Cell viability was calculated using Equation 2.

$$Cell\ Viability\ (\%) = \frac{initial\ cell\ number - death\ cell\ number}{initial\ cell\ number} \quad (2)$$

2.6. Moisture Content Analysis of Microcapsule Powder

Moisture content was analyzed according to Wardani *et al.* (2007). Several grams of each sample were dried in an oven for 1 hour at 105°C. After heating, the samples were desiccated for 30 minutes and weighed using an analytical balance. Moisture content was calculated by Equation 3.

$$Moisture\ Content\ (\%) = \frac{initial\ sample\ weight - sample\ weight\ anfter\ drying}{initial\ sample\ weight} \quad (3)$$

2.7. Hygroscopicity Analysis of Microcapsule Powder

Hygroscopicity was analyzed by desiccating one gram of powder sample at 25°C with saturated NaCl solution. Weighing was carried out until equilibrium was reached. Hygroscopicity (%) was expressed in 1 gram of adsorbed moisture per 100 grams of dry solid (g/100 g) using Equation 4.

$$Hygroscopicity\ (\%) = \frac{\Delta m/(M+Mi)}{1+\Delta m/M} \quad (4)$$

Where Δm is the increase in mass after equilibrium (g), M is moisture content (%), and Mi is the initial moisture content (g).

2.8. Color Analysis of Microcapsule Powder

L*, a*, b* color parameters were measured using the CIE system using a CHN Spec Colorimeter. Total color difference was calculated by comparing spray-dried powder with commercial probiotic powders using Equation 5.

$$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{0,5} \quad (5)$$

Where ΔE is total color difference, L* is brightness value, a* is red/green color value, and b* is blue/yellow color value.

2.9. Powder Morphology

Morphology analysis was conducted using a scanning electron microscope (JEOL JSM-6510A, Japan). Probiotic powder was sputtered with gold and analyzed using magnifications of 400x, 1000x, 2000x, dan 5000x.

2.10. Production of Probiotics Ice Cream and Probiotic Cell Viability Analysis

Instant vanilla-flavored ice cream dough was added with the best variety of encapsulated probiotic powder. Ice cream powder was weighed and mixed with 150 mL of cold water. Then, the dough was shaken for 5-10 minutes at high speed and the probiotic powder was added before freezing. In addition, probiotic ice cream without encapsulation was also made with the addition of bacteria by mixing ice cream dough with liquid bacteria culture to compare cell viability. All handling and processes have complied with food safety standards and have been done in a sterile, non-contaminated food engineering laboratory in accordance with FSSC 22000 standards. The ice cream was stored for 14 days of storage. During storage, the number of cells in probiotic ice cream with and without encapsulation was counted to determine the probiotic cell viability (Arepally *et al.*, 2019). The schematic diagram in Figure 2 illustrates the whole probiotic ice cream production process.

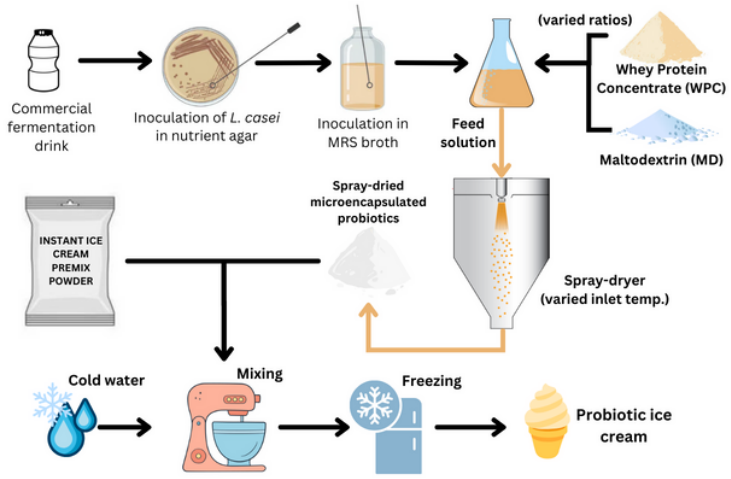

Figure 2 Schematic diagram of probiotic ice cream production.

2.11. Statistical Analysis

The data in this study were obtained from two replicates for each experiment and are presented as mean ± standard deviation. Differences were tested for significance by ANOVA (p-value $\leqslant$ 0.05).

3. Results and Discussion

3.1. Effect Comparison of Coating Materials on the Number of The Probiotic Cell in Feed Solution

The effect of the amount of coating materials on the number of cells in the feed solution were compared and shown in Figure 3. No significant difference in probiotics amounts was observed between the use of 5:1 MD:WPC ratio versus 5:3 (p-value > 0.05). MD has the capability to form a viscous glass, which can protect probiotics from damage. It also enhances the stability of probiotics by trapping them within an amorphous microstructure. Moreover, MD serves as a coating material that contains nutrients that can modify the intestinal microflora and aid in the growth of probiotics (Yadav *et al.*, 2022). When combined with MD, WPC acts as a film-forming emulsifier and increases the mechanical stability of the capsule. It can be concluded that a higher concentration of MD in the coating material solution leads to a greater number of probiotics being trapped within the MD microstructure. The probiotic cell counts in the feed solution, with variations in the ratio of MD to WPC of 5:1 and 5:3, were measured to be 7.562±0.05 and 7.53±0.03

log cells/mL, respectively. These results indicate that the initial cell count exceeded 7 log cfu/mL cells, meeting the standards for probiotic products.

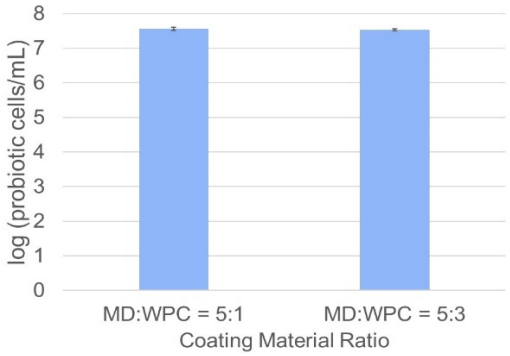

Figure 3 The amount of probiotics in the sample solution (log cell/mL) before spray drying variations depending on the ratio of the coating material MD:WPC.

3.2. Effect of Operating Conditions on the Yield of Probiotic Microcapsule Powder

The yield of powder was shown in Figure 4, ranging from 16.77±0.32% - 21.65±3.34%. No blocking phenomenon was seen in the spray dryer nozzle, which meant the powder particles did not stick together. Langrish *et al.* (2013) showed that higher temperature of the spray dryer inlet may increase the yield. However, yield can also be affected by glass temperature (Tg) and moisture content. The use of MD and WPC coating materials influences the Tg of the feed solution mixture, where an increase in the temperature of the inlet can increase the Tg of the coating material, accelerating water evaporation and reducing moisture content of the powder so that powder production can increase.

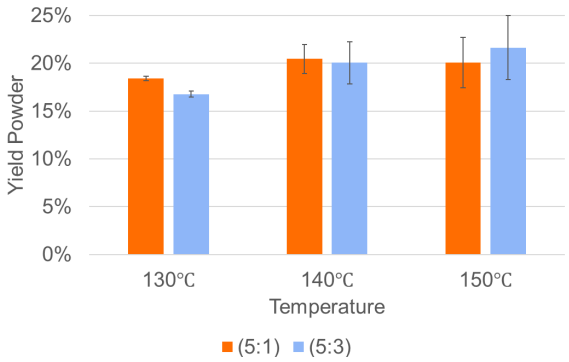

Figure 4 Yield (%) results from spray drying varied by the spray dryer inlet temperature and the ratio of coating materials MD:WPC.

The addition of probiotics in the feed solution might also increase powder solubility, thereby increasing powder yield during spray drying (Sundararajan *et al.*, 2023). Increasing WPC concentration in the feed solution can increase powder yield due to higher solids content. The addition of WPC in the feed solution also influences the ability of the particles to stick together due to the nature of WPC which can form protein networks that hold particles together (Chen *et al.*, 2020).

3.3. Effect of Operating Conditions on the Color of Probiotic Microcapsule Powder

The *L** index of all variations is shown in Figure 5a. The *L** index ranged from 93.48±1.19 - 96.49±0.49. The L* index is a color parameter that states dark with L* =0 and light with L*=100. A higher L* value indicates a whiter color (Kang, 2011), and white powder is desired among food additives because it does not tamper much with the

product's original color. Based on the data, all variations have an index in the range of 90-100, showing that the powder produced has a near-perfect white color.

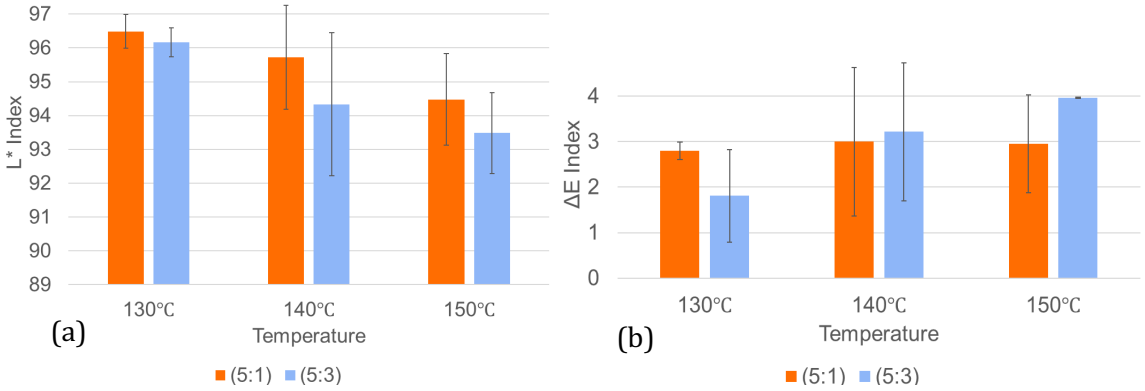

Figure 5 L* Index results **(a)** and ΔE Index results **(b)** from spray drying varied by the spray dryer inlet temperature and the ratio of coating materials MD:WPC.

Increasing the inlet temperature of the spray dryer decreased the whiteness of the powder because heating caused non-enzymatic Maillard browning (Ghosal *et al.*, 2022). In addition, the presence of a larger amount of MD coating material can result in the powder having a higher L* index because MD with white color has a higher composition. However, in this study, the difference in the amount of MD did not cause any significant color deviation (p-value > 0.05). In addition, the calculation of the total color difference (ΔE) was carried out to see the difference between the powder produced and commercial probiotic powder. The ΔE index of all variations is shown in Figure 5b. The ΔE index for the sample in the variation of temperature inlet spray dryer 130°C and MD:WPC ratio of 5:3 has values in the range 1-2, which indicate a distinguishable difference through close observation in the color, indicating color similarity towards the commercial powder.

3.4. Effect of Operating Conditions on the Moisture Content of Probiotic Microcapsule Powder

The moisture content percentage of all variations is shown in Figure 6. The moisture content values ranged from 3.60±0.48% - 5.26±0.80%. The moisture content of the powder falls in the range of 2-6%, in accordance with the moisture content of powders in general (Jin *et* al., 2019). From Figure 6, an increase in the inlet temperature is shown to decrease the moisture content of the powder (p-value < 0.05) because it is easier for water to evaporate at higher temperatures. In addition, the presence of a larger amount of WPC coating material can decrease the moisture content. The use of WPC as coating material can reduce the water content due to its ability to form films that prevent moisture from entering the powder. Additionally, WPC also improves thermal stability and film strength, thereby extending shelf life and powder quality (Sun *et al.*, 2020).

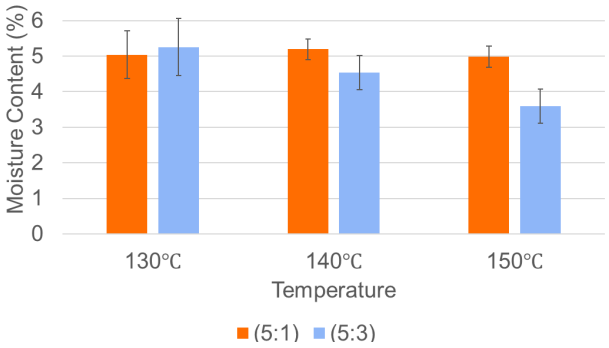

Figure 6 Moisture content (%) results from spray drying varied by the spray dryer inlet temperature and the ratio of coating materials MD:WPC.

3.5. Effect of Operating Conditions on the Hygroscopicity of Probiotic Microcapsule Powder

The hygroscopicity percentage of all variations is shown in Figure 7. The hygroscopicity values ranged from 6.20±0.73% - 9.82±0.40%. Increasing the spray dryer inlet temperature decreased the hygroscopicity and lowered the moisture left in the powder. In addition, the presence of a larger amount of WPC coating material can increase hygroscopicity. This happened because WPC can form a thin film that protects and maintains the chemical and physical stability of the powder. In addition, WPC has a high protein content, therefore increasing the affinity of water on the powder surface and increasing its hygroscopicity (Wang *et al.*, 2019).

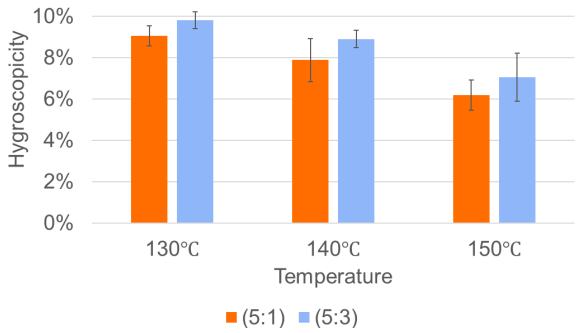

Figure 7 Hygroscopicity (%) results from spray drying varied by the spray dryer inlet temperature and the ratio of coating materials MD:WPC.

3.6. Scanning Electron Microscopy (SEM) of Probiotics Microcapsule Powder

Analysis of the surface morphological structure of the powder was carried out for sample in a variation of temperature inlet spray dryer 140℃ and MD:WPC ratio of 5:3. The result of SEM is shown in Figure 8.

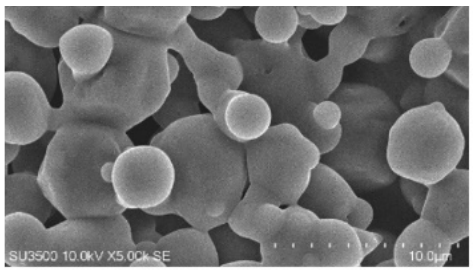

Figure 8 Surface morphological structure of probiotic microcapsule powders with 5000x magnification.

SEM imaging showed that the powder tends to be spherical in shape with smooth surfaces connected to each other, forming a water bridge phenomenon which indicates the presence of water molecules that form between the powder particles (Teixeira *et al.*, 2021). A water bridge can cause the morphology of the powder to be affected by the forces between adjacent particles, causing an increase in the forces between the particles and resulting in agglomeration (Shofinita *et al.*, 2021). MD has hygroscopic properties which cause the powder to agglomerate and stick when exposed to hot air, while WPC has adhesive properties which cause particles to stick together. The presence of protein in WPC can also form a gel or protein network on the particle surface which can also cause agglomeration. Based on the picture, there are no visible cracks on the surface of the powder due to the spray drying process, resulting in a dry and structurally strong outer layer of the powder. Hydrolized starches such as MD have properties that can maintain powder stability and avoid cracking by inducing faster skin formation and increasing glass

temperature (Siccama *et al.*, 2021), while WPC as a protein material can help bind particles so that the agglomeration becomes more stable (Mishra *et al*., 2019).

3.7. Effect of Operating Conditions on the Viability Cell of Probiotic Microcapsule Powder

Cell viability results were shown in Figure 9. In this study, the analysis of probiotic cell viability in the powder involved comparing the number of viable cells before and after the spray drying process. The probiotic cell viability ranged from 44.58±0.33 - 82.92±4.30 %. Increasing the spray dryer inlet temperature resulted in decreased cell viability, while a higher ratio of WPC increased viability (p-value < 0.05).

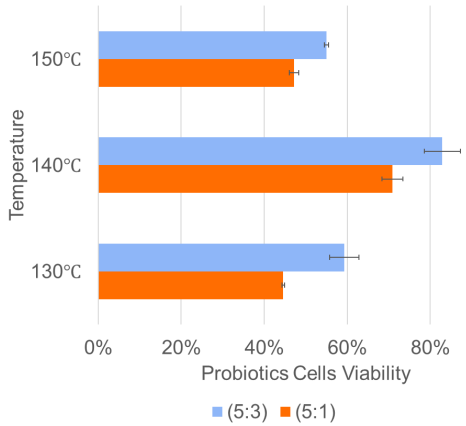

Figure 9 Cell viability (%) results from spray drying varied by the spray dryer inlet temperature and the ratio of coating materials MD:WPC.

The best protection for microencapsulation was achieved with 5:3 ratio of MD:WPC. The formulation of probiotic microcapsule powder by complexing MD and WPC led to higher viability and emulsification ability of whey proteins. (Meena *et al*., 2021). WPC, which contains milk fat, also contributed to the support of cell viability. A higher ratio of WPC in the coating material provided better protection for the MD matrix and increased heat resistance. The viability of probiotic cells was affected by spray dryer inlet temperature, with higher temperatures leading to reduced viability due to thermal inactivation and dehydration. On the other hand, higher temperatures increased water evaporation and improved yield. Moisture content played a role in cell viability, with low moisture causing dehydration and high moisture promoting undesired microorganism growth. The spray dryer inlet temperature of 140℃ resulted in the best viability and stable moisture content. Additionally, all variations exceeded the required 7 log cells/mL, indicating successful protection of probiotics during spray drying.

3.8. Effect of Probiotic Microcapsule Powder Coating on Viability Cell During Ice Cream Storage Period

The viability of probiotic cells was tested by incorporating the spray-dried powder as an ice cream additive. The analysis focused on the best variety of the powder (MD:WPC=5:1, 140℃). The viability of probiotic cells in the ice cream was evaluated over a 14-day period, with testing conducted twice per week. In addition to ice cream with encapsulated probiotic powder, probiotic ice cream without encapsulation was also prepared. The viability of probiotic cells without encapsulation was 44.63±0.21%, while cells with encapsulation reached 80.56±0.80%. The viability of non-encapsulated probiotic cells significantly decreased during the 14-day storage period, whereas encapsulated probiotic cells maintained a relatively constant viability level despite a slight decrease, as shown in Figure 10.

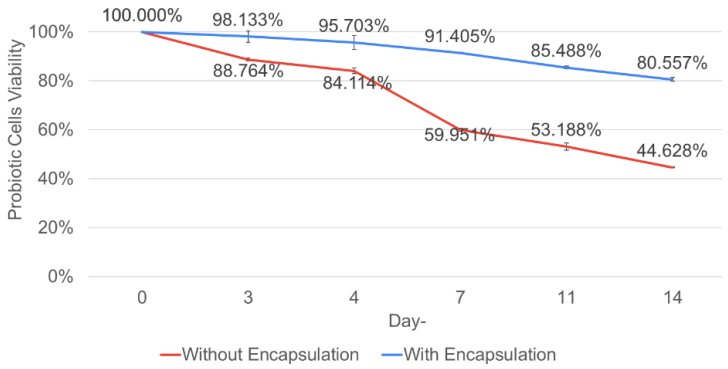

Figure 10 Viability curve of probiotic cells (%) in probiotic ice cream

The viability of probiotic cells in ice cream was higher when encapsulated because encapsulated probiotic cells possess a protective wall that stabilizes cellular structures and reduces environmental stress by limiting molecular movement, significantly increasing stability and survivability (Rodrigues *et al.*, 2020). Furthermore, the viability of probiotic cells during storage is greatly influenced by temperature (Jannah *et al*., 2022). Encapsulated probiotic microcapsule powder stored at lower temperatures exhibited better viability and stability as adverse chemical reactions occur at a slower rate. Additionally, storage below the glass transition temperature improves cell survival due to limited probiotic proliferation and chemical reactions within the cells, lowering internal mobility and oxygen diffusion. The low permeability of the coating material below glass temperature prevents oxygen ingress and preserves the core material. Below the glass transition temperature, food is in a stable state for a long time (glassy state). Above the glass transition temperature, the viscosity of the matrix decreases so that the glass and organic polymers become soft (rubbery state) (Shofinita *et al.*, 2023). Since probiotic ice cream is stored in a freezer with temperatures below the glass transition temperature of MD and WPC, it aligns with the data suggesting that encapsulated probiotic ice cream has prolonged shelf life, demonstrating excellent viability even after 14 days of storage. A comparison of probiotic cells viability in various research is presented in Table 1. The findings of Olivares *et al*. (2019) show a substantially higher percentage of probiotic viability after storage with Na-alginate as encapsulating agent compared to the results of this study. This may be due to the use of vibration which created a thicker layer of encapsulating agent around the probiotic, preserving its viability. However, vibration encapsulation was unable to produce powder at large quantities and using viscous encapsulating agent (Pisani *et al*., 2020; Whelehan and Marison, 2011), unlike spray drying. Thus, for industrial purposes, spray drying is far more favorable and effective with minimal viability trade-off.

Table 1 Comparison of encapsulation method, encapsulant, probiotic, and probiotic viability in various research.

Research	Encapsulant	Method of Encapsulation	Bacteria	Viability after 14 days storage
Pourjafar *et al.* (2020)	Chitosan	Extrusion	*L. acidophilus*	6.29 ± 3.91%
	Chitosan	Extrusion	*L. rhamnosus*	8.29 ± 5.33%
Olivares *et al*. (2019)	Na-Alginate	Vibration	*L. casei*	>91%
Yonekura *et al*. (2014)	HPMC	Spray Drying	*L. acidophilus*	1.06%
	Na-Alginate	Spray Drying	*L. acidophilus*	2.54%
This research	MD and WPC	Spray Drying	*L. casei*	80.56±0.80%.

4. Conclusions

This research has successfully created a stable microencapsulated probiotic powder to be used as ice cream additive. All operating variations in this research have no detrimental effect on probiotic powder yield, physical properties, and viability. Microencapsulation using MD and WPC saw an almost two-fold increase of probiotic viability during storage compared to no encapsulation, proving its feasibility in application and worthwhile to be studied further.

Acknowledgement

This work was supported by the Faculty of Industrial Technology, Institut Teknologi Bandung (ITB), Indonesia through The Research, Community Service, and Innovation Program (Program Penelitian, Pengabdian Masyarakat, dan Inovasi ITB).

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